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Asian Journal of Plant Science and Research, 2015, 5(5):34-46

ISSN : 2249-7412
CODEN (USA): AJPSKY

In-vitro antioxidant and hemorrhoidal potential of hydroethanolic leaf extracts of *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi*

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ABSTRACT

The antioxidant properties of hydroethanolic extract of Bergenia species namely Bergenia ciliata, Bergenia ligulata and Bergenia stracheyi were analyzed using ten antioxidant assays. The absorbance for ferric thiocyanate assay and Thiobarbituric acid (TBA) assay was 0.058 nm and 0.082 nm. The half-maximal inhibitory concentration (IC_{50}) for Phosphomolybdenum assay, Nitric oxide radical inhibition assay, ABTS radical scavenging assay, Hydroxyl radical scavenging activity, Peroxynitrite scavenging assay, Singlet oxygen scavenging activity, Hypochlorous acid scavenging activity, Deoxyribose assay was 110.71 µg/ml, 64.79µg/ml, 19.45 µg/ml, 1.931µg/ml,39.25 µg/ml, 3053 µg/ml, 76.86 µg/ml, 5.661µg/ml respectively. This experiment presents total phenolics content (0.081 mg GAE/g) and flavonols content (0.079 mg TAE/g). A non-significant relationship between antioxidant capacity and total phenolics content, total flavonols content indicates stoichiometry of reaction, between antioxidant compounds in the extracts and the various radical. This may be inferred as a reason for the difference in their scavenging potential.

Keywords: Antioxidant assay, hemorroidal activity, Himalayan herb, astringency.

INTRODUCTION

Saxifragaceae is a family of herbs or shrubs, rarely trees or vines. The family includes about 80 genera and 1250 species worldwide. Most members of the *saxifragaceae* family are herbs. There are three species of *Bergenia* found in Himalayan region of India. These are *Bergenia ligulata*, *Bergenia ciliata*, *Bergenia stracheyi*. These are in combined popularly known as *Pashanbheda* in Indian system of medicine. *Bergenia* species is used in traditional ayurvedic medicine for the treatment of diuretic activity, antilithic activity, anti-bradikinin activity, antibacterial activity, antiviral avtivity, anti-inflammatory activity, antipyretic activity, hepatoprotective activity, etc. in Nepal, India, Pakistan and Bhutan [1-4].

Berginia species have a number of secondary metabolites. These are Bergenin, Tannic acid, Gallic acid, Stigmesterol, β-Sitosterol, Catechin, (+)-Afzelechin, 1,8-cineole, Isovalaric acid, (+)-(6S)-parasorbic acid, Arbutin, Phytol, Caryophyllene, Damascenone, β-eudesmol, 3-methyl-2-buten-1-ol, (Z)-asarone, Terpinen-4-ol, Paashaanolactone [5].

Out of these, Gallic acid, Stigmesterol and Bergenin are already reported for antioxidant activity in different part of different species of B*erginia* [5].

Oxidative stress plays an important role in the pathogenesis of various diseases such as piles, heart disease cancer etc. Oxidative stress is initiated by reactive oxygen species (ROS), such as superoxide anion, perhydroxy radical and hydroxyl radical. These radicals are formed by a one electron reduction process of molecular oxygen. ROS can easily initiate the lipid peroxidation of the membrane lipids, causing damage of the cell membrane of phospholipids, lipoprotein by propagating a chain reaction cycle. Thus, antioxidants defense systems have co-evolved with aerobic metabolism to counteract oxidative damage from ROS. Most living species have efficient defense system to prevent themselves against oxidative stress inducing by ROS.

Recent investigation have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human disease and aging process [6-10].

So in the present study we streamline the comparative *in-vitro* antioxidative properties of hydroethanolic leaf extracts of *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi*.

MATERIALS AND METHODS

2.1) Collection of Plant: Plant *Bergenia ligulata*, *Bergenia ciliata* and *Bergenia stracheyi* were collected from *Danulti* and *Mussorie* (Himalaya Region) of Uttarakhand, India, in the month of June 2012 and 2013, May and July 2014.

2.2) Authentication of plant: Plant was authenticated by Dr. Manisha sarkar deputy director in Homoeopathic Pharmacopeia laboratory Ghaziabad, UP India and Botanical survey of India, Dehradun on 7th Dec 2012. Accetion no 114536-38 was given for *B.ligulata*, *B.ciliata* and *B. stracheyi* respectively.

2.3) Plant part used: Leaf of all three species.

2.4) Physico-Chemical Evaluation: Plant was evaluated for following physical property as describe in Indian pharmacopoeia [11].

1. Loss on drying (LOD), 2. Ash Value (Total ash value, Acid insoluble ash, water soluble ash), 3. Extractive Value, 4. Percentage Yield, 5. Inorganic Analysis.

