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MEMBRANE STABILIZING ACTIVITY AND ANTIOXIDANT EFFECT OF *SIDA CORDATA* (BURM F.) BIOSS AND *RHODODENDRON ARBOREUM* SM. LEAVES

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ABSTRACT:

Purpose: To investigate membrane stabilizing activity and antioxidant activity off different extracts of leaves of *Sida cordata* and *Rhododendron arboreum*.

Methods: Preliminary phytochemical analysis of different extracts of leaves of *Sida cordata* and *Rhododendron arboreum* were performed for presence of phytoconstituents. *In vitro* membrane stabilizing activity was done by using RBC membrane stabilising activity and antioxidant activity was done by using DPPH method.

Results: Preliminary phytochemical analysis showed that methanol extract are very rich in phytoconstituents as they showed the presence of alkaloid, carbohydrate, glycosides, proteins and amino acid, Phenolic compounds. All extract are investigated for their *in vitro* membrane stabilizing activity and antioxidant effect. Methanol and acetone extract of *Sida cordata* and *Rhododendron arboreum* showed maximum effect in comparison with other extracts.

Conclusion: From the above study it could be concluded that the leaves of *Sida cordata* and *Rhododendron arboreum* showed protective effect on inflammation and oxidant.

INTRODUCTION: *Sida cordata* (Burm F.) Bioss is a small weed found throughout India, usually on the road sides and other waste places. A procumbent, diffuse, much branched hairy herb with a very short main stem and long slender trailing branches that occasionally root at places of contact with the soil; leaves long-petioled, cordate to roundish with stellate hairs; flowers yellow, solitary or in pairs in the axils; fruits schizocarp located within the persistent calyx; seeds brownish, glabrous.

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The roots are sweet, sour, astringent, bitter thermogenic and tonic and useful in fever, uropathy and arthritis. The bark of root is used in leucorrhoea and gonorrhoea and hyperdiuresis. The leaves are good for diarrhoea. The flower and ripe fruits refrigerant and are useful in relieving burning sensation [1]. *Rhododendron arboreum* is one of the most stately and impressive rhododendron species. It is extremely variable in stature, hardness, flower color and leaf characteristics. Its species named Arboreum means tree like. Originally discovered in north central India, the plant is found in the Himalayas from Kashmir to Bhutan & in the hills of Assam & Manipur at altitudes of 1200-400 m. It grows at elevations of 4500 to 10,500 ft & grows up to 40 to 50 ft high sometimes attaining over 100 ft.

This is an evergreen much branched tree up to 14 m in height & 2.4 m in girth. Flowering season is from March- April/ June-September bearing deep red or crimson to pale pink flowers. Rhododendron arboreum is an evergreen shrub or small tree with a showy display ofbright red flowers. Rhododendron is the national flower of Nepal & is known as (Laligurans) & the state tree of Uttarakhand (India). It is called 'Burans, Bras, Buras or Barah ke-phool' in local dialect. It is widely popular for the processed juice of its flowers which havegained 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 & is used for the treatment of various ailments [2].

The selection of these plants for evaluation was based on their traditional usages. From literature survey it indicated that few works are carried out on both plants.

Therefore in present study, we investigated the Phytochemical, membrane stabilizing activity and antioxidant activity of *Sida cordata(Burm F.)Bios* and *Rhododendron arboreum Sm.* Leaves.

EXPERIMENTAL

Collection & identification of leaves of *Sida cordata* (Bern. F.) Bioss.: Leaves of *Sida codata (Burn.f.)Bioss.* were collected from locality of Dehradun (India). Plant material was authenticated by S. K. Srivastava (Scientist D/HOD), in Botanical Survey of India, Northern regional centre, Dehradun (BSI). Authenticated specimen no is- Acc. no.113678.

Leaves of *Rhododendron arboreum* Sm were collected from Dhanaulti road, Mussoorie, Dehradun (India). Plant material was authenticated by S. K. Srivastava (Scientist D/HOD), in Botanical Survey of India, Northern regional centre, Dehradun (BSI). Authenticated specimen no is- Acc. No. 113680.

Extraction and Phytochemical analysis: The collected plant Material was washed with water to remove other undesirable material and dried under shade. The air-dried leaves (100 gm) of *Sida cordata* and *Rhododendron arboreum* were crushed.

The crushed leaves extracted with different solvents of increasing polarity viz. petroleum ether, chloroform, acetone, methanol by hot percolation method using Soxhlet Apparatus. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure.

