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R. ARBOREUM FLOWER AND LEAF EXTRACTS: RP-HPTLC SCREENING, ISOLATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

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ABSTRACT

In the present study, preliminary i*n-vitro* anticancer screening of the alcoholic extracts of leaves (LE) and flowers (FE) of *Rhododendron arboreum* was performed against Crown Gall tumor and MCF-7 breast cancer cell lines. Both the extracts showed prominent inhibition in the development of Crown Gall tumor in potato discs while only LE was found to be significantly effective against MCF-7. Three terpenoids **(1-3)**, one sterol **(4)** and two flavonoids **(5-6)** were isolated from the LE of the *Rhododendron arboreum*. The flavonoidal compounds, quercetin and rutin were simultaneously identified from the flowers and leaves using high-performance thin-layer chromatography (HPTLC). Precoated silica-gel RP-18 F 254 S plates were used with a mobile phase of methanol: water: formic acid (55: 42: 03 v/v/v) and densitometric determination of these compounds were carried out at 254 nm in absorbance mode. Structures of all the isolated compounds were elucidated by spectroscopic methods like IR, PMR, CMR and Mass spectrometry.

Keywords: *R. arboreum*; Terpenoids, Quercetin, Rutin, Anticancer, HPTLC

INTRODUCTION

Rhododendron, the largest genus of the Ericaceae family, includes 1200 species distributed throughout Northeast Asia and Eurasia, Western Europe and North America[1]. Dried flowers of *R. arboreum* were used in the treatment of diarrhea and blood dysentery[2], and squash of the flowers was used for the treatment of mental retardation[3-4]. Traditionally its leaves were used in the treatment of gout and rheumatism[5]. *R. arboreum* possess various pharmacological activities like oxytocic, oestrogenic, prostaglandin synthetase inhibiting activity[6] and CNS depressant[7]. It has also been reported that this plant possesses hepatoprotective, antidiabitic, anti-inflammatory and antimicrobial activities[8-12]. Shaifulla *et al.* have isolated flavone glycoside, 5, 2′-dihydroxy-7-methoxy-4′-O-glucoside and dimethyl ester of terphthalic acid, from the leaves[13]. β-sitosterol, ursolic acid, quercetin and friedelin have been reported in the leaves and bark of the plant[14]. Harborne *et al.* identified uniform flavonoid pattern and quantitated various flavonoids such as gossypetin, kaempferol, myricetin, azaleatin, caryatin, dihydromyricetin, dihydroquercetin, dihydrokaempferol and coumarins in the leaf survey of 206 *Rhododendron* species, subspecies and varieties[15]. Harborne studied natural distribution of flavonol 5-methyl ethers in the leaves and petals of 50 species of *Rhododendron*[16]. Flavonoids constitute one of the most ubiquitous groups of all plant phenolics. They generally occur bound to sugars as glycosides. Due to their phenolic nature, they are easily detected on chromatogram or in solution due to change in colour, when treated with base or ammonia. Flavonoids contain conjugated aromatic systems and thus show intense absorption bands in the UV and visible regions of the spectrum [17-18]. Many clinical studies conducted in the last two decades have shown that flavonoids exert positive influence on the treatment of many serious diseases like cancer, oxidative stress, cardiovascular, microbial and viral infections, diabetes, etc. Many flavonoids containing

plants act as diuretics, hepatoprotectives, antioxidant, antiallergic, antithrombotic, antifungal, antiulcer, etc [19].

EXPERIMENTAL

Materials and methods

Leaves and flowers of *R. arboreum* were collected, identified and authenticated from Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (NBRI, CSIR), Lucknow, India (NBRI/CIF/72/2009). The authentic markers of quercetin and rutin were procured from S.D. Fine Chemicals, India. The TLC plates RP-18 F254 S (10 x 10 cm) (E. Merck, Germany) were used as such. All the chemicals and solvents used were of analytical and HPLC grade. Melting ranges were determined by melting point apparatus. IR spectra were recorded on Shimadzu FTIR-8400S in the range of 400-4000 cm^{-1} with KBr pellets. NMR spectra were obtained on a Bruker ADVANCE DRX 300 MHz spectrometer with TMS as an internal standard at room temperature (δ in ppm, *J* in Hz). ESIMS and DARTMS were carried out on a JEOL SX 102/DA-600 mass spectrometer. Camag, Linomat IV applicator was used for sample application. Camag Twin trough glass chamber (20 x10 cm) was used for the development of plates and Camag TLC scanner-3 equipped with win CATS 1.4.4.6337 version software was used for interpretation of data. Fractions were monitored by TLC (silica gel $60F_{254}$, E. Merck, Darmstadt, Germany) and spots were visualized using iodine vapors and UV light. Solvents were distilled prior to use.