2.5) Preparation of Extracts: 250 gm of each plant material was packed in an air tight container for five days with petroleum ether (40:60) as solvent with regular shaking at intervals. After five days solvent was filtered under vacuum and marc was again washed with petroleum ether (40:60). Marc of plants was dried at room temperature. Extract was dried using rotary vacuum evaporator at 40°C till no further decrease in volume was observed. Dried, defatted marc was further treated with ethanol: water solvent system in a ratio of 70:30 for five days with regular shaking at interval. After five days solvent was removed under vacuum and marc was again washed with ethanol: water (70:30) solvent system.

2.6) Qualitative Test: These tests include test for Carbohydrates, Proteins, Steroids, Glycosides, Flavonoids, Alkaloids and Amino Acids [11].

2.7) Quantitative chemical test: These tests include test for Total Phenol, Tannins, Total flavonoid[15].

2.8) *In-Vitro* hemorrhoidal activity: To screen this activity following procedures have been followed.
A. *In-vitro* antioxidant parameters: 1: Qualitative DPPH Radical scavenging activity [16-17], 2: Reducing power assay [18], 3: Nitric oxide radical inhibition assay [19-20], 4: β-Carotene bleaching assay [21], 5: Hydrogen peroxide scavenging activity [22], 6: ABTS radical scavenging assay [23], 7: Phosphomolybdenum assay (Total Antioxidant activity) [24], 8: p-NDA assay [25], 9: Frap assay (Ferric reducing antioxidant power) [26], 10: Peroxynitrite scavenging assay [27-28], 11: Superoxide anion radical scavenging activity [29], 12: Singlet oxygen scavenging activity [30-31], 13: Metal chelating activity [32], 14: Hypochlorous acid scavenging assay activity [33-34], 15: Hydroxyl radical scavenging activity [35], 16: Alkaline DMSO assay [36], 17: Deoxyribose assay [37].

B. Determination of Lipid Peroxidation: **a.** Lipid peroxidation assay [38], **b.** Ferric thiocynate method (FTC) method [39], **c.** Thiobarbituric acid (TBA) method [40].

C. Determination of Astringency [41]:

RESULTS AND DISCUSSION

2.9) Results from Physiochemical Evaluation

a. Loss on drying (LOD): Loss on drying in different part of *Bergenia* species have been given in Table 1.

Table: 1 Loss on drying of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

Leaf	Loss on Drying (%)	Extractive value (%)	Yield (%)
B.ciliata	6.63	*12.70*	23.94
B. ligulata	13.14	23.00	13.62
B. stracheyi	13.67	41.50	22.75

b. Ash Value: Ash values in different part of *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi* are given in Table 2.

Table: 2 Ash values of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

Ash values (%)	*B. ciliata*	*B. ligulata*	*B.stracheyi*
Total water ash	*14.95*	48.90	56.50
Water insoluble ash	13.30	38.35	17.60
water soluble ash	01.65	10.55	38.90
Total acid soluble ash	*14.45*	50.00	75.00
Acid insoluble ash	03.30	37.55	56.25
Acid soluble ash	11.15	12.45	18.75

c. Extractive value: Extractive values of *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi* in each part like given in Table 1.

d. Percentage yield: Percentage yield in different part of *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi* has been given in Table 1.

e. Inorganic analysis: Inorganic analysis of different part of *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi* are given in Table 3.

Table: 3 Inorganic analysis of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

Test name	*B. ciliata*	*B. ligulata*	*B. stracheyi*
Calcium test	+	+	+
Magnesium test	-	-	-
sodium	+	+	+
potassium	-	-	-
iron	+	+	+
sulphate	+	+	+
phosphate	-	-	-
chloride	+	+	+
carbonate	+	+	+
nitrate	+	+	+

2.10) Results from Qualitative analysis: Qualitative analysis of different part of *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi* are given in Table 4.

Table: 4 Qualitative analysis of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

Chemical Test	*B. ciliata*	*B. ligulata*	*B. stracheyi*
Alkaloid	-	-	-
Amino acid	-	-	-
Carbohydrate (selivanoff) For ketone	+	+	+
Flavonoid			
Shinoda	+	+	+
Alkaline reagent zinc	+	+	+
Hydrochloride	+	+	+
Glycosides			
General test a	+	+	+
General test b	+	+	+
Foam test	+	+	+
Tannins			
Lead acetate	+	+	+
Ferric chloride test	+	+	+
Gelatin test	+	+	+
Catechin test	+	+	+
Chlorogenic test	+	+	+
Protein test	-	-	-
Steroid and triterpenoids			
Salkovaski	+	+	+
Libermen	+	+	+
Sulfer powder	+	+	+

2.11) Results from Quantitative analysis

Results from all the parameters have been expressed as mean ± SD(n=3) and variances and determined in respect of two way ANOVAs with P****** < 0.0001 by graph pad prism. Results are given in Table 5.

2.12) Physico-Chemical Evaluation

2.13) Qualitative Test: There is presence of the presence calcium, sodium, iron, sulphate, chloride, carbonate and nitrate in all three species (Table 3-4).

2.14) Quantitative chemical test: Again all three species show the presences of carbohydrates, Flavonoids, glycoside, tannins, steroid and terpenoid (Table5).

