European Journal of Medicinal Plants
15(1): 1-10, 2016, Article no.EJMP.25370
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antioxidant, Lipid Peroxidation and Astringency Study of Hydroethanolic Root Extracts of *Bergenia ligulata, Bergenia ciliata* and *Bergenia stracheyi*

Rajani Chauhan[1*], Km Ruby[1] and Jaya Dwivedi[2]

[1]Department of Pharmacy, Banasthali University, Rajasthan, India.
[2]Department of Chemistry, Banasthali University, Rajasthan, India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/25370
Editor(s):
(1) Daniela Rigano, Department of Chemistry of Natural Compounds, University Federico II of Naples, Italy.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Roberto Martínez Beamonte, Universidad de Zaragoza, Spain.
(2) Anonymous, Université de Gabès, Tunisia.
(3) Caili Fu, Fuzhou University, China.
(4) Sahar Mohamed Kamal Shams El Dine, Ain Shams University, Cairo, Egypt.
(5) Ana Carolina Oliveira da Silva Gambôa, FACHO, Brazil.
(6) B. Ravishankar, SDM Centre for Research in Ayurveda and Allied Sciences, India.
Complete Peer review History: http://sciencedomain.org/review-history/14562

Original Research Article

Received 29th February 2016
Accepted 22nd April 2016
Published 11th May 2016

ABSTRACT

Aims: Hemorrhoid is a painful disorder both physically and mentally. So in the present research article anti-hemorrhoidal activity of hydroethanolic root extract of *Bergenia* species namely *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi* were studied by antioxidant, lipid peroxidation and astringency parameters.
Methods: For this UV-spectroscopy method was used.
Place and Duration of Study: Work was done in department of Pharmacy, Banasthali University, India, July 2013-July 2015.
Methodology: To perform the antioxidant, lipid peroxidation and astringency parameters, total 3 samples of plant extract and 4 standards were selected. All samples were studied at maximum respective absorbance. IC_{50} value (inhibition at 50% concentration), R^2 (regression value) and

**Corresponding author: E-mail: rajanichauhan_pharma@yahoo.com;*

probability of extracts and standards were calculated at constant and different concentration.
Results: Results showed that extracts are best free radical inhibitor at higher concentration and best mechanism to control the free radical is metal chelating activity.
Conclusion: We have successfully tried to find out solution for hemorrhoid by observing the results responsible for this.

Keywords: Hemorroidal activity; Himalayan herb; pashanbheda; pattherchat; community health.

1. INTRODUCTION

1.1 Rationale to Present Study

Oxidative stress plays an important role in the pathogenesis of various diseases such as piles, heart problems, 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 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. This results in generation of piles or hemorrhoid, due to failure of system to defend the ROS. Thus, antioxidants defense systems have co-evolved with aerobic metabolism to counteract oxidative damage from ROS. Lipid peroxidation and astringency study help us to identify whether plant is active to hemorrhoid or not. First, greater the antioxidant activity, greater the number of ROS will decrease and growth of hemorrhoid will also decrease. Second, greater the astringency effect that is protein (blood) precipitating effect, greater will be healing of developed and ruptured hemorrhoid [1-5].

Some organic and inorganic elements like flavonols and calcium respectively are also responsible to treat the hemorrhoid problem by increasing the blood flow and preventing the clotting of blood in veins. So here we also studied presence of variety of organic and inorganic compounds.

Hemorrhoid is the problem of mouth of rectum. To treat this problem so many steroids are available in the market. But these steroids are expensive and full of side effects. So patients to keep him/her self free from side effects, have to go for surgery. This treatment is very painful also and there may be chances of relapsing. So here we are trying to find out an alternative medication for hemorrhoid by studying elements present in it and antioxidant, lipid peroxidation and astringency effect.

So in the present study we have carried out the comparative *in-vitro* antioxidative activity of hydroethanolic root extracts of *B. ciliata* (Haw.), *B. ligulata* (Wall) Engl) and *B. stracheyi* (JD Hooker & Thomson & Hooker) Engler.

1.2 Plant Description

Bergenia is plant of family *Saxifragaceae.* It is herbs or shrubs and rarely trees or vines. This family includes about 80 genera and 1250 species worldwide. Genus *Bergenia* is found in Himalayan region of India. This genus has three species. These are *B. ligulata, B. ciliata* and *B. stracheyi.* Commonly these are known as *Pashanbheda* and *pattherchat,* in Indian system of medicine.