The extracts of leaves of *Sida cordata* and *Rhododendron arboretum* undergo various qualitative phytochemical tests. They showed their presence and absence in the different solvent systems. The different extracts of leaves of *Sida cordata* and *Rhododendron arboreum* were tested for various phytoconstituents viz., alkaloid, carbohydrate, sterols and Terpenoids, Phenolic compound and tannins, Protein and amino acid, saponin etc.

Membrane stabilizing activity and antioxidant activity:

Membrane stabilizing activity of different extracts: The membrane stabilizing ativity of the extracts was determined according to the method of Shinde *et al* and Sikdar *et al* [3, 4].

Effect on haemolysis: Erythrocyte suspension: Whole blood was collected from goat under ether anesthesia. NIH was used to prevent clotting. NIH solution was made by adding 5.5 g Trisodium citrate, 2 g citric acid, 6.125 g dextrose in 250 ml distilled water. The blood was washed three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4). which contained in 100ml of distilled water: $NaH_2PO_4.2H_2O$, 0.26 g; Na_2HPO_4 , 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer). The isotonic buffer solution was composed of 154 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4).

Hypotonic solution-induced haemolysis: Stock erythrocyte suspension (30 µl.) was mixed with 5 ml of the hypotonic solution containing the different extracts of *Sida cordata* at concentrations of 1000 µg/ml, 1500 µg/ml, 2000 µg/ml and 2000 µg/ml, 2500 µg/ml, 3000 µg/ml for *Rhododendron arboreum* , while the control sample was mixed with drug free solution. The mixtures were incubated for 10 min at room temperature, and centrifuged at 3000 rpm for 10 min.

All the experiments were performed in triplicates and the absorbance (O.D.) of the supernatant was measured at 560 nm.

Acetyl salicylic acid (Aspirin) was used as a reference standard at a concentration 1000 µg/ml, 1500 µg/ml and 2000 µg/ml.

Calculation: The percentage inhibition or acceleration of haemolysis in tests and standard was calculated according to the equation:

% acceleration or inhibition of hemolysis = 100x $\left[\frac{OD1-OD2}{OD1}\right]$

Where, OD_1 = Optical density of hypotonic saline solution + blood (control) and

OD_2 = Optical density of test sample in hypotonic saline solution + blood

Antioxidant activity of leaves extract:

Antioxidant activity [5, 6]:

DPPH Method:

- **Mechanism of DPPH method:** The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH:**1**) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (**2**) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).

- **Methodology of DPPH method:**

  Preparation of DPPH: DPPH is a highly oxidisable compound. It oxidized in light, so DPPH is prepared in dark. Weigh accurately 20 mg DPPH and dissolved in 100 ml of solvent. Generally Methanol and for some cases Ethanol is used as a solvent for DPPH.

  Preparation of test and standard ascorbic acid solution: Ascorbic acid is a strong antioxidizing agent. It is taken as standard. Standard solution of ascorbic acid and test sample of different extracts of leaves of *Sida cordata and Rhododendron arboreum* is prepared viz. 50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml. 3 ml of different concentration of test sample of different extracts of *Sida cordata and Rhododendron arboreum* and standard solution of ascorbic acid were mixed with 1 ml of DPPH solution in dark. The prepared solution of test sample and standard were incubated for 30 minutes then measure absorbance with the help of U.V. Spectrophotometer at 517 nm.

Calculation: We calculate the % activity of individual concentration of individual extract from the following formula:

%Activity=

$$\frac{\text{Abs. of Control} - \text{Abs. of Individual Conc.}}{\text{Abs. of Control}} \times 100$$

Abs. = Absorbance.

RESULT AND DISCUSSION: From phytochemical analysis, there is presence of steroids in petroleum ether extract; Saponin is present in petroleum ether and chloroform extract. Alkaloid, carbohydrate and Phenolic compounds showed in acetone extract. Methanol extract was the richest extract for phytoconstituents. It contains all tested phytoconstituents viz. Alkaloids, Carbohydrate, glycosides, proteins and amino acid, Phenolic compounds.