Preparation of extracts

Alcoholic (ethanol) extracts of flowers and leaves of *R. arboreum* were prepared by continuous hot percolation (8 hrs, 70-80 ºC) method using soxhlet apparatus. The solvent of both the extracts was evaporated under vacuum using rotary evaporator (IKA-RV 10 basic), and were stored in a desiccator for further use.

Preliminary phytochemical investigation

The alcoholic extract of the leaves of *R. arboreum* was subjected to preliminary phytochemical tests to identify the various phyto-constituents like saponins, phytosteroids, flavanoids, carbohydrates and amino acids[20-21].

Crown Gall Tumor inhibition assay (Potato disc anti-tumor assay)

Crown gall is a neoplastic disease of plants, which is induced by a gram-negative bacterium *Agrobacterium tumefaciens*. The bacteria possess large Ti (tumor inducing) plasmids which carry genetic information (T-DNA) that transform normal, wounded, plant cells in to autonomous tumor cells. Since, the mechanism of tumor induction is similar to that in animals, this test system has been used to evaluate and pre-screen the antitumor/cytotoxic properties of natural products.

A loop full of *Agrobacterium tumefaciens* was inoculated into sterile autoclaved Yeast Mannitol broth, in a screwed test tube and incubated at 30°C for 48 hours. Yeast Mannitol Agar medium (as per MTCC) was used for the activation of *Agrobacterium tumefaciens*.

Test sample preparation

About 16 mg of FE (flower extract) and LE (Leaves extract) were dissolved in 2 ml sterile water for injection in a sterile test tube separately and each was filtered through a millipore filter into another sterile tube. Then, 0.5 ml of these solutions was added to 1.5 ml sterile water for injection. Thus, final concentration of 2 mg/ml of FE and LE were prepared. Further, 1 mg/ml of FE and LE were prepared by double dilution with sterile water. Sterile water for injection was used as control.

Method

Fresh potato tubers of moderate size were surface sterilized by immersion in liquid bleach (Clorox) for 20 minutes; a core cylinder of tissue was removed from the potato by means of a surface sterilized (ethanol and flame) cork borer (6 mm diameter). The 2 cm ends of each potato cylinder were discarded and the remainder of the cylinder was cut into discs of uniform thickness with surface-sterilized knife. The discs were then transferred to 1.5% agar plate (1.5 g of agar was dissolved in 100 ml of distilled water, autoclaved and 20 ml poured into each sterile petri dish). Each plate contained 4 discs and 3 petri plates were prepared for each experimental sample. About 2 ml of broth culture of *Agrobacterium tumefaciens* (a 48 hours culture containing 5x 10^{9} cells/ml) was added aseptically in 2 ml of each test sample. Control

was prepared in the same manner but sterile water for injection was used in the place of test sample. One drop (0.05ml) from each sample as well as control tubes was used to inoculate the respective potato discs and was spread over the disc surface with the help of disposable micro tips fitted with micropipette.
The plates were incubated at room temperature for twelve days, the lid were sealed to minimize the moisture loss. After twelve days of inoculation, the tumors were counted after staining with Lugol's solution, under a dissecting microscope. The tumor cells were lacked starch. The number of tumors in the control was used as a reference for determining the activity[22].

Evaluation

$$\% \text{ Tumor} = \frac{\text{Mean No. of tumor (Test sample)}}{\text{Mean No. of tumor (Control)}} \times 100$$

$$\% \text{ Tumor Inhibition} = 100 - \% \text{ Tumor}$$

Sulforhodamine B (SRB) assay for anti-cancer activity[23]

Sulforhodamine B (SRB) is a bright pink aminoxanthene dye. Under mildly acidic conditions, SRB binds to basic amino acid residues of TCA fixed proteins. It provides a stable end-point that does not have to be measured within any fixed period of time. Once stained and air dried, plates can be kept for months before solubilization and reading. This assay has proven particularly useful in large scale anti cancer drug screening.