1.3 Medicinal Use

Bergenia species have been in use in Ayurvedic medicine system for diuretic activity, antilithic activity, anti-bradikinin activity, antibacterial activity, antiviral activity, anti-inflammatory activity, antipyretic activity, hepatoprotective activity, etc. in Nepal, India, Pakistan and Bhutan [6-9].

1.4 Chemical Compounds

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 [9]. Out of these, Gallic acid, Stigmesterol and Bergenin have already been reported for antioxidant activity from different part of B*erginia* species [10].

1.5 Justification of Present Work

We have selected four standards abbreviated AA, BHT, QU, Vit E and EDTA for Ascorbic acid, Butyl hydroxyl toluene, Qurcetine, Tocopherol and Ethylene diamine tetra acetic acid. These standards are used against three extracts J, M and P. These term stands for root extract of *B. ciliata, B. ligulata, B. stracheyi.* These standards are most potent antioxidant in the market. So we have selected these standards to verify that extracts are whether superior to standard or not. We have already published similar work for hydroethanolic leaf extract [10]. But the rationale of the present work is to screen the antioxidant, lipid peroxidation and astringency in roots, because we want to search, species of this genus having most effective results to treat the hemorrhoid.

2. MATERIALS AND METHODS

2.1 Collection of Plant

B. ligulata, *B. ciliata* and *B. stracheyi* were collected from *Danulti-Mussorrie,* Uttrakhand, India, in the month of June 2012 & 2013 and May & July 2014 [11].

2.2 Authentication of Plant

Plant was authenticated by Dr. Manisha Sarkar, deputy director, Homoeopathic Pharmacopeia laboratory, Ghaziabad, UP, India and "Botanical survey of India", Dehradun, Uttrakhand, India, dated on 7th Dec 2012. The Accession numbers 114536 to 38 were allotted for *B. ligulata, B. ciliata* and *B. stracheyi* respectively [11].

2.3 Physico-Chemical Evaluation [12]

Plant was evaluated for following physical property as describe in Indian pharmacopoeia.

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

2.4 Preparation of Extracts

Recently locals of Uttrakhand, India, use this plant just by cruising and directly applying on hemorrhoid to treat the problem. So in continuation we want to study hydroethanolic extracts also. So extract J, M, P and standards AA, BHT, QU, Vit E and EDTA were prepared [11].

2.5 Qualitative Test [12]

These tests include test for Carbohydrates, Proteins, Steroids, Glycosides, Flavonoids, Alkaloids and Amino Acids.

2.6 Quantitative Chemical Test [13-16]

These tests include test for Total Phenol, Tannins, Total flavonoid.

2.7 *In-vitro* Antioxidant Activity

Antioxidant parameters were studied by two ways. First by keeping concentration of extract constant to calculate the IC_{50} value and in second way percentage of inhibition of free radicals by all extracts were studied at different concentration [11]. Following parameters have been adopted for in-vitro anti-oxidant activity. These parameters were studied by UV-Visible spectroscopy.

1. Qualitative DPPH Radical scavenging activity [17,18], 2. Reducing power assay [19], 3. Nitric oxide radical inhibition assay [20,21], 4. β-Carotene bleaching assay [22], 5. Hydrogen peroxide scavenging activity [23], 6. ABTS radical scavenging assay [24], 7. Phosphomolybdenum assay (Total Antioxidant activity) [25], 8. p-NDA assay [26], 9. Frap assay (Ferric reducing antioxidant power) [27], 10. Peroxynitrite scavenging assays [28,29], 11. Superoxide anion radical scavenging activity [30], 12. Singlet oxygen scavenging activity [31,32], 13. Metal chelating activity [33], 14. Hypochlorous acid scavenging assay activity [34,35], 15. Hydroxyl radical scavenging activity [36], 16. Alkaline DMSO assay [37], 17. Deoxyribose assay [38],

2.8 Determination of Lipid Peroxidation

a. Lipid peroxidation assay [39], **b.** Ferric thiocynate method (FTC) method [40], **c.** Thiobarbituric acid (TBA) method [41].

2.9 Determination of Astringency [42]

Astringency was studied by using Odukaoya OA et al. method.

3. RESULTS

Only those tables and figures have been presented here having comparable to best readings to standards used.

3.1 Physiochemical Evaluation

Loss on drying, extractive values, Percentage yields (Table 1) and Ash values (Table 2) of hydroethanolic root extract of *Bergenia* species have been observed.