The membrane stabilizing action of different extracts of leaves of *Sida cordata and Rhododendron arboreum* observed in this model, experiments were performed on the erythrocyte membrane. *Sida cordata and Rhododendron arboreum* was found to inhibit the haemolysis of erythrocytes induced by hypotonic solution showed in **Tables 1-4.**

TABLE 1: ABSORBANCE OF DIFFERENT EXTRACT OF *SIDA CORDATA* LEAVES ON HEMOLYSIS

Concentration (µg/ml)	Absorbance of Extracts at 560 nm				Standard drug
	Pet. ether	Chloroform	Acetone	Methanol	Acetyl Salicylic acid (Aspirin)
1000	1.003±0.002	0.809±0.003	0.597±0.002	0.462±0.003	0.442±0.002
1500	0.925±0.003	0.755±0.003	0.567±0.004	0.430±0.005	0.361±0.002
2000	0.914±0.002	0.725±0.004	0.524±0.006	0.412±0.007	0.342±0.001
Control	1.051±0.001	0.936±0.005	1.030±0.008	0.847±0.006	0.919±0.002

The average value of three calculations are presented as mean±SD

TABLE 2: EFFECT OF DIFFERENT EXTRACTS OF LEAVES OF *SIDA CORDATA* ON INHIBITION OF HEMOLYSIS

Concentration (µg/ml)	% Inhibition of hemolysis of Extracts				Standard drug
	Pet. ether	Chloroform	Acetone	Methanol	Acetyl Salicylic acid (Aspirin)
1000	4.56±0.19	13.5±0.38	41.9±0.34	45.4±0.41	51.9±0.29
1500	11.9±0.39	19.3±0.35	44.9±0.45	49.2±0.62	60.71±0.19
2000	13±0.23	22.5±0.23	49.1±0.62	51.3±0.94	62.78±0.20

The average value of three calculations are presented as mean±SD

TABLE 3: ABSORBANCE OF DIFFERENT EXTRACT OF *RHODODENDRON ARBOREUM* LEAVES ON HEMOLYSIS

Concentration (µg/ml)	Absorbance of Extracts at 560 nm			
	Pet. Ether	Chloroform	Acetone	Methanol
2000	0.947±0.008	0.773±0.006	0.720±0.007	0.921±0.018
2500	0.909±0.010	0.682±0.005	0.546±0.013	0.858±0.009
3000	0.876±0.003	0.656±0.009	0.524±0.015	0.802±0.005
Control	1.076±0.012	1.078±0.014	1.060±0.008	1.060±0.005

The average value of three calculations are presented as mean±SD

TABLE 4: EFFECT OF DIFFERENT EXTRACTS OF LEAVES OF *RHODODENDRON ARBOREUM* ON INHIBITION OF HEMOLYSIS.

Concentration (µg/ml)	% Inhibition of hemolysis of Extracts			
	Pet. Ether	Chloroform	Acetone	Methanol
2000	11.98±0.73	28.29±0.51	32.09±0.64	13.11±1.75
2500	15.61±0.89	36.82±0.30	48.58±1.21	19.15±0.67
3000	18.68±0.33	39.23±0.92	50.56±1.38	24.33±0.48

The average value of three calculations are presented as mean±SD

Methanol extract of *Sida cordata* showed maximum membrane stabilizing activity 51.3% at a concentration of 2000 µg/ml whereas acetone extract of *Rhododendron arboreum* showed maximum membrane stabilizing activity 50.56 at a concentration of 3000 µg/ml in comparison to standard drug acetyl salicylic acid (Aspirin) which showed maximum membrane stabilizing effect 62.78 % at a concentration 2000 µg/ml. A possible explanation for the stabilizing activity of *Sida cordata and Rhododendron arboreum* leaves extract could be an increase in the surface area/volume ratio of the cells which could be brought about by an expansion of membrane or shrinkage of the cell, and an interaction with membrane proteins[3]. The present study showed the posible mechanism for membrane stabilizing activity of methanol extract of *Sida cordata* and acetone extract of *Rhododendron arboreum* play an important role in anti-inflammatory activity.

Antioxidant activity of methanol extract of leaves of *Sida cordata* showed maximum antioxidant activity of 81.93% at 500 µg/ml and acetone extract of *Rhododendron arboreum* showed maximum antioxidant activity 78.60 % at 500 µg/ml (results are shown in **Tables 5-9**).

TABLE 5: ABSORBANCE AND % ANTIOXIDANT ACTIVITY OF DIFFERENT CONCENTRATION OF ASCORBIC ACID AT 517nm

S. No.	Concentration (µg/ml)	Ascorbic acid (Abs. at 517 nm)	%
1	Control	0.959±0.009	-
1	50	0.032±0.003	96.66±0.31
2	100	0.032±0.003	96.66±0.31
3	200	0.031±0.005	96.76±1.16
4	300	0.032±0.004	96.66±1.28
5	400	0.034±0.006	96.45±0.69
6	500	0.030±0.002	96.87±0.23

