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Preliminary Phytochemical Screening of Flowers, Leaves, Bark, Stem and Roots of *Rhododendron arboreum*

[1]*Mohammad Nisar,* [1]*Sajid Ali and* [2]*Muhammad Qaisar*

[1]Institute of Chemical Sciences, University of Peshawar, Peshawar - 25120, Pakistan
[2]Medicinal Botanic Centre, PCSIR Laboratories Complex, Peshawar - 25120, Pakistan

Abstract: *Rhodendron arboreum* is a plant of northern area of Pakistan was screened for its active chemical ingredients. Preliminary phytochemical screening of various parts viz flowers, leaves, bark, stem and roots, using conventional natural products identification tests indicated the presence of different classes of secondary metabolites such as alkaloids, steroids, flavonoids, terpenoids, anthraquinones, phlobatanins, saponins, glycosides, tannins and reducing sugars. These secondary constituents vary in type in different parts of the plant. The presence of these secondary metabolites signifies the potential of *Rhodendron arboreum* as a source of therapeutic agent. Infrared spectroscopic analysis of the methanolic extract of flowers leaves, bark, stem and roots of *Rhodendron arboreum* indicated the presence of O-H, C=O, C-H, C=O, C=C, NH def, NO_2 and C-O-C bond stretching. The medicinal values of *Rhodendron arboreum* are due to the presence the detected metabolites.

Key words: *Rhodendron arboreum* · Phytochemical screening · Infrared spectroscopic

INTRODUCTION

Plants consist of a number of biologically active ingredients therefore they are used for the treatment of a large number of infectious diseases [1-5]. These biologically active ingredients are alkaloids, flavonoids, steroids, glycosides, Terpenes, tannins and phenolic compounds [6-10]. *Rhododendron arboreum* a member of the family Ericaceae is one of medicinally important plant. The genus *Rhododendron* consists of 1000 species which are distributed throughout the world, mostly concentrated in China, India, Malaysia and Nepal [11, 12]. *Rhododendron arboreum* has a number of uses in folk medicine. The dried flowers of *R. arboreum* are highly efficient in the treatment of diarrhea and blood dysentery [13]. The young leaves of *R. arboreum* are poisonous to some extent but still they have medicinal properties and are used in alleviating headache when applied on forehead [14]. Flowers of *R. arboreum* are used for the preparation of squash and heart wood is grind to make 'khukri ', handles etc [15].

Chemical analysis of the leaves of *R. arboreum* revealed the presence of four biologically active compounds viz epicatechin, synergic acid, quercitine-3-O-galactoside and quercitirine by using validated HPTLC method [16]. Recently three biologically active phenolic compounds viz quercitine, rutin and coumaric acid were reported in the flowers of *R. arboreum* by using high performance thin layer chromatography (HPTLC) [17].

In the present communication the preliminary phytochemical screening and IR spectroscopic data of the methanolic extract of flowers, leaves, bark, stem and root of this plant is described to find the various active chemical ingredients.

MATERIALS AND METHODS

Plant Material: The plant *R. arboreum* was collected from Seran valley (Khyber Pakhtoonkhwa, Pakistan) in the month of February 2011. A voucher specimen for this collection has been deposited in the National Herbarium of Peshawar University, for future reference.

Extraction: The shade dried plant material such as flowers (1Kg), leaves (1Kg), bark (5Kg), stem (5Kg) and roots (5Kg) were crushed into small pieces and finally pulverized into fine powder. The plant materials were soaked in methanol with occasional shaking, at room

Corresponding Author: Mohammad Nisar, Institute of Chemical Sciences, University of Peshawar, Peshawar - 25120, Pakistan. Tel: +92-91-5854660, Fax: +92-91-9216652.

temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at 40°C temperature using rotary evaporator to give thick syrup that constituted the plant crude methanolic extract.

Phytochemical Screening: Chemical tests were carried out on the diethyl ether, ethyl acetate, chloroform and water extracts of the seeds *R.arboreum* using standard procedures to identify the constituents as described by Sofowora [8], Trease [9] and Evans and Harborne [10].

Alkaloids: About 0.2g of the extracts was wormed with 2% H_2SO_4 for two minutes. It was filtered and a few drops of Dragondorff reagent were added. Orange red precipitate indicated the presence of alkaloids.

Tannins: A small quantity of each extracts were mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added to the filtrate. A dark green solution indicates the presence of tannins.

Anthraquinones: About 0.5 g of the extract was boiled with 10 % HCl for few minutes in water bath. It was filtered and allowed to cool. Equal volume of CHCl3 was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Formation of rose-pink color indicates the presence of anthraquinones.

Glycosides: The extracts were hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of glycosides.

Reducing Sugars: The extracts were shaken with distilled water and filtered. Then boiled with few drops of Fehling, s solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

Saponins: About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Flavonoids: Extract of about 0.2 g was dissolved in diluted NaOH and Hcl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

Phlobatanins: The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

Steroids: 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H_2S0_4. The colour changed from violet to blue or green in some samples indicate presence of steroids.

Terpenoids (Salkowski Test): 0.2 g of the each extract was mixed with 2 ml of chloroform ($CHCl_3$) and concentrated H_2SO_4 (3ml) was carefully added form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

IR Spectroscopy Analysis: Infrared Spectroscopy of Shimadzu Corporation of model IR prestige 21 was used. The extracts were scanned in accordance with ASTM 1252-98. A drop of each extract was applied on a sodium chloride cell to obtain a thin layer. The cell was mounted on the FT IR and scanned through the IR region.