3.2 Qualitative Analysis

Qualitative analysis of different part of *Bergenia* species have been given in Table 3 and Table 4.

3.3 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 method (Version: GraphPad InStat). Results have been given in Table 5. The regression for results has been found above 0.98.

4. DISCUSSION

4.1 Physico-Chemical Evaluation

Least loss on drying in *B. ciliata* plant indicates least moisture content. This data show that crude dried plant can be easily protected from microbial growth during storage. But the highest extractive value of *B. ligulata* extract indicates greater amount of the organic and inorganic chemicals in the extract and we have succeeded to get highest percentage yield of *B. stracheyi* Table 1.

4.2 Qualitative Test

Water insoluble ash and acid soluble ash are highest for *B. stracheyi* indicating that this plant extract is full of organic compounds and multivalent inorganic compounds respectively Table 2. This thinking has been supported by presence of inorganic compounds, like calcium, sodium, iron, sulphate and chloride and organic functional group like carbohydrate, flavonoids, glycosides, tannins, steroid and triterpenoids Table 3 and Table 4. Presence of calcium and iron in the plant indicates that plant may be good hemorrhoid agent. Because calcium has tendency to prevent the clotting of blood in veins. Clotting of blood in vein is one of the major reasons for hemorrhoid. Flavonoids are well known to treat hemorrhoid by increasing the flow of blood in veins and plant of this research have flavonoids. So this plant is helpful to treat the hemorrhoid.

4.3 Quantitative Chemical Test

B. ligulata root extract is highly rich in phenolic content. This chemical is also responsible to increase blood flow in blood vessels and decreases the chances of hemorrhoids. The second major chemical group is flavonols found in *B. ciliata* and B*. ligulata.* Again flavonoids increases the flow of blood in veins. Table 5.

4.4 Antioxidant Activity

4.4.1 In the first method

All the extracts were studied at constant concentration to calculate the IC_{50} value. Best results have been found out for metal chelating and hydroxyl radical scavenging activity Table 8. Here all three extracts are superior to standards used. Best metal chelating property indicates that these may bind with iron atom of heam (protein) and may stop the free flow of blood by precipitating it. This may fasten the healing of worsen condition of hemorrhoid. Hydroxyl radical scavenging activity came as most used mechanism by extracts to reduce the number of ROS and maintain cells of blood vessels.

B. stracheyi has been showed best result for DPPH scavenging activity Table 6. *B. ciliata* has been showed best results for total antioxidant activity and singlet oxygen scavenging activity Table 7.

Reviewing all above data we concluded that best result has been obtained from B*. ciliata* for singlet oxygen scavenging activity. This extract has activity far beyond the marketed antioxidant preparations (standards).

4.4.2 In second method

We have calculated percentage inhibition of free radicals by extracts at concentration of 10, 20, 40, 60, 80 and 100 µg/ml. Again metal chelating effect has been find out at ranging from 10-100 µg/ml. So application of this result as discussed just in previous (first) method can be obtained at very low concentration. These extracts are powerful metal chelating agent and can replace

EDTA (a powerful marketed chelating agent) in future. This is the major finding of our research Fig. 5. Next major finding of these extract is reducing power assay of *B. ligulata* to inhibit the free radical at very low concentration that is 10 µg/ml Fig 2.

