IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY OF FLOWER PLANT OF VARIOUS EXTRACT OF RHODODENDRON ADBOREUM LINN.

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Abstract

Aim: The study was designed to examine the *in vitro* antioxidant activities of pet.ether, ethyl acetate and ethanolic extract of plant flower of *Rhododendron arboreum* Linn. **Method:** The shade dried flower plant powder was extracted with pet.ether, ethyl acetate and ethanol by continuous hot percolation method using Soxhlet apparatus. **Result:** The antioxidant activity was determined by DPPH assay, Superoxide anion scavenging activity, iron chelating activity, hydroxyl radical scavenging activity, nitric oxide scavenging activity, FRAP assay, total antioxidant activity (phosphomolybdic acid method) at four different doses 125 to 1000 µg/ml with reference natural standard rutin, quercetin, ascorbate and EDTA respectively and total phenolic and total flavonoid content was analysed. **Conclusion:** An IC_{50} value was found that ethanolic extract of *Rhododendron arboreum* is more effective all antioxidant activity. These *in-vitro* assays indicate that this plant extract are better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: Antioxidant, *Rhododendron arboreum, In-vitro* antioxidant, Total flavonoid content, Total phenolic content.

Introduction

Rhododendron arboreum Linn is an evergreen shrub or small tree with a showy display of bright red flowers. The name 'RHODODENDRON' is derived from the Greek word 'RHODO' means rose & 'DENDRON' means tree. Rhododendron is the national flower of Nepal & is known as (Laligurans) & the state tree of Uttarakhand. It is called 'Burans, Bras, Buras or Barahke-phool' in local dialect. It is widely popular for the processed juice of its flowers which have gained market popularity as rhodojuice /

sharbat. The plant is found in the Himalayas from Kashmir eastwards to Nagaland. Various parts of the plant exhibited medicinal properties & it is used for the treatment of various ailments.

Rhododendron arboreum Linn plant belongs to genus *Rhododendron* and family *Ericaceae*. Commonly it is known as "Billi" and "Buransh" in hindi. The flowers of *R. arboreum* range in color from a deep scarlet, to red with white markings, pink to white. Bearing up to twenty blossoms in a single truss this rhododendron is a spectacular sight when in full bloom. It is reported that the bright red forms of this rhododendron are generally found at the lower elevations (Orwa *et al*., 2009). Flowers are showy, red in dense globose cymes (Chauhan, 1999). Calyx- fine cleft, Corolla-tube spotted funnel shaped, Stamens-hypozygnous declining, Filaments filiform, Anthers-ovate, Style-capitate (Paxton, 1834). It is one of the most traditional system of medicine in Ayurvedic and Siddha.

Sub species

- *Rhododendron arboreum* spp. Arboreum (red or rose red flowers) found in Western Himalayas.
- *Rhododendron arboreum* spp. Cinnamomeum (white, pink or red flowers) found in Central Himalayas.
- *Rhododendron arboreum* spp. Delavayii (red flowers) found in Eastern Himalayas.
- *Rhododendron arboreum* spp. Nilagiricum (red flowers) found in Nilgiri.
- *Rhododendron arboreum* spp. Zeylancium (orange red flowers) found in Sri Lanka.

Petals are eaten for their sour-sweet taste. Flowers are offered to deities in almost all religious functions. Fresh leaves are burnt along with leaves of species of **Juniper**/**Thuja**/ **Pinus**, for making smoke that is believed to be sacred and help in purifying air in West Kameng and Tawang districts of Arunachal Pradesh. Young leaves are applied to the forehead for alleviating headache. Flowers and bark are used to cure digestive and respiratory disorders.

Materials and Methods

a) Collection and authentication of plant material

The leaves plant of *Rhododendron arboreum* was collected from Himachel Pradesh, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamilnadu, India. The plant powdered materials were put away in a hermetically sealed holder. The flowers were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

b) Preparation of plant extract

The flower powder samples of *Rhododendron arboreum* were extracted with pet.ether, ethyl acetate and ethanol at temperature between 60-70ºC by using soxhlet extractor. The solvent was evaporated by rotavapor to obtained viscous semi solid masses.