Cell culture

MCF-7 (Human Breast Cancer Cell line) cells obtained from national center for Cell Science, Pune, India and being maintained in Tissue Culture department of Central Drug Research Institute, Lucknow, (India) were cultured in DMEM pH-7.4, containing penicillin (100 U/ml), streptomycin (100 µg/ml) and gentamicin (60 µg/ml), supplemented with 10% FCS and 10 mM HEPES was used for culturing the cell. The cells were cultured and sub cultured in T-25 tissue culture flasks at appropriate intervals in a humidified atmosphere of 5% CO_2 at 37°C in a CO_2 incubator.

Method

A fully confluent flask of MCF-7 cells were trypsinized and 10^4 cells/well were plated in a 96-well flat bottom plate in 200µl DMEM, pH 7.4, and allowed to attach for 24 hours at 37°C in a humidified CO_2 incubator. Subsequently, sample A and sample B dissolved in sterile Phosphate buffered saline (pH-7.4) and temoxifen (Standard) in ethanol were added at specified concentrations and further incubated for 48 h as before. The cells were then fixed in 50 µl cold 50% TCA and incubated for 1 h at 4°C. The supernatant was discarded and the plate was washed five times with deionized water and air-dried. Hundred microliters of 0.4% w/v sulforhodamine B (SRB) in 1% acetic acid was added to each well and incubated at room temperature for 30 min. Unbound SRB was removed by five washes with chilled 1% acetic acid and the plate was air-dried. Two hundred microliters of unbuffered 10 mM Tris base was added to solubilize the bound stain for 5 min at room temperature and OD (Optical Density) was read at 560 nm in a plate reader[24].

Fractionation, isolation and characterization

Alcoholic extract (100 g) of the leaves was suspended in distilled water (200ml) and then extracted in a separatory funnel with n-hexane, chloroform and n-butanol. All the fractions were concentrated under vacuum, using a rotary evaporator, and then placed in a desiccator at reduced pressure for complete drying. n-Hexane fraction (25 g) was subjected to silica gel column chromatography (60-120 mesh) and eluted with gradient n-hexane-ethyl acetate (100:0 to 90:10). A total of 84 fractions of 10 ml each were collected and similar fractions were pooled after monitoring their TLC. This afforded four pure compounds. Sub fraction one (95:05) was further purified by petroleum ether to obtain compound **1** (3.7 mg). Compounds **2** (2.6 mg) and **3** (16 mg) were obtained with the same composition of the mobile phase. The next sub fraction (90:10) was further purified by repeated silica gel column chromatography

($CHCI_3$:CH_3OH, 99:01) to get compound **4** (5.4 mg). Chloroform fraction (13 g) was subjected to silica gel column chromatography (60-120 mesh) and eluted with gradient chloroform-methanol (100:0 to 85:15). A pure compound (**5**, 3.2 mg) was obtained at 85:15 (chloroform-methanol) as light greenish yellow amorphous powder. Another pure compound (**6**, 14.7 mg) was isolated from aqueous fraction of the alcoholic extract of the leaves by solvent-solvent extraction method.

Table-1: Inhibition of Crown Gall tumor development by FE and LE of *R. arboreum.*

Samples (mg/ml)/ disc	Mean no. of tumor cells ± SD	% Tumors	% Inhibition of tumors
Control	84.83 ± 17.42	---	--------
FE			
2	42.33 ±15.1	50.49	49.51***
1	54 ± 14.72	64.41	35.59***
LE			
2	26 ± 9.3	31.01	68.99***
1	33.33 ± 4.8	39.75	60.25***

Crown Gall Tumor Inhibition effect of the LE and FE of *R. arboreum*. Values are Mean ± SD. The comparison of control Vs. extracts treated discs. *** $p < 0.001$. Each sample tested on 3 plates with 4 discs. n = 12

Ursolic acid (1)
White colored gummy amorphous powder, Yield 3.7 mg, mp 283°C; IR (KBr) cm^{-1}:3448, 3425, 2920, 2850, 1708, 1618 , 1461, 1251. ^{1}H-NMR ($CDCI_3$) δ: 0.922-1.975 (m), 2.25-2.39 (s), 3.76-3.78 (m), 5.30-5.40 (m). ESI-MS m/z: 457.0 (M+1), 455.0.