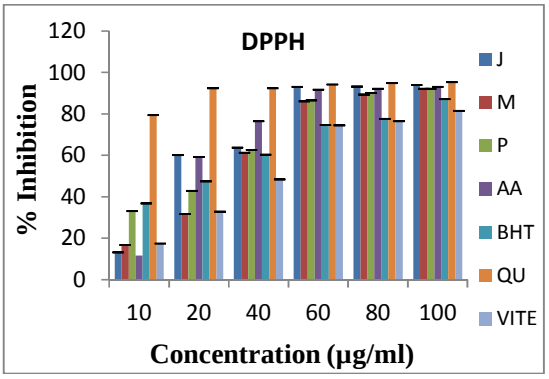

Fig. 1. DPPH scavenging activity

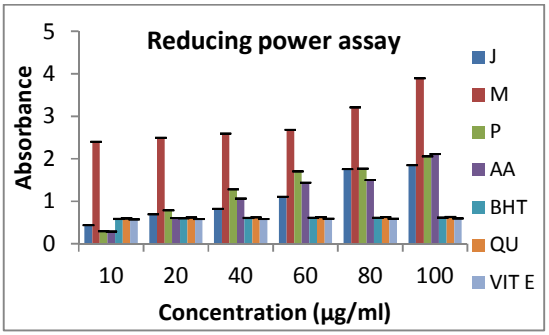

Fig. 2. Reducing power assay

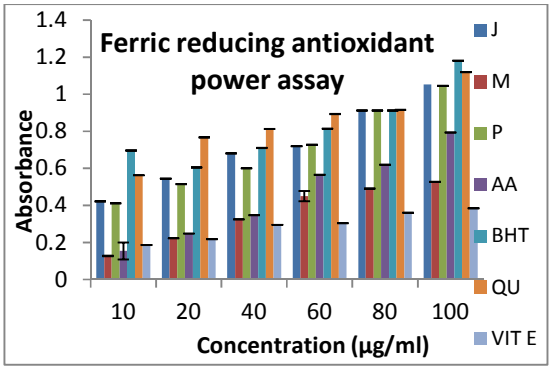

Fig. 3. Ferric reducing antioxidant power assay

4.4.3 R^2 (regression) values

Regression value is a statistical method to measure the closeness of data. In throughout the study, we have find out R^2 nearer to 100% in all the parameters. So these values represent that experiment for antioxidant activity, fits to this model and data are accurate. Tables 6-9.

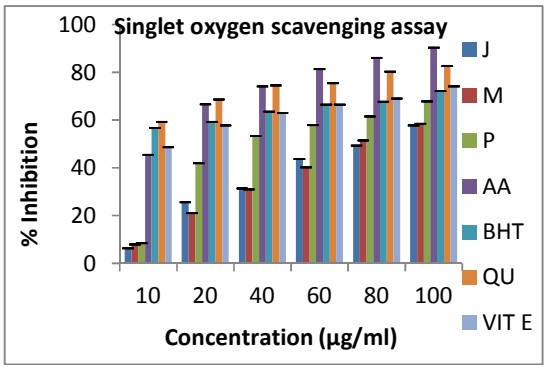

Fig. 4. Singlet oxygen scavenging activity

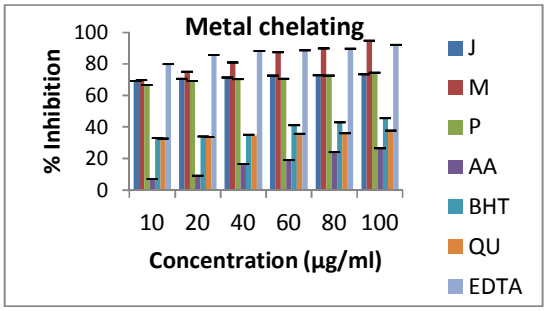

Fig. 5. Metal chelating activity

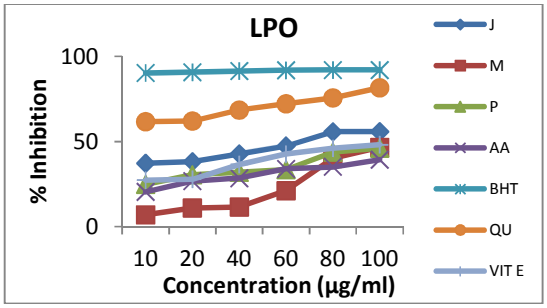

Fig. 6. Lipid peroxidation assay

4.5 Determination of Lipid Peroxidation

B. ligulata has given best results for ferric thiocynate assay and for thiobarbituric assay and *B. ciliata* root hydroethanolic extract has given comparable result to standards for lipid peroxidation assay Figs. 6-8. The end products of lipid peroxidation are reactive aldehydes and these have hemolytic effect by rupturing blood cells and cause lypolysis of lipid membrane of

veins. So for smooth treatment of hemorrhoid, it is necessary to protect the blood cells and veins and extracts of *B. ligulata* may help in this problem.