***In vitro* antioxidant studies**

1. DPPH radical scavenging effect

The DPPH assay of flower part of the plant was measured using the method described by (Mensor *et al.*, 2001). The ethanolic extract was taken in different concentrations varying between 125 to 1000µgmL and results showed that the antioxidant activity, the percentage of inhibition. The absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging\ activity\ (\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

2. Nitric oxide radical scavenging activity

The ability of the plant extracts to scavenge the nitric oxide radical activity were determined by the method described by Green *et al.*, (1982). Nitric oxide radical generated from sodium nitroprusside in aqueous solution at optimum pH conditions interacts with oxygen to produce nitrile ions which can be estimated by the use of Griess Ilosvay reaction at 540 nm.

Scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100

3. Iron chelating activity

The iron chelating assay of flower plant extracts of *Rhododendron arboreum* were described by (Benzie and Strain, 1996). The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100

4. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging properties of ethanolic extract were determined by (Elizabeth and Rao, 1990). The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-Ascorbate-EDTA-H_2O_2 system (Fenton reaction). The scavenging activity on hydroxyl radical was calculated as follows:

Scavenging activity (%) = (1 - absorbance of sample/absorbance of control) × 100

5. Superoxide radical scavenging activity

The assay for superoxide anion radical scavenging activity was supported by riboflavin-light-NBT system (Winterbourne *et al.*, 1975). Ascorbic acid was used as standard. The scavenging ability of the plant extract was determined by the following equation:

Scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100

6. Total antioxidant activity (Phosphomolybdic acid method)

The antioxidant activity of the ethanolic extract of *Rhododendron arboreum* was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto *et al.*, 1999). Ascorbic acid was used as standard. The antioxidant capacity was estimated using following formula:

$$Totalantioxidant\ activity\,(\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

7. FRAP assay

The FRAP assay of whole plant extracts of *Rhododendron arboreum* were described by modified method of (Benzie and Strain, 1996). Readings of the coloured product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results were expressed in µM (Fe (II) /g) dry mass and compared with that of ascorbic acid.

8. Estimation of total phenol content

Total phenolic content of aerial part of the plant was measured using the method described by Mallick and Singh *et al.*, (1980).

9. Estimation of total flavonoids content

Total flavonoid content of aerial part of the plant was measured using the method described by Cameron *et al.*, (1943).

Results and Discussion

Invitro Antioxidant

1) DPPH Photometric Assay

The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge DPPH photometric assay was calculated as % inhibition and was compared with rutin used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **42.96%** in pet.ether, **60.35%** in ethyl acetate and **67.33%** in ethanol when compared to Rutin **69.83%** which is statistically significant at same concentration. The IC_{50} value was found to be **1190 µg/ml** for pet.ether, **610 µg/ml** for ethyl acetate and **455 µg/ml** for ethanolic extract of *Rhododendron arboreum* and for rutin it was **480 µg/ml**.

Table 1: Effect of various extracts of *Rhododendron arboreum* on DPPH assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	**Sample (Ethyl acetate extract)**	**Sample (Ethanolic extract)**	**Standard (Rutin)**
1	125	16.22 ± 0.051	18.08±0.014	38.56 ± 0.058	18.85 ± 0.076
2	250	30.36 ± 0.014	25.58±0.033	48.12 ± 0.076	22.08 ± 0.054
3	500	34.55 ± 0.012	43.64±0.041	51.98 ± 0.052	52.21 ± 0.022
4	1000	42.96 ± 0.070	60.35±0.020	67.33 ± 0.044	69.83 ± 0.014
		IC_{50} = 1190 µg/ml	**IC_{50} = 610µg/ml**	**IC_{50} = 455 µg/ml**	**IC_{50} = 480 µg/ml**

*All values are expressed as mean ± SEM for three determinations.

2. Superoxide anion scavenging activity

The ability of pet.ether, ethyl acetate and ethanolic extracts to superoxide anion scavenging activity was calculated as % inhibition and was compared with quercetin used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **51.34%** in pet.ether, **72.58%** in ethyl acetate and **78.65%** in ethanol when compared to quercetin **98.01%** which is statistically significant at same concentration. The IC_{50} value was found to be **930 µg/ml** for pet.ether, **340 µg/ml** for ethyl acetate and **200 µg/ml** for ethanolic extract of *Rhododendron arboreum* and for quercetin it was **60 µg/ml**.