Compound (2)
A single compound was obtained from the eluent of n-hexane: ethyl acetate (95:05) as colorless fine crystals, after storing it overnight. These crystals were separate and washed with chilled n-hexane. Yield-2.6 mg. IR (KBr) cm^{-1}:3552, 3477, 3415, 2920, 2850, 1685, 1618 and 1461. ^{1}H-NMR ($CDCI_3$) δ: 0.598-2.196 (m), 3.09-3.18 (m), 4.49-4.61 (d, *J*=35.25), 5.06-5.11 (s). ESI-MS m/z: 708.2, 665.3, 664.3, 663.2, 409.2, 391.

3, 10-Epoxyglutinane (3)
Greenish white amorphous powder, Yield- 16 mg, mp 199 ºC, IR (KBr) cm^{-1}: 2921, 1461, 1190. ^{1}H-NMR ($CDCI_3$) δ: 0.740-2.016 (s), 3.66 (m). ESI-MS m/z: 426.44 (M^+), 425.43, 410.45, 409.45.

β-Sitosterol (4)
White-gummy amorphous powder, Yield- 5.4 mg, Melting range: 146-150 ºC; IR (KBr) cm^{-1}: 3406, 2920, 2852, 1618, 1381, 1033. ^{1}H-NMR ($CDCI_3$) δ: 0.650-2.032 (m), 2.191-2.283 (m), 3.485-3.556 (m), 5.343-5.357 (s). ^{13}C-NMR ($CDCI_3$) δ: 12.09-12.22 (d, *J*=39), 19.02 (s), 19.29-19.62 (m), 20.04 (s), 21.34 (s), 23.34 (s), 24.54 (s), 26.40 (s), 28.48 (s), 29.45 (s), 31.93 (s), 32.17 (s), 34.22 (s), 36.39-36.76 (s), 37.52 (s), 40.04 (s), 42.58 (s),46.12 (s), 50.41 (s), 72.06 (s), 121.94 (s), 141.02 (s). ESI-MS m/z: 416.1 (M+2), 415, 414, 412.

Quercetin (5)
Light greenish yellow amorphous powder, mp: >300 ºC, IR (KBr) cm^{-1}: 3417, 1662, 1612, 1560, 1521, 1458. ^{1}H-NMR ($CDCI_3$) δ: 3.346 (s), 6.180- 6.186 (s), 6.400-6.406 (s), 6.86-6.89 (d, *J*=8.4), 7.519-7.553 (s), 9.30-9.58 (s), 12.489 (s). ^{13}C-NMR ($CDCI_3$) δ: 93.80 (s), 98.62 (s), 103.46 (s), 115.51 (s), 116.05 (s), 120.42 (s), 122.40 (s), 136.19 (s), 145.51 (s), 147.25 (s), 148.15 (s), 156.58 (s), 161.17 (s), 164.33 (s), 176.29 (s). ESI-MS m/z: 304.09 (M+2), 303.08.

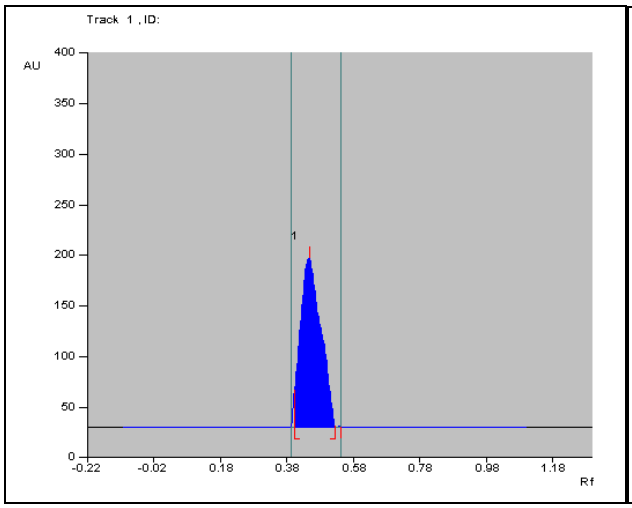

Fig. -1: Chromatogram of standard quercetin.

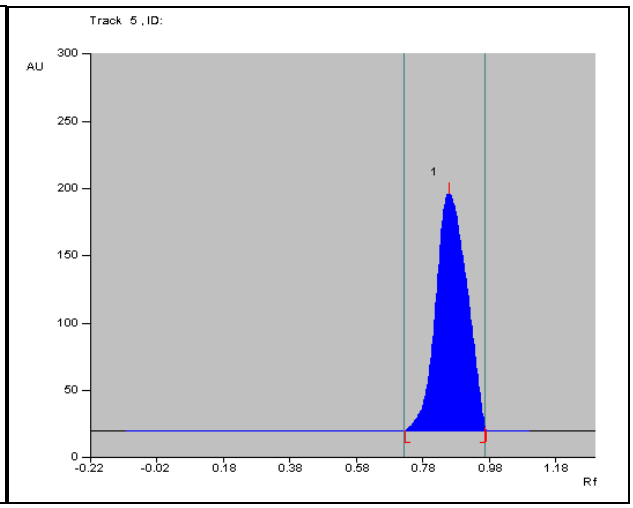

Fig. -2:Chromatogram of standard rutin.