Table: 5 Total Phenolic content of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

Leaf	Phenolic content (gallic acid equivalent)mg/g	Tannin content (tannin acid equivalent) mg/g	Flavonoid content (quercetin equivalent) mg/g	Flavonols content (qurcetine equivalent) mg/g
B.ciliata	0.83660 ±0.00081	***0.0085± 0.00170***	0.65150± 0.00047	0.2030± 0.00262
B.ligulata	0.81660±0.000816	0.1150± 0.00216	0.07523± 0.00471	0.7415± 0.00249
B.stracheyi	0.08100 ±0.002160	0.1063± 0.00124	0.07900± 0.00081	0.0600± 0.00094

2.15) *In-Vitro* hemorrhoidal activity

3.7.1) Antioxidant Activity

a) DPPH Radical scavenging activity: These have been compared with IC_{50} value of standards ascorbic acid, butyl hydroxyl toluene, qurcetine, Vit E. These standards have values as 1.275µg/ml, 4.49µg/ml, 1.111µg/ml and 5.35µg/ml respectively. This test indicates that *B. ciliata*- rhizome-ethanolic extract is equivalent to ascorbic acid and qurcetine (Fig. 1, Table 6).

Table: 6 IC_{50} and r^2 values of DPPH scavenging activity of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant parts of *Bergenia*	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	9.850	0.871
O	*B. ligulata*	3.109	0.752
R	***B. stracheyi***	**9.890**	**0.812**
AA	Ascorbic acid	1.275	0.773
BHT	Butyl hydroxyl toluene	4.490	0.994
QU	Qurcetine	1.111	0.745
Vit E	Tocopherol	5.350	0.974

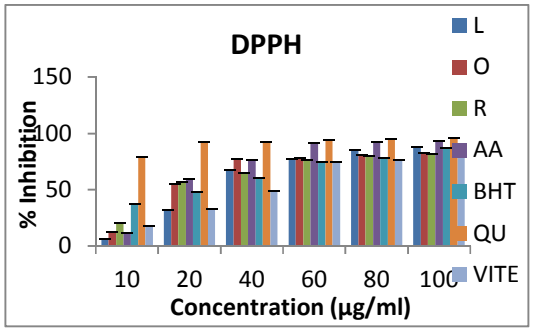

Fig. 1: DPPH scavenging activity

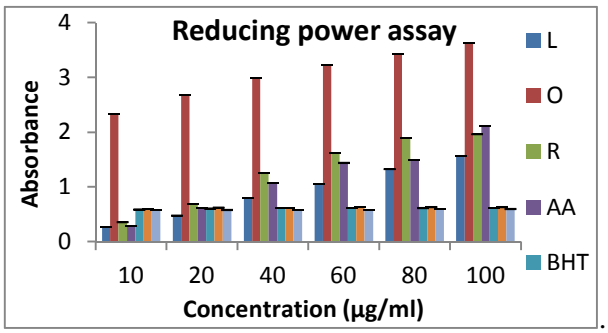

Fig. 2: Reducing power assay

b)Reducing power assay: Where it is compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine and Vit E and the values of these standards are 2.115µg/ml, 0.615µg/ml, 0.63µg/ml, and 0.596333µg/ml (Fig.2).

c) Nitric oxide radical inhibition assay: Compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine and Vit E. These standards have values as 2.86, 24.63, 22.57, 27.93 µg/ml respectively. *B. ciliata*-rhizome-ethanolic extract show better result than all standers used except ascorbic acid (Fig. 3, Table 7).

Table: 7 IC_{50} and r^2 values of nitric oxide radical inhibition assay of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	***B. ciliata***	**82.26**	**0.985**
O	*B. ligulata*	75.18	0.964
R	*B. stracheyi*	64.79	0.905
AA	Ascorbic acid	2.86	0.987
BHT	Butyl hydroxyl toluene	24.63	0.985
QU	Qurcetine	22.57	0.974
Vit E	Tocopherol	27.93	0.990

RL= Representing letter; Values expressed in mean ±SD; n=3*

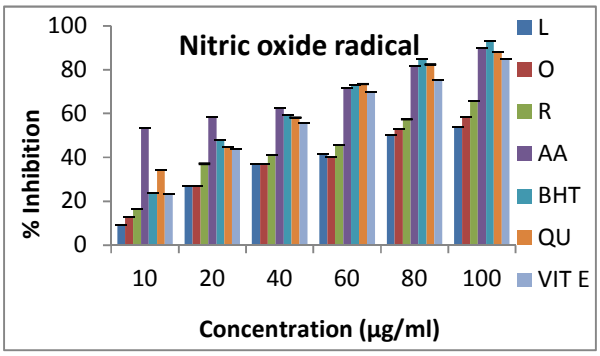

Fig.3: Nitric oxide radical inhibition assay (Leaf-hydoethanolic extract)