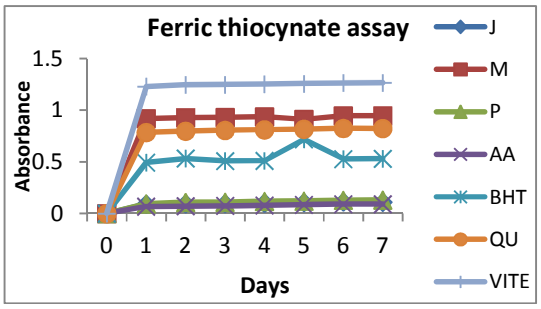

Fig. 7. Ferric thiocynate assay

Table 1. Loss on drying of root extracts of *Bergenia* species

Root	Loss on drying (%)	Extractive value (%)	Yield (%)
B.ciliata	*5.684*	18.84	29.50
B. ligulata	*17.92*	25.00	29.40
B. stracheyi	14.68	12.15	*42.00*

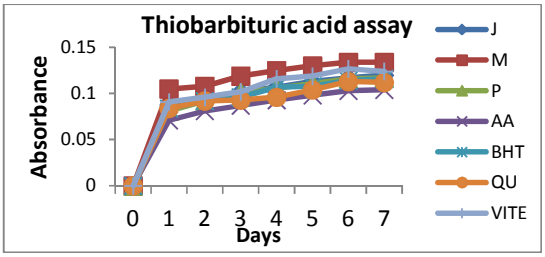

Fig. 8. Thiobarbituric acid assay

Table 2. Ash values of root extracts *of Bergenia* species

Ash values (%)	*B. ciliata*	*B. ligulata*	*B. stracheyi*
Total ash	*14.95*	10.75	26.00
Water insoluble ash	10.10	10.10	20.10
Water soluble ash	*00.65*	*00.65*	*05.90*
Total ash	10.91	10.91	39.00
Acid insoluble ash	01.95	01.95	03.45
Acid soluble ash	11.15	08.96	35.55

4.6 Determination of Astringency

Results from Table 9 indicates that all extracts are lead for protein precipitation. Again protein precipitation power reduces the unwanted flow of blood from ruptured hemorroidal veins and prevents the condition of hemorrhoid to be worse.

Table 3. Inorganic analysis of root extracts *of Bergenia* species

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

Table 4. Qualitative analysis of root extracts *of Bergenia* species

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			

Table 5. Total phenolic content of root extracts *of Bergenia* species

Root extract	Phenolic content (gallic acid equivalent)ng/g Abs:765 nm	Tannin content (tannin acid equivalent) ng/g, Abs: 700 nm	Flavonoid content (quercetin equivalent) ng/g Abs: 415	Flavonols content (qurcetine equivalent) ng/g, Abs: 440
J	11.03 x 10^4±0.01087	00.85±x 10^4 0.00170	85.84 x 10^4±0.00047	73.07 x 10^4±0.01633
M	94.08 x 10^4±0.00329	00.82 x 10^4±0.00205	84.61 x 10^4±0.00124	00.62 x 10^4±0.00244
P	11.04 x 10^4±0.00408	00.63 x 10^4±0.00205	8.64 x 10^4±0.00124	73.07 x 10^4±0.01414

Table 6. IC_{50} values of different root extract *of Bergenia* species

Root extract *Bergenia*	DPPH scavenging activity	nitric oxide radical inhibition assay	β-Carotene bleaching assay	Hydrogen peroxide scavenging activity	ABTS radical scavenging activity
J	3.150	44.65	96.78	69.33	81.77
M	9.790	49.69	81.61	55.81	38.39
P	1.890	78.87	76.24	62.24	37.22
AA	1.275	2.86	4.43	24.68	19.45
BHT	4.490	24.63	26.86	24.68	16.62
QU	1.111	22.57	17.81	22.48	34.44
Vit-E	5.350	27.93	61.43	35.23	54.83

Table 7. IC_{50} values of different root extract *of Bergenia* species

Plant parts of *Bergenia*	Phospho-molybdenum (total Antioxidant activity)	for p-NDA assay	Peroxy nitrite scavenging assay	Superoxide anion radical scavenging activity	Singlet oxygen scavenging activity
J	81.53	84.85	81.28	3.576	0.235
M	210.79	141.63	73.69	1.349	79.38
P	139.63	200.13	42.77	2.570	42.01
AA	15.95	20.55	27.49	6.10	10.58
BHT	29.90	29.63	22.57	7.00	4.039
QU	38.22	27.66	1.64	4.22	1.12
Vit-E	82.93	27.57	33.87	9.56	2.320