Table 2: Effect of various extracts of *Rhododendron arboreum* on Superoxide anion scavenging activity

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	**Sample (Ethyl acetate extract)**	**Sample (Ethanolic extract)**	**Standard (Quercetin)**
1	125	30.43±0 .012	31.52 ± 0.021	40.68 ± 0.032	73.81 ± 0.006
2	250	37.81 ± 0.035	48.46 ± 0.033	52.86 ± 0.026	91.31 ± 0.011
3	500	43.98 ± 0.018	65.76 ± 0.042	65.12 ± 0.040	92.99 ± 0.024
4	1000	51.34 ± 0.022	72.58 ±0.018	78.65 ± 0.028	98.01 ± 0.012
		IC_{50} = 930 µg/ml	**IC_{50} = 340 µg/ml**	**IC_{50} = 200 µg/ml**	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

3. Iron chelating activity

The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge iron chelating activity was calculated as % inhibition and was compared with EDTA used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **51.78%** in pet.ether, **59.46%** in ethyl acetate and **63.42%** in ethanol when compared to EDTA **97.90%** which is statistically significant at same concentration. The IC_{50} value was found to be **970 µg/ml** for pet.ether, **490 µg/ml** for ethyl acetate and **280 µg/ml** for ethanolic extract of *Rhododendron arboreum* and for EDTA it was **65 µg/ml**.

Table 3: Effect of various extracts of *Rhododendron arboreum* on iron-chelating method

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	**Sample (Ethyl acetate extract)**	**Sample (Ethanolic extract)**	**Standard (EDTA)**
1	125	19.80 ± 0.022	26.68 ± 0.018	29.37 ± 0.035	58.68 ± 0.007
2	250	28.91 ± 0.018	39.12 ± 0.022	48.56 ± 0.028	65.87 ± 0.018
3	500	34.32 ± 0.040	50.23 ± 0.026	56.98 ± 0.020	83.83 ± 0.012
4	1000	51.78 ± 0.026	59.46 ± 0.030	63.42 ± 0.018	97.90 ± 0.019
		IC_{50} = 970 µg/ml	**IC_{50} = 490 µg/ml**	**IC_{50} = 280 µg/ml**	**IC_{50} = 65 µg/ml**

*All values are expressed as mean ± SEM for three determinations

4. Hydroxyl radical scavenging activity

The ability of pet.ether, ethyl acetate and ethanolic extracts to hydroxyl radical scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **52.08%** in pet.ether, **64.12%** in ethyl acetate and **68.80%** in ethanol when compared to ascorbate **55.23%** which is statistically significant at same concentration. The IC_{50} value was found to be **940 µg/ml** for pet.ether, **520 µg/ml** for ethyl acetate and **470 µg/ml** for ethanolic extract of *Rhododendron arboreum* and for ascorbate it was **410 µg/ml**.

Table 4: Effect of various extracts of *Rhododendron arboreum* on Hydroxyl radical scavenging activity

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	**Sample (Ethyl acetate extract)**	**Sample (Ethanolic extract)**	**Standard (Ascorbate)**
1	125	19.80 ± 0.048	26.34 ± 0.018	35.22 ± 0.056	26.87 ± 0.076
2	250	30.32 ± 0.026	40.44 ± 0.035	45.65 ± 0.044	30.30 ± 0.054
3	500	39.90 ± 0.032	49.78 ± 0.030	52.78 ± 0.038	60.64 ± 0.022
4	1000	52.08 ± 0.052	64.12 ± 0.028	68.80 ± 0.035	55.23 ± 0.014
		IC_{50} = 940 µg/ml	**IC_{50} = 520 µg/ml**	**IC_{50} = 470 µg/ml**	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

5. Nitric oxide scavenging activity

The ability of pet.ether, ethyl acetate and ethanolic extracts to nitric oxide scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **52.08%** in pet.ether, **60.10%** in ethyl acetate and **64.92%** in ethanol when compared to ascorbate **55.23%** which is statistically significant at same concentration. The IC_{50} value was found to be **920 µg/ml** for pet.ether, **690 µg/ml** for ethyl acetate and **430 µg/ml** for ethanolic extract of *Rhododendron arboreum* and for ascorbate it was **410 µg/ml**.

Table 5: Effect of various extracts of *Rhododendron arboreum* on Nitric oxide scavenging activity

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	**Sample (Ethyl acetate extract)**	**Sample (Ethanolic extract)**	**Standard (Ascorbate)**
1	125	24.56 ±0 .018	27.60 ± 0.048	42.38 ± 0.018	26.87 ± 0.076
2	250	30.18 ± 0.052	38.43 ± 0.033	48.89 ± 0.034	30.30 ± 0.054
3	500	38.52 ± 0.028	46.65 ± 0.028	58.66 ± 0.028	60.64 ± 0.022
4	1000	52.08 ± 0.035	60.10 ± 0.035	64.92 ± 0.032	55.23 ± 0.014
		IC_{50} = 920 µg/ml	**IC_{50} = 690 µg/ml**	**IC_{50} = 430 µg/ml**	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