RESULTS

The extractive values and the percent yield of the methanolic extracts of different parts of *R.arboreum,* such as flowers, leaves, bark, stem and roots are given in Table 1. The phytochemical screening of different extracts of *R.arboreum* are listed in Table 2.

The IR spectroscopic analysis of the crude extracts of flowers, leaves, bark, stem and roots of *R.arboreum* gave the following characteristic absorption peaks as shown in Table 3.

DISCUSSION

The result of the extractive value of different parts such as flowers, leaves, bark, stem and roots of *R.arboreum* showed that methanolic extract of bark (33%) contain a greater proportion by mass of the component compounds. The rest of the parts have percent extractive value in the order as follow flowers (24.35%), leaves (11.3%), roots (5.92%) and stem (4.4%) as shown in Table 1.

The preliminary phytochemical screening results of *R.arboreum* extracts of various parts such as flowers, leaves, bark, stem and roots showed the presence of

Table1: Extractive values of the different parts of *R.arboreum* in methanol

Parts	Weight of Crude extracts (g)/ 20g plant material	% yield
Flowers	4.870	24.350
Leaves	2.260	11.300
Bark	6.600	33.000
Stem	0.880	4.400
Roots	1.185	5.925

Table 2: Phytochemical screening of the different parts of *R.arboreum*

Chemical components	Flowers	Leaves	Bark	Stem	Roots
Alkaloids	-	-	+	+	+
Steroids	+	+	+	+	+
Terpenoids	+	+	+	+	+
Flavonides	+	+	-	-	-
Anthraqunones	-	+	-	+	+
Tannins	+	+	+	+	+
Phlobatanins	-	-	-	-	-
Saponins	-	-	+	+	+
Glycoside	-	-	-	+	+
Reducing sugars	-	-	+	+	+

Key = - = absent, + = Present

Table 3: IR Spectroscopic data different extract of *R.arboreum*

	Region (cm^{-1})				
Components	Flowers	Leaves	Bark	Stem	Roots
OH	3296.35	3381.21	3365.78	3290.59	3323.35
CH	2920.23	2924.09	2926.09	2926.01	2924.09
C=O	-----	1728.22,1683.86	1683.86	1732.08, 1687.71	1730.15,1883.86
C=C	1604.77	1614.42	1608.63	1606.70	1606.70
NH def	1558.48	1558.48	1558.48	1558.48	1558.48
NO2	1435.04,1361.74	1456.26,1377.17	1444.68,1375.25	1444.68,1373.32	1444.68,1373.32
C-O-C	1199.72,1029.99	1070.49,1039.63	1105.21,1033,85	1041.56	1064.71,1043.49

various bioactive secondary metabolites constituents (Table 2). Phytochemical study of the flower extract showed that flowers contained flavonoids, steroids, terpenoids and tannins while alkaloids, anthraquinones, phlobatanins, saponins, glycoside and reducing sugar were absent. Leaves comprised of flavonoids, steroids, terpenoids, anthraquinones and tannins while alkaloids, phlobatanins, saponins, glycoside and reducing sugar were absent. Bark of *R. arboreum* consisted of alkaloids, steroids, terpenoids, tannins, saponins and reducing sugar while flavonoids, anthraquinones, phlobatanins and glycosides were absent. Similarly stem of *R. arboreum* contained alkaloids, steroids, terpenoids, anthraquinones, tannins, saponins, glycosides and reducing sugar while flavonoids and phlobatanins were absent. The extract of root contained alkaloids, steroids, terpenoids, anthraquinones, tannins, saponins, glycosides and reducing sugar while flavonoids and phlobatanins were absent.

These components are well known to have curative activity against several human problems such as diuretic, choleretic, spasmodic, chronic eczema, diarrhea, dysentery and menstrual disorders [18, 19] and therefore could suggest the folk use of this plant.

The presence of the secondary metabolites functional groups in each extract was confirmed by IR spectroscopy analysis [20], the results suggested the presence of functional groups. The IR spectrums (Table 3) exhibited strong absorption bands at 3296.35, 3381.21, 3365.78, 3290.59 and 3323.35 cm^{-1} for flowers, leaves, bark, stem

and roots, respectively this finding indicated to the presence of O-H stretching, Hydroxyl groups; 2920.23, 2924.09, 2926.09, 2926.01and 2924.09 cm^{-1} for flowers, leaves, bark, stem and roots, respectively also, the results indicated to the presence of saturated C-H stretching; 1728.22, 1683.86, 1683.86, 1732.08, 1687.71 and 1730.15,1883.86 cm^{-1} for leaves, bark, stem and roots respectively. In addition the results indicated to the presence of C=O stretching; 1604.77, 1614.42, 1608.63, 1606.70 and 1606.70 cm^{-1} for flowers, leaves, bark, stem and roots respectively. The data obtained pointed to the presence of C=C stretching; 1558.48, 1558.48, 1558.48, 1558.48 and 1558.48 cm^{-1} for flowers, leaves, bark, stem and roots, respectively. The results revealed to the presence of NH stretching; 1435.04- 1361.74, 1456.26-1377.17, 1444.68-1375.25, 1444.68-1373.32 and 1444.68-1373.32 cm^{-1} for flowers, leaves, bark, stem and roots respectively indicated the presence of NO stretching and 1199.72-1029.99, 1070.49-1039.63, 1105.21-1033.85, 1041.56 and 1064.71-1043.49 cm^{-1} for flowers, leaves, bark, stem and roots respectively indicated the presence of C-O-C bond stretching.

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