Table 8. IC_{50} values of different root extract *of Bergenia* species

Plant parts of *Bergenia*	Metal chelating activity	for Hypochlorous acid scavenging activity	Hydroxyl radical scavenging activity	Alkaline DMSO assay	Deoxyribose assay
J	2.542	78.25	2.34	1824.00	91.45
M	3.280	50.95	3.99	150.87	152.322
P	1.098	79.37	2.71	189.34	81.61
AA	6.632	10.206	3.43	23.68	16.48
BHT	5.568	10.51	1.71	41.63	42.49
QU	9.23	5.08	5.98	20.62	28.76
Vit-E	2.07	135.69	2.34	185.46	89.56
EDTA	1.233	78.25	3.99	1824.00	91.45

Table 9. IC_{50} values of different root extract *of Bergenia* species

Plant parts of *Bergenia*	Astringency value in tannic acid equivalent (ng/g)
J	37.39 x 10^4±0.002494
M	30.53 x 10^4±0.000471
P	38.40 x 10^4±0.004546

5. CONCLUSION

Bergenia species or *Pashanbheda* possesses many medicinal properties which need to be exploited. One of the health conditions is hemorrhoidal/ piles problem. To study this model, we first try to solve the cause of this problem. Ayrveda also tell to finish the root cause of any diseases. So here we studied the main cause of

hemorrhoid, such as free radical, astringency and lipid peroxidation activity. The rigorous study of plant extracts concluded that extracts are very potent chelating agent and can replace the use of EDTA in future. These extracts also are best astringent candidate, to precipitate the protein (blood) during the bleeding from already developed hemorrhoid/piles. From these studies we have become able to conclude that all these extracts act as antioxidant candidate mainly by DPPH scavenging activity, hydroxyl radical scavenging activity, superoxide anion radical scavenging activity, ferric reducing antioxidant power assay and metal chelation method.

Extracts are best suited for lipid peroxidation mechanism by stopping the rupturing of veins.

All these activity are due to chemicals present in this plant for example flavonoids, inorganic multivalent elements (calcium and iron) are present.

All this study has given an idea to explore this plant so that it can be available for the society to set them free from painful problem of hemorrhoid.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford: Oxford University Press; 1998.
2. Yazdanparast R, Ardestani A. *In vitro* antioxidant and free radical scavenging activity of *Cyperus rotundus*. J Med Food. 2007;10:667-674.
3. Yazdanparast R, Bahramikias S, Ardestani A. *Nasturtium oficinale* reduces oxidative stress and enhances antioxidant capacity in hypercholesterolaemic rats. Chem Biol Interact. 2008;172:176-184.
4. Packer L, Ong ASH. Biological oxidants and antioxidants: Molecular mechanisms and health effects. AOCS Press, Champaign, Illinois, USA; 1998.
5. Jovanovic SV, Simic MG. Antioxidants in nutrition. Ann NY Acad Sci. 2000;899:326-334.
6. Ruby K, Chauhan R, Dwivedi J. Phytochemistry and pharmacology of some indian medicinal plants. LAP-LAMBERT; Acedemic Pubilisher. 2012; 978-3-659-16710-2.
7. Chauhan R, Ruby K, Dwivedi J. Natural product–Part I, Berginia: A complete study. LAP-LAMBERT; Acedemic Pubilisher. 2012;978-3-659-11299-7.
8. Chauhan R, Ruby K, Dwivedi J. *Berginia ciliata* mine of medicinal properties: A review. Int J Pharmaceutical Sci Reas Review. 2012;15(2):20-23.
9. Chauhan R, Ruby K, Dwivedi J. Pashanbheda a golden herb of Himalaya: A review. Int J Pharmaceutical Sci Reas Review. 2012;15(2):24-30.
10. Chauhan R, Ruby KM, Dwivedi J. Secondary metabolite found in *Bergenia* species: A compendious review. Int J Pharm Pharm Sci. 2013;5(1):9-16.
11. Ruby K, Dwivedi J, Chauhan R. *In-vitro* antioxidant, lipid peroxidation and astringency screening of hydroethanolic leaf extracts of *Bergenia* species. Int. J. Medicinal Plant. 2016;110:723-734.
12. Harborne JB. Phytochemical methods. London Chapman and Hall Ltd. 1973;49-88.
13. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001;73:73-84.
14. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965;16:144-158.
15. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 2002;10:178-182.
16. Kumaran A, Karunakaran RJ. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. Food Chem. 2006;97:109-14.
17. Molyneux P. The use of the stable free radical diphenylpicryl hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol. 2004;26: 211–219.