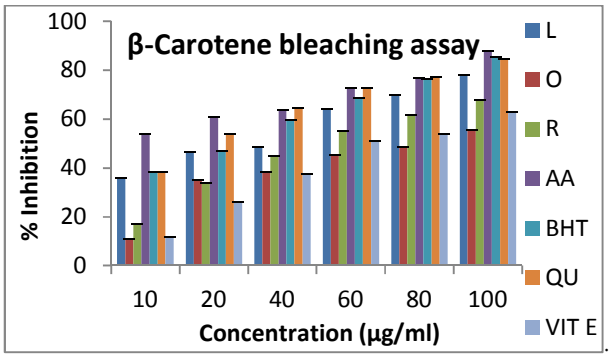

Fig.4: β-Carotene bleaching assay (Leaf-hydroethanolic extract)

d)β-Carotene bleaching assay: Compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine and Vit E. These standards have values as 4.43, 26.86, 17.81, 61.43 µg/ml respectively (Fig. 4, Table 8).

Table: 8 IC_{50} and r^2 values of β-Carotene bleaching assay of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant parts of *Bergenia*	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	35.77	0.969
O	*B. ligulata*	75.41	0.926
R	*B. stracheyi*	45.92	0.994
AA	Ascorbic acid	4.43	0.923
BHT	Butyl hydroxyl toluene	26.86	0.984
QU	Qurcetine	17.81	0.993
Vit E	Tocopherol	61.43	0.988

RL= Representing letter; Values expressed in mean ±SD; n=3*

e) Hydrogen peroxide scavenging activity: Compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine and tocopherol. Values of standards are 24.68, 24.68, 22.48, 35.23µg/ml respectively (Fig 5, Table 9).

Table: 9 IC_{50} and r^2 values of Hydrogen peroxide scavenging activity of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant parts of *Bergenia*	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	94.16	0.771
O	*B. ligulata*	64.47	0.923
R	*B. stracheyi*	114.43	**0.992**
AA	Ascorbic acid	24.68	0.987
BHT	Butyl hydroxyl toluene	24.68	0.943
QU	Qurcetine	22.48	0.980
Vit E	Tocopherol	35.23	0.985

RL= Representing letter; Values expressed in mean ±SD; n=3*

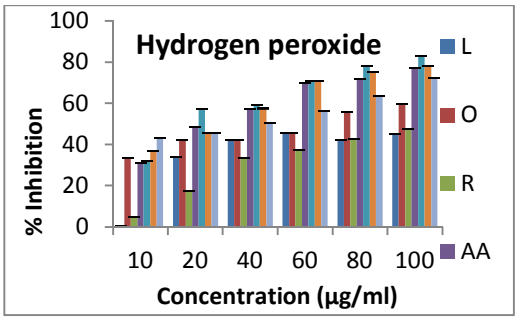

Fig.5: Hydrogen peroxide scavenging activity

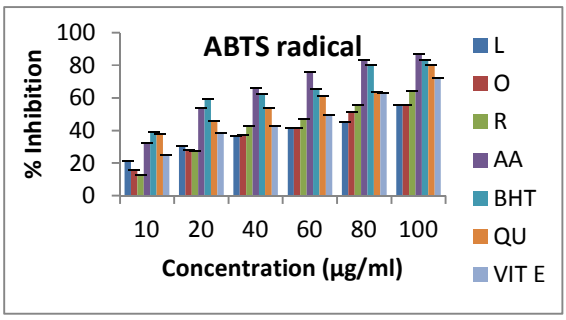

Fig.6: ABTS radical scavenging activity

f) ABTS radical scavenging assay: When compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine, tocopherol. These standards have values as 19.45, 16.62, 34.44, 54.83, µg/ml respectively (Fig. 6, Table 10).

Table: 10 IC_{50} and r^2 values of ABTS radical scavenging activity of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant parts of *Bergenia*	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata* (Leaf-hydroethanolic)	55.58	0.955
O	*B. ligulata* (Leaf-hydroethanolic)	30.63	0.981
R	*B. stracheyi* (Leaf-hydroethanolic)	19.45	0.986
AA	Ascorbic acid	19.45	0.991
BHT	Butyl hydroxyl toluene	16.62	0.917
QU	Qurcetine	34.44	0.959
Vit E	Tocopherol	54.83	0.967

RL= Representing letter; Values expressed in mean ±SD; n=3*

g)Phosphomolybdenum assay (Total Antioxidant activity): Extract L and O are potent content. Extract L, O and R are comparable to Vit E *B. stracheyi*-leaf-ethanolic extract have most significant IC_{50} value is 19.92 µg/ml and *B. ligulata*-rhizome-hydroethanolic extract have least significant IC_{50} value is 220.081 µg/ml when compared with

standards ascorbic acid, butyl hydroxyl toluene, qurcetine and tocopherol values are 15.95, 29.90, 38.22, 82.93 µg/ml respectively(Fig. 7, Table 11).