6. Total antioxidant activity (Phosphomolybdic acid method)

The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge Superoxide anion scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **53.14%** in pet.ether, **58.76%** in ethyl acetate and **67.08%** in ethanol when compared to ascorbate **55.23%** which is statistically significant at same concentration. The IC_{50} value was found to be **840 µg/ml** for pet.ether, **620 µg/ml** for ethyl acetate and **450 µg/ml** for ethanolic extract of *Rhododendron arboreum* and for ascorbate it was **410 µg/ml**.

Table 6: Total antioxidant activity of various extracts of *Rhododendron arboretum* (Linn.)

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	**Sample (Ethyl acetate extract)**	**Sample (Ethanolic extract)**	**Standard (Ascorbate)**
1	125	18.65 ± 0.035	21.30 ± 0.028	34.18 ± 0.018	26.87 ± 0.076
2	250	25.60 ± 0.020	34.61 ± 0.036	46.95 ± 0.016	30.30 ± 0.054
3	500	37.69 ± 0.012	45.54 ± 0.022	56.26 ± 0.030	60.64 ± 0.022
4	1000	53.14 ± 0.030	58.76 ± 0.016	67.08 ± 0.023	55.23 ± 0.014
		IC_{50} = 840 µg/ml	**IC_{50} = 620 µg/ml**	**IC_{50} = 450 µg/ml**	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

7. FRAP assay

The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge Superoxide anion scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be **51.43%** in pet.ether, **58.64%** in ethyl acetate and **72.11%** in ethanol when compared to ascorbate **98.07%** which is statistically significant at same concentration. The IC_{50} value was found to be **930 µg/ml** for pet.ether, **510 µg/ml** for ethyl acetate and **275 µg/ml** for ethanolic extract of *Rhododendron arboreum* and for ascorbate it was **50 µg/ml**.

Table 7: FRAP assay of various extracts of *Rhododendron arboreum*

S. No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	**Sample (Ethyl acetate extract)**	**Sample (Ethanolic extract)**	**Standard (Ascorbate)**
1	125	22.63 ± 0.032	29.70 ± 0.018	38.72 ± 0.038	72.04 ± 0.014
2	250	34.52 ± 0.018	35.36 ± 0.026	48.55 ± 0.016	82.05 ± 0.034
3	500	42.95 ± 0.026	48.88 ± 0.020	59.34 ± 0.024	86.04 ± 0.026
4	1000	51.43 ± 0.020	58.64 ± 0.034	72.11 ± 0.030	98.07 ± 0.041
		IC_{50} = 930 µg/ml	**IC_{50} = 510 µg/ml**	**IC_{50} = 275 µg/ml**	**IC_{50} = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

8. Estimation of total phenol content

TPC showed a sharp pet.ether extract range of **3.726 mg/g**, ethyl acetate extract range of **5.21 mg/g** and ethanolic extract range of **8.06 mg/g** as concentration of plant extract varied from 50µg/ml to 1000µg/ml.

Table 8: The total phenolic content of various extracts of flower of *Rhododendron arboreum*

S. No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1	Petroleum ether extract of *Rhododendron arboreum*	3.726 ± 0.022
2	Ethyl acetate extract of *Rhododendron arboreum*	5.21 ± 0.038
3	Ethanolic extract of *Rhododendron arboreum*	8.06 ± 0.041

*All values are expressed as mean ± SEM for three determinations

9. Estimation of total flavonoids content

The TFC showed a sharp pet.ether extract range of **0.010 mg/g**, ethyl acetate extract range of **0.298 mg/g** and ethanolic extract range of **2.024 mg/g** as concentration of plant extract varied from 50µg/ml to 1000µg/ml.

Table 9: The total flavonoids content of various extracts of flower of *Rhododendron arboreum*

S. No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Petroleum ether extract of *Rhododendron arboretum*	0.010 ± 0.005
2	Ethyl acetate extract of *Rhododendron arboretum*	0.298 ± 0.003
3	Ethanolic extract of *Rhododendron arboretum*	2.024 ± 0.020

*All values are expressed as mean ± SEM for three determinations

CONCLUSION

The present study was clearly indicated ethanolic extract of *Rhododendron arboretum* Linn showed strong antioxidant activity when compared with pet.ether and ethyl acetate extracts. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the ethanolic plant extract.

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