18. Blois MS. Antioxidant determinations by the use of stable free radicals. Nature. 1958;26:1199-1200.
19. Oyaizu M. Studies on products of the browning reaction. Antioxidative activities of browning reaction products prepared from glucosamine. Jpn. J. Nutr. 1986; 44(6):307-315.
20. Marcocci I, Marguire JJ, Droylefaiz MT, Packer L. The nitric oxide scavenging properties *Ginkgo biloba* extract. Biochemical and Biophysical Research Communication. 1994;201:748-755.
21. Green JC, Hibberd DJ, Pienaar RN. The taxonomy of Prymnesium (Prymnesiophyceae) including a description of a new cosmopolitan species, *P. patellifera* sp. nov. and further observation on *P. parvum*. N. Carter. Br. Phycol. J. 1982;17:363-382.
22. Sun T, Ho CT. Antioxidant activities of buckwheat extracts. Food Chem. 2005;90: 743–74.
23. Ihami GI, Haci AA, Mehmet C. Determination of *in vitro* antioxidant and radical scavenging activities of Propofol. Chem Pharmacol Bull. 2005;53(3):281-285.
24. Re R, Pellegrini N, Proteggente A, Pannala A, Yong M, Rice-Evas C. Antioxidant activity applying an improved ABTS radical cation decolorusation assay. Free Rad Biol Med. 1999;26(9/10):1231–1237.
25. Preito P, Pineda M, Aguilar M. Spectrphotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999;269:337-341.
26. Kerri E, Rao MNA. Oxygen radical scavenging activity of curcumin. Int. J. Pharm. 1990;58:237–240.
27. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem. 1996;239:70-76.
28. Beckman JS, Chen H, Ischiropulos H, Crow JP. Oxidative chemistry of peroxynitrite. Methods Enzymol. 1994;233: 229-240.
29. Bailly F, Zoete V, Vamecq J, Catteu JP, Bernier JL. Antioxidant actions of ovothiol-derived 4-mercaptoimidazoles: glutathione peroxidase activity and protection against peroxynitrite- induced damage. FEBS Lett. 2000;486:19-22.
30. Liu F, Ooi VEC, Chang ST. Free radical scavenging activity of mushroom polysaccharide extracts. Life Science. 1997;60:763-771.
31. Chakraborty N, Tripathy BC. Involvement of singlet oxygen in 5- aminolevulinic acid-induced photodynamic damage of cucumber (*Cucumbis sativus* L.) chloroplasts. Plant Physiol. 1992;98:7-11.
32. Pedraza-Chaverrí J, Barrera D, Maldonado PD, Chirino YI, Macías- Ruvalcaba NA, Medina-Campos ON, Castro L, Salcedo MI, Hernández-Pando R. S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen *in vitro* and attenuates gentamicin induced oxidative and nitrosative stress and renal damage *in vivo*. Clin Pharmacol. 2004;4:5.
33. Dinis TCP, Madeira VMC, Almeidam LM. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and peroxyl radical's scavengers. Arch Biochem and Biophy. 1994;315:161-169.
34. Aruoma OI, Halliwell B. Action of hypochlorous acid on the antioxidant protective enzymes superoxide dismutase, catalase and glutathione peroxidase. Biochem J. 1987;248:973-976.
35. Pedraza-Chaverrí J, Arriaga-Noblecía G, Medina-Campos ON. Hypochlorous acid scavenging capacity of garlic. Phytother Res. 2007;21:884-888.
36. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Analytical Biochemistry. 1987; 165:215-219.
37. Govindarajan R, Vijaya Kumar M, Rawat AKS, Mehrotra S. Free radical scavenging potential of *Picrrorhiza kurroa* Royle ex Benth. Indian J. Exptl. Biol. 2003;41:875.
38. Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 1984;219:1-14.
39. Ruberto G, Baratta MT, Deans SG, Dorman HJD. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. Planta Medica. 2000;66:687–693.

40. Kikuzaki H, Nakatani N. Antioxidant effects of some ginger v constituents. J. Food Sci. 1993;58(6):1407-1410.
41. Ottolenghi A. Interaction of ascorbic acid and mitochondria lipids. Arch. Biochem. Biophy. 1959;179:355-363.
42. Odukaoya OA, Sofiyada MO, Ilori OO, Gbededo MO, Ajadotuigwe JO, Olleye OO. Hemorrhoid therapy with medicinal plants: Astringency and inhibition of lipid peroxidation as key factors. Int. J. Bio. Chem. 2009;3(3):111-118.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14562