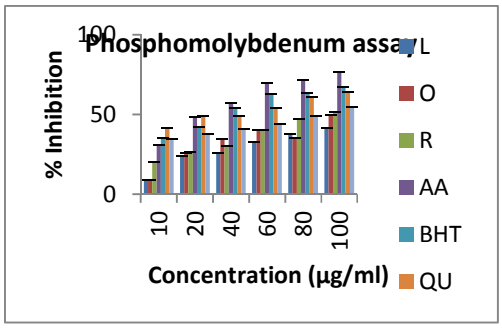

Fig. 7: Total Antioxidant activity (Leaf- hydroethanolic extract)

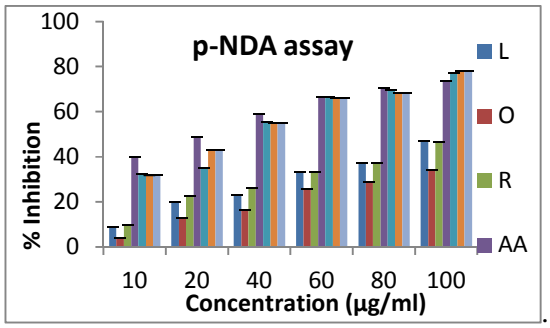

Fig.8: p-NDA assay (Leaf- hydroethanolic extract)

h)p-NDA assay: Extract L, O and R are comparable to standards.When compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine, tocopherol 20.55, 29.63, 27.66, 27.57µg/ml respectively (Fig. 8, Table 12).

i) Frap assay (Ferric reducing antioxidant power): Where it is compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine and Vit E. (0.794±0.000816, 1.181± 0.000471, 1.119± 0.000471, 0.385 ±0.000471) (Fig. 9).

Table: 11 IC_{50} and r^2 values of Total Antioxidant activity of *Bergenia species* leaf (*ciliata, ligulata* and *stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	211.45	0.956
O	*B. ligulata*	127.86	0.899
R	*B. stracheyi*	110.71	0.927
AA	Ascorbic acid	15.95	0.971
BHT	Butyl hydroxyl toluene	29.90	0.982
QU	Qurcetine	38.22	0.954
Vit E	Tocopherol	82.93	0.982

RL= Representing letter; Values expressed in mean ±SD; n=3*

Table: 12 IC_{50} and r^2 values of p-NDA assay of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	109.13	0.961
O	*B. ligulata*	423.68	0.959
R	*B. stracheyi*	172.77	0.942
AA	Ascorbic acid	20.55	0.996
BHT	Butyl hydroxyl toluene	29.63	0.954
QU	Qurcetine	27.66	0.983
Vit E	Tocopherol	27.57	0.983

RL= Representing letter; Values expressed in mean ±SD; n=3*

j) Peroxynitrite scavenging assay: When compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine and tocopherol. These standards have values as 27.49, 22.57, 1.64, 33.87µg/ml respectively (Fig. 10, Table 13).

k) Superoxide anion radical scavenging activity: Compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine, tocopherol. These standards have values as 6.10, 7.00, 4.22, 9.56µg/ml respectively. This is 4-9 times potent than all standards used (Fig 11, Table 14).

Table: 13 IC_{50} and r^2 values of Peroxynitrite scavenging assay of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	49.00	0.975
O	*B. ligulata*	83.58	0.952
R	*B. stracheyi*	39.25	0.974
AA	Ascorbic acid	27.49	0.981
BHT	Butyl hydroxyl toluene	22.57	0.967
QU	Qurcetine	1.64	0.989
Vit E	Tocopherol	33.87	0.874

RL= Representing letter; Values expressed in mean ±SD; n=3*

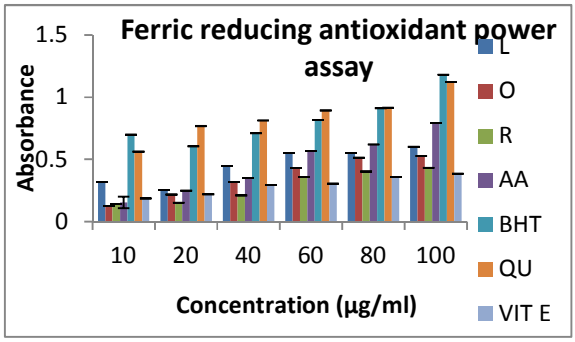

Fig.9: Ferric reducing antioxidant power assay (Leaf-hydroethanolic extract)

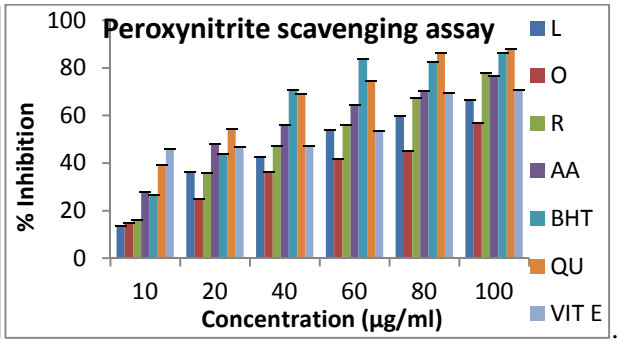

Fig.10: Peroxynitrite scavenging assay (Leaf-hydroethanolic extract)