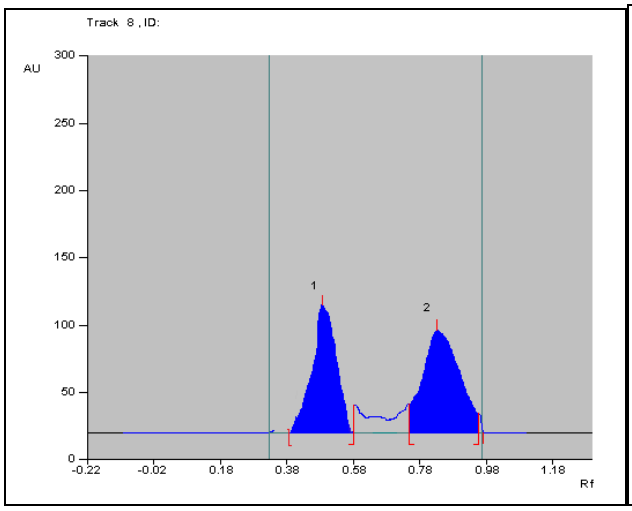

Fig. -3: Chromatogram of the LE of *R. arboreum.*

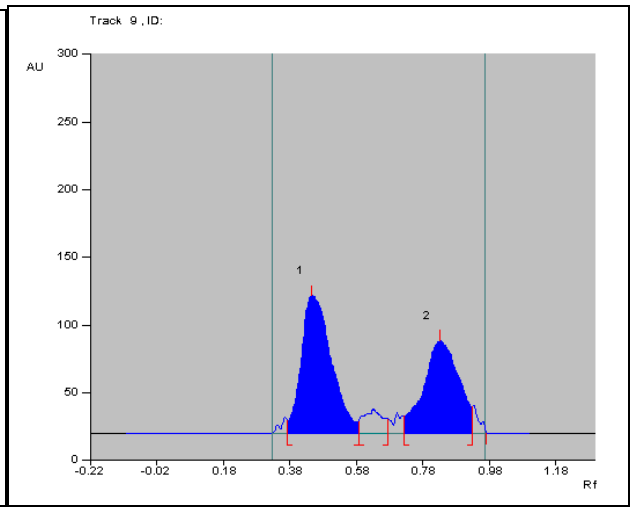

Fig. -4: Chromatogram of the FE of *R. arboreum.*

Simultaneous identification of quercetin and rutin in the alcoholic extract of the flowers and leaves by HPTLC

Preparation of standard solutions and samples

Standard stock solutions of quercetin (10 mg/ml) and rutin (1 mg/ml) were prepared by dilution in ethanol. FE and LE (100mg) of *R. arboreum* were taken and dissolved in ethanol (10 ml) and then 1 ml of each was diluted to 10 ml separately and filtered through 0.45µ Millipore filters. Working solutions of quercetin (1000, 500, 250, 125 and 62.5µg/ml) and rutin (100, 10, 1, 0.1 and 0.01 µg/ml) were prepared separately by serial dilution of stock solution using ethanol as the solvent.

Optimized chromatographic conditions

The standards and samples (1µl each) were applied using a manual TLC sampler on 10x10 cm TLC plate at 20 mm from X and 10 mm from Y axis and 5 mm spaces were left between adjacent spots. TLC plates were developed in a glass tank, which was pre-saturated with developing solvent system (methanol: water: formic acid, 55: 42: 03 v/v/v) up to the height of 7.5cm. The composition of the solvent system was optimized on the basis of the resolution of spots. After development, the plate was removed; air dried

and scanned in a TLC scanner at 254 nm with a slit width of 6 x 0.4mm and scanning speed 20mm/s. The evaluation of the plate was performed in the absorbance mode.