The average value of three calculations are presented as mean±SD

TABLE 6: ABSORBANCE AND % ANTIOXIDANT ACTIVITY OF DIFFERENT CONCENTRATION OF PETROLEUM ETHER EXTRACT AT 517NM

S. No.	Concentration (µg/ml)	Pet. Ether extract of *Sida cordata* (Abs. at 517 nm)	% Antioxidant activity	Pet. Ether extract of *Rhododendron arboreum* (Abs. at 517 nm)	% Antioxidant activity
1	Control	0.588±0.011	-	0.507±0.010	-
2	50	0.541±0.008	7.99±1.41	0.501±0.009	1.11±1.78
3	100	0.511±0.005	13.09±0.80	0.498±0.011	1.17±2.81
4	200	0.487±0.013	17.18±1.22	0.496±0.017	2.17±3.40
5	300	0.465±0.004	20.92±0.39	0.486±0.012	4.14±2.28
6	400	0.451±0.006	23.30±0.99	0.474±0.015	6.50±3.01
7	500	0.444±0.008	24.49±1.03	0.467±0.014	7.89±2.79

The average value of three calculations are presented as mean±SD

TABLE 7: ABSORBANCE AND % ANTIOXIDANT ACTIVITY OF DIFFERENT CONCENTRATION OF CHLOROFORM EXTRACT AT 517nm

S. No.	Concentration (µg/ml)	Chloroform extract of *Sida cordata* (Abs. at 517 nm)	%	Chloroform extract of *Rhododendron arboreum* (Abs. at 517 nm)	%
1	Control	0.596±0.005	-	1.695±0.005	-
2	50	0.483±0.007	18.95±1.22	1.444±0.003	14.81±0.18
3	100	0.391±0.009	34.39±1.50	1.231±0.009	27.37±0.54
4	200	0.387±0.006	35.06±1.01	1.101±0.006	35.04±0.35
5	300	0.379±0.007	36.40±1.20	1.001±0.004	40.94±0.30
6	400	0.344±0.011	42.28±1.98	0.989±0.014	41.65±0.89
7	500	0.341±0.012	47.81±3.10	0.969±0.015	42.83±0.61

The average value of three calculations are presented as mean±SD

TABLE 8: ABSORBANCE AND % ANTIOXIDANT ACTIVITY OF DIFFERENT CONCENTRATION OF ACETONE EXTRACT AT 517nm

S. No.	Concentration (µg/ml)	Acetone extract of *Sida cordata* (Abs. at 517 nm)	%	Acetone extract of *Rhododendron arboreum* (Abs. at 517 nm)	%
1	Control	0.525±0.010	-	1.122±0.004	-
2	50	0.341±0.019	35.04±3.63	0.70±0.004	37.61±5.22
3	100	0.334±0.012	36.38±2.30	0.65±0.012	42.06±9.98
4	200	0.241±0.016	54.09±3.05	0.45±0.009	59.35±8.61
5	300	0.208±0.005	60.38±0.95	0.25±0.008	77.80±7.04
6	400	0.194±0.011	63.04±2.10	0.25±0.003	77.98±2.45
7	500	0.180±0.007	65.71±1.34	0.24±0.005	78.60±4.50

The average value of three calculations are presented as mean±SD

TABLE 9: ABSORBANCE AND % ANTIOXIDANT ACTIVITY OF DIFFERENT CONCENTRATION OF METHANOL EXTRACT AT 517NM

S. No.	Concentration (µg/ml)	Methanol extract of *Sida cordata* (Abs. at 517 nm)	%	Methanol extract of *Rhododendron arboreum* (Abs. at 517 nm)	%
1	Control	0.548±0.006	-	0.959±0.016	-
2	50	0.355±0.010	35.25±1.80	0.811±0.007	15.43±0.73
3	100	0.309±0.010	43.60±1.84	0.801±0.005	16.47±0.60
4	200	0.178±0.009	67.51±1.65	0.790±0.009	17.62±0.94
5	300	0.146±0.012	73.35±2.21	0.779±0.018	18.76±2.02
6	400	0.113±0.016	79.37±2.97	0.768±0.012	19.91±1.26
7	500	0.099±0.003	81.93±0.61	0.770±0.003	19.70±0.29

The average value of three calculations are presented as mean±SD

Antioxidants protect cells against damage caused by molecules known as free radicals the antioxidant effects of plant extracts are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes Phenolic are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants or plant products.[7]

CONCLUSION: From the above studies it could be concluded that acetone and methanol extracts of leaves of *Sida cordata and Rhododendron arboreum* have maximum invitro anti-inflammatory and antioxidant activity. Further studies are needed for their active principle to elucidated exact mechanism for the protective effect on inflammation and oxidant effect.

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