Table: 14 IC_{50} and r^2 values of ethanolic and hydroethanolic extracts of Superoxide anion radical scavenging activity of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	3.298	**0.840**
O	*B. ligulata*	2.192	0.972
R	*B. stracheyi*	6.10	0.896
AA	Ascorbic acid	6.10	0.943
BHT	Butyl hydroxyl toluene	7.00	0.974
QU	Qurcetine	4.22	0.967
Vit E	Tocopherol	9.56	0.927

RL= Representing letter; Values expressed in mean ±SD; n=3*

l) Singlet oxygen scavenging activity: When compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine, tocopherol. These standards have values as 10.58, 4.039, 1.12, 2.320 µg/ml respectively.This is 5-50 times potent to standards used (Fig. 12, Table 15).

Table: 15 IC_{50} and r^2 values of ethanolic and hydroethanolic extracts of Singlet oxygen scavenging activity of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	**115.35**	0.975
O	*B. ligulata*	36.59	0.956
R	*B. stracheyi*	30.53	0.961
AA	Ascorbic acid	10.58	0.970
BHT	Butyl hydroxyl toluene	4.039	0.951
QU	Qurcetine	1.12	0.968
Vit E	Tocopherol	2.320	0.979

RL= Representing letter; Values expressed in mean ±SD; n=3*

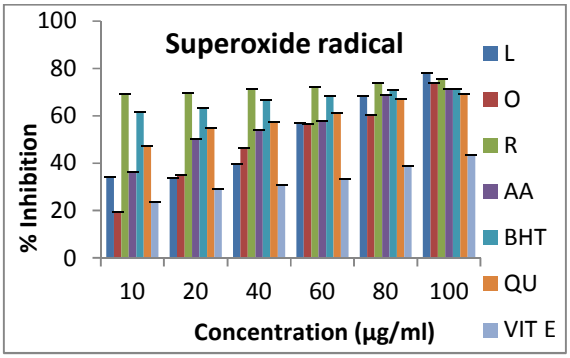

Fig.11: Superoxide anion radical scavenging activity (Leaf-hydroethanolic extract)

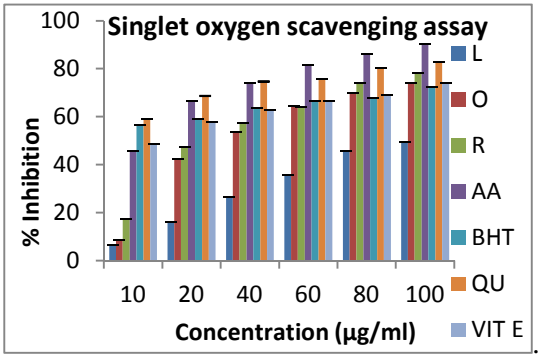

Fig.12: Singlet oxygen scavenging activity (Leaf-hydroethanolic extract

m) Metal chelating activity: When compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine, tocopherol and EDTA. These standards have values as 6.632, 5.568, 9.23, 2.07, 1.233 µg/ml respectively. This is 9 times more potent then qurcetine (Fig. 13, Table 16).

n)Hypochlorous acid scavenging activity: When compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine, tocopherol. These standards have values as 10.206, 10.51, 5.08, 135.69 µg/ml respectively (Fig. 14, Table 17).

Table: 16 IC_{50} and r^2 values of ethanolic and hydroethanolic extracts of Metal chelating activity of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*Bergenia ciliata*	2.680	0.918
O	*Bergenia ligulata*	**8.512**	0.931
R	*Bergenia stracheyi*	3.74	0.978
AA	Ascorbic acid	6.632	0.984
BHT	Butyl hydroxyl toluene	5.568	0.858
QU	Qurcetine	9.23	0.936
Vit E	Tocopherol	2.07	0.897
EDTA	Ethylene diamine tetra acetic acid	1.233	0.934

RL= Representing letter; Values expressed in mean ±SD; n=3*

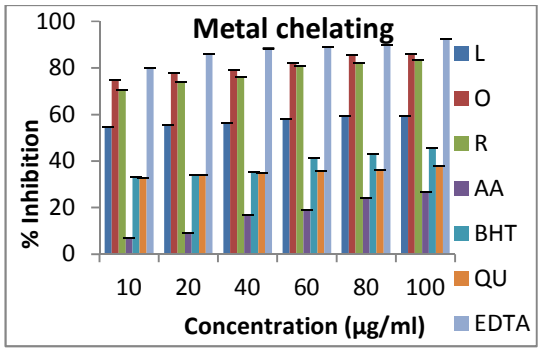

Fig.13: Metal chelating activity (Leaf- hydroethanolic extract)