Rutin (6)

The 50 ml water soluble fraction of the alcoholic extract was concentrated under reduced pressure up to 20 ml and then refrigerated. Greenish-yellow amorphous powder settled down in the beaker, which was filtered and washed with chilled water. It was further purified with hot water[25], Yield- 14.7 mg; melting range: 192-195 ºC. IR (KBr) cm^{-1}: 3434, 2902, 1677, 1585, 1498, 1282. ^{1}H-NMR ($CDCI_3$) δ: 0.984 (s), 3.248 (s), 3.387 (s), 6.183 (s), 4.368-4.384 (s), 5.332 (s), 6.373 (s), 6.834-6.849 (s), 7.513-7.535 (s), 8.340 (s), 12.595 (s). ^{13}C-NMR ($CDCI_3$) δ: 17.81 (s), 67.06, 67.15, 68.31 (s), 70.02, 70.42, 70.59, 71.88, 74.12, 75.93, 76.48, 79.24, 100.81, 101.27 (s) 93.70 (s), 98.84 (s), 103.85 (s), 115.28 (s), 116.28 (s), 121.18 (s), 121.64 (s), 133.31 (s), 144.83 (s), 148.52 (s), 156.50 (s), 156.61 (s), 161.24 (s), 164.56 (s), 177.35 (s). ESI-MS m/z: 611.4 (M^+), 610.4, 609.5, 300.7.

RESULTS AND DISCUSSION

In the present study, extraction of the leaves and flowers of *R. arboreum* was carried out. The yields of alcoholic extracts of leaves and flowers were found to be 4.01%w/w and 6.9% w/w respectively. Both the extracts tested positive for the presence of various phyto-constituents like saponins, phytosteroids, flavanoids, carbohydrates and amino acids. *In-vitro* anti-cancer screening of the alcoholic extracts of the leaves and flowers of *R. arboreum* was first performed against crown gall tumor. Since the mechanism of tumor induction in potatoes is similar to that in animals. The FE and LE demonstrated dose-dependent significant activity against *A. tumefaciens* induced tumors on potato discs. In context of inhibition of tumor initiation and tumor development, LE has greater antitumor activity at each concentration than FE (Table-1). The results suggest that the potato disc assay is a safe, simple, rapid and inexpensive in-house screening method for natural products with antitumor activity. The anti-cancer effect of both the extracts against MCF-7 breast cancer cell lines using Sulphorhodamine assay method was found to dose-dependent (Table -2, 3). LE showed significant anti-cancer activity in comparison with TAM, while FE showed some decline in the OD (optical density) but effect was found to be statistically insignificant.

The simultaneous identification of quercetin and rutin from the leaves and flowers of *R. arboreum* was done by RP-HPTLC. Four pure compounds were isolated from n-hexane fraction of alcoholic extract of the leaves. Three of those were identified as terpenoids and the fourth one was identified as a phytosterol. One flavonoid was isolated from the chloroform fraction and one flavonoid from the aqueous fraction. All the compounds, except **4,** from n-hexane fraction showed positive Liebermann-Burchard test, indicating their terpenoidal nature. Compounds isolated from chloroform and aqueous fractions showed positive tests for flavonoid (Pew's and Shinoda tests). All the compounds were characterized by melting range, IR, PMR, CMR and Mass spectrometry.

Compound **1** was a white amorphous powder. The IR spectrum of **1** indicated the presence of OH (3425 cm^{-1}), C=C and C-O str. of COOH. ^{1}HNMR data showed the presence of carboxylic, hydroxyl and cycloalkane protons. Mass spectrum showed the M+1 peak. These data were similar to those of the constituent isolated previously[26].

Compound **2** was obtained as colourless crystals. This compound could not be identified, due to low yields to further spectral studies. It showed a positive test for terpenoids (Liebermann-Burchard test).

Compound **3** was a greenish-white amorphous powder. IR spectrum showed peaks at 2921 and 1190 cm^{-1} for C-H and C-O-C str. respectively. ^{1}HNMR data showed the presence of cycloalkane protons and the evidence for the oxide bridge between carbon atom 3 and 10, was provided by the chemical shift of the proton α to the cyclic ether. In the five membered ring systems, these protons gave a signal at δ 3.66. Since in the compound **3**, the signal for the lone proton appeared at δ 3.66, the oxygen bridge should be between C-3 and C-10 and thus the compound was identified as 3, 10-epoxy glutinane[27].