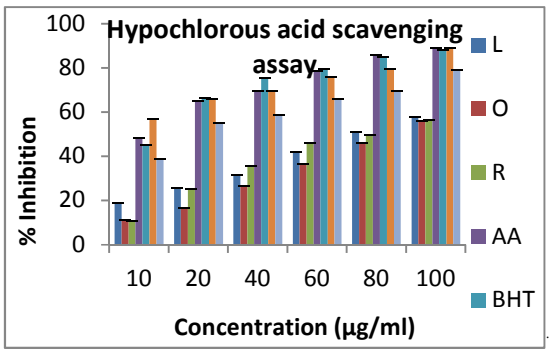

Fig.14: Hypochlorous acid scavenging activity (Leaf-hydroethanolic extract)

Table: 17 Inhibitory concentration fifty and r^2 values of hydroethanolic extracts of Hypochlorous acid scavenging activity of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	80.06	0.994
O	*B. ligulata*	87.79	**0.999**
R	*B. stracheyi*	76.86	0.993
AA	Ascorbic acid	10.206	0.972
BHT	Butyl hydroxyl toluene	10.51	0.957
QU	Qurcetine	5.08	0.954
Vit E	Tocopherol	135.69	0.897

RL= Representing letter; Values expressed in mean ±SD; n=3*

o)Hydroxyl radical scavenging activity: When compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine, tocopherol and the values are 24.68, 24.68, 22.48, 35.23µg/ml respectively (Fig 15, Table 18).

Table: 18 IC_{50} and r^2 values of hydroethanolic extracts of Hydroxyl radical scavenging activity of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	5.83	0.894
O	*B. ligulata*	3.74	**0.763**
R	*B. stracheyi*	1.93	0.976
BHT	Butyl hydroxyl toluene	3.43	0.839
QU	Qurcetine	1.71	0.888
Vit E	Tocopherol	5.98	0.942

RL= Representing letter; Values expressed in mean ±SD; n=3*

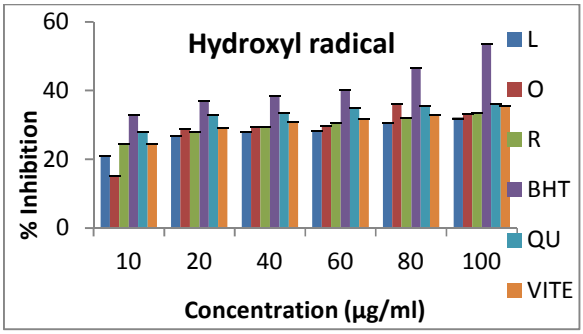

Fig.15: Hydroxyl radical scavenging activity (Leaf-hydroethanolic extract)

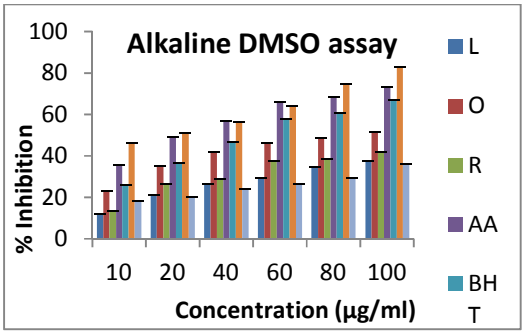

Fig. 16: Alkaline DMSO assay (Leaf-hydroethanolic extract)

p)Alkaline DMSO assay: When compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine, tocopherol. These standards have values as 23.68, 41.62, 20.62 and 185.46µg/ml respectively (Fig. 16, Table 19).

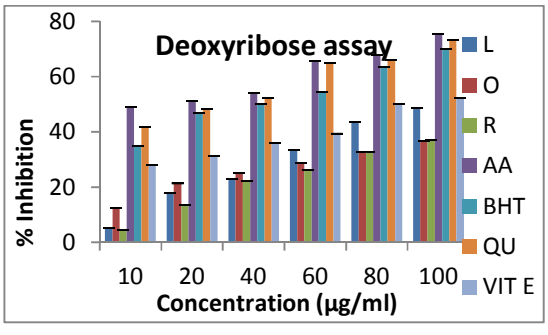

Fig.17: Deoxyribose assay

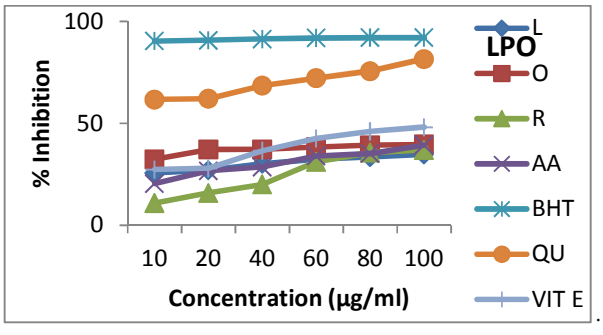

Fig.18: Lipid peroxidation assay

Table: 19 Inhibitory concentration fifty and r^2 values of hydroethanolic extracts of Alkaline DMSO assay of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	373.53	0.985
O	*B. ligulata*	83.84	0.984
R	*B. stracheyi*	200.13	0.961
AA	Ascorbic acid	23.68	0.992
BHT	Butyl hydroxyl toluene	41.63	0.989
QU	Qurcetine	20.62	0.992
Vit E	Tocopherol	185.46	0.968

RL= Representing letter; Values expressed in mean ±SD; n=3*

q)Deoxyribose assay: When compared with standards ascorbic acid, butyl hydroxyl toluene, qurcetine, tocopherol. These standards have values as 16.48, 42.49, 22.48, 89.56 µg/ml respectively (Fig 17, Table 20).

Table: 20 Inhibitory concentration fifty and r^2 values of hydroethanolic extracts of Deoxyribose assay of *Bergenia* species leaf (*ciliata, ligulata and stracheyi*)

RL*	Plant	IC_{50} (µg/ml)	r^2 Values
L	*B. ciliata*	97.91	0.971
O	*B. ligulata*	511.322	0.976
R	*B. stracheyi*	**5.661**	0.987
AA	Ascorbic acid	16.48	0.966
BHT	Butyl hydroxyl toluene	42.49	0.949
QU	Qurcetine	28.76	0.961
Vit E	Tocopherol	89.56	0.970

RL= Representing letter; Values expressed in mean ±SD; n=3*

4.4.2) Determination of Lipid Peroxidation

a)Lipid peroxidation assay: When compared with the leaf of *B. ligulata* and *B. stracheyi* whereas standard Ascorbic acid, BHT, Qurcetine and Vit E was 39.34, 92.05, 81.58, 48.13 % respectively(Fig. 18).

b)Ferric thiocynate assay (FTC): When compared with the root of *B. ciliata* and *B. ligulata*. *B. ciliata* rhizome ethanolic 0.057 nm when compared with *B. ligulata* and *B. stracheyi* rhizome. *B. stracheyi* leaf ethanolic 0.085 nm when compared with the *B. ciliata* and *B. ligulata* leaf respectively (Fig.19).

c) Thiobarbituric acid assay (TBA): Fig. 20

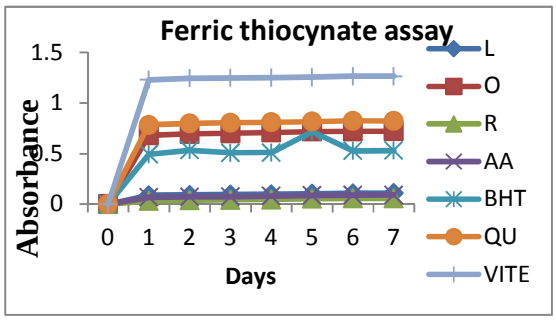

Fig. 19: Ferric thiocynate assay (Leaf-hydroethanolic extract)

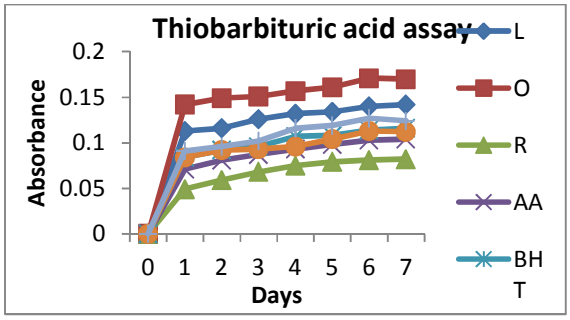

Fig. 20: Thiobarbituric acid assay (Leaf-hydroethanolic extract)

4.4.3) Determination of Astringency: We have checked out astringency for *Bergenia* species and the results have been found for all the extracts. *B. ligulata* (Rhizome-hydroethanolic) have least power to precipitate the protein that 0.20142 whereas most potent protein precipitator is *B. ligulata* (root-ethanolic). The value is 0.55371 equivalents to tannic acid. *B ciliata* (rhizome-ethanolic) also gave comparable result to above said potent extract (Table 21).

CONCLUSION

Bergenia species or *Pashanbheda* possesses many medicinal properties which need to be exploited; therefore the main attempt of the present study was based on pharmacological (Hemorrhoidal activity) and phytochemical comparison of *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi*. Hydroethanolic and ethanolic extracts of root, rhizome and leaf all of the *Bergenia* species were studied for this, Since from this study we can conclude that root of *Bergenia ligulata*, rhizome of *Bergenia ciliata* and leaf of *Bergenia stracheyi* show the best activity in most of the parameters so these are further taken for isolation

The main big reason for the comparison of all the species of *Bergenia*, is that it have medicinal properties, just because of secondary metabolite in their parts. So we have done *in-vitro* antioxidant study related to hemorrhoidal activity. The study done by comparative method to identify best part of the plant for isolation of compounds and to find out their *in-vitro* related hemorrhoidal activity.

These studies suggest that root from *Bergenia ligulata*, rhizome from *Bergenia ciliata* and leaf from *Bergenia stracheyi* have been shown best result (in-vitro hemorrhoidal activity).

So these extract further adopted for isolation of chemicals and their in-vitro hemorrhoidal activity.

Acknowledgement
Work has been done under the major research project sanctioned by University Grant Commission, Delhi, India, F.N. 42-678/2013(SR). Authors are thankful to UGC and Department of Science and Technology, Delhi, India, for providing instruments for analysis.

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