Compound **4**, a white gummy amorphous powder showed a positive test for sterol (Salkowski test). IR spectrum of **4** indicated the presence of OH (3406 cm^{-1}), CH (2920, 2852 cm^{-1}) and C=C (1618 cm^{-1}) ^{1}HNMR showed the signals for the protons of cycloalkane, hydroxyl and the proton α to the hydroxyl

group. ^{13}CNMR gave the signals of 29 carbons. Mass spectrum showed the M^+, M+1 and M+2 peaks at m/z 414, 415 and 416 respectively. Spectral data were found to be similar as reported in literature[28].

Table-2: Antiproliferative Effect of FE and LE of *R. arboreum* on MCF-7 cells using SRB assay

S. No.	Concentration of sample	Mean OD ± SEM
	TAM (µM/ml)	
1	1	1.66 ± 0.1129
2	5	1.67 ± 0.0401
3	10	1.53 ± 0.0660
4	15	1.21 ± 0.0387
5	20	0.92 ± 0.0712
6	25	0.19 ± 0.0040
	FE (µg/ml)	
7	1	2.16 ± 0.0394
8	10	1.72 ± 0.0714
9	25	1.70 ± 0.0788
10	50	1.63 ± 0.0958
11	75	1.56 ± 0.0354
12	100	1.45 ± 0.1020
	LE (µg/ml)	
13	1	1.97 ± 0.1142
14	10	1.78 ± 0.0841
15	25	1.75 ± 0.0814
16	50	1.50 ± 0.0693
17	75	1.46 ± 0.1141
18	100	1.41 ± 0.0165

Table-3: Linear regression analysis of SRB assay of FE and LE of *R. arboreum.*

Compounds	Regression Eq.	Correlation	p value
TAM	Y= 1.960 – 0.060 C	-0.916	<0.01
FE	Y= 1.60 – 0.0028 C	-0.424	NS
LE	Y= 1.832 – 0.0041C	-0.618	<0.01

The concentrations of TAM, FE and LE were subjected to obtained the best-fit regression line eq. Y=a+bc where a= intercept and b= the rate of change in O.D. w.r.t. the concentrations. In the table, TAM and LE have significant fit indicating that the O.D. and concentrations are linearly related. The –ve slope indicates the O.D. decrease with increase in concentrations. This was determined by comparing the slope of the regression lines the slopes of TAM and LE, that were significantly different with $p<0.01$.

Compound **5** was **a** light greenish yellow amorphous powder. IR spectrum of **5** indicated the presence of bonded OH (3417 cm^{-1}), C=O (1662 cm^{-1}), aromatic ring (1612 cm^{-1}) and C=C of cycloalkene at 1560, 1521and 1458 cm^{-1}. ^{1}HNMR spectrum showed the signals for the protons of phenolic and enolic hydroxyl groups and for the protons of aromatic rings. ^{13}CNMR gave the signals of 15 carbons. Mass spectrum gave the M+1 and M+2 peaks at m/z 303 and 304 respectively.

Rutin **(6)** and quercetin were simultaneously identified from the alcoholic extracts of the flowers and leaves using HPTLC. The method was a RP-HPTLC method. It makes use of silica gel RP-18 F 254 S stationary phase precoated on aluminium sheet. The mobile phase comprised of methanol: water: formic acid (55: 42: 03 v/v/v) which gave good separation between quercetin (Rf =0.43) and rutin (Rf=0.85). Figures 1-4 depict typical densitograms showing the resolution between them. Isolation and characterization of quercetin from flowers has been reported [12]. Rutin is being reported for the first time in the leaves. It was isolated from the aqueous fraction and characterized. IR spectrum of **6** indicated

the presence of bonded OH (3434 cm^{-1}), C=O (1677 cm^{-1}), aromatic ring (1585 cm^{-1}) and C=C of aromatic ring at 1498 cm^{-1}. ^{1}HNMR spectrum showed the signals for the protons of phenolic and enolic hydroxyl groups and for the protons of aromatic rings. ^{13}C-NMR gave the signals of the carbons of aglycone and sugar moiety. Mass spectrum gave the M+, M-1 and M-2 peaks at m/z 611, 610 and 609 respectively.

Thus the study reports that the leaves of *R. arboreum* contain terpenoids, phytosterols and flavonoids. Quercetin and rutin were successfully identified by RP-HPTLC in the leaves and flowers of the plant. Rutin is being reported for the first time in the leaves. These components may be responsible for the promising anticancer activity of the extracts, which has been determined by the crown gall tumor and SRB assays.

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