Available online on 15.05.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Extraction, Qualitative and Quantitative Determination of Secondary Metabolites of *Bergenia Ciliata* (How) Sternb Rhizome

Mudassir Kanth*[1], Ahatsham Hussain[1], Pradeep K Shrivastva[1], Manik Sharma[2], Jagrati Tripath[3], Mohammed Azaz Khan[4]

[1] Department of Limnology, Barkatullah University Bhopal, India

[2] Department of Zoology Carrier College Bhopal, India

[3] Dept of Botany LBS College Sironj, India

[4] Pinnacle Biomedical Research Institute (PBRI), Bhopal, (M.P), 462003, India

ABSTRACT

In the last few years, there has been an exponential growth in the field of herbal medicine and gaining popularity both in developing and developed countries because of their natural origin and less side effects. Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. *Bergenia ciliata* Sternb (*B. ciliate*, Saxifragaceae) commonly known as Paashaanbhed is a rhizomatic herb with fleshy leaves, growing upto 30 cm tall, having a stout creeping rhizomatous rootstock with scars and intermittent axillary buds. Plant is quite hardy and able to survive frost during winter turning reddish in colour. It is evergreen and flowers in April to June. Its flowers are white-pink and purple in colour. Stem is short and the rhizome comes out from the cervices of rocks and hangs in the air in sloppy areas. Leaves are 5-30 cm long, glabrous, sparsely hairy in margins, broadly obovate or elliptic, finely or sparsely denticulate or shallowly sinuatedenate. It is also reported to cure fevers, diarrhoea, boils ophthalmia and cough. It is also used to dissolve kidney and bladder stones and forms an important ingredient in various kidney curative herbal drugs. The important phytochemicals of this plant include bergenin, gallic acid, (+)-catechin, Paashanolactone, sitoindoside, quercitin, (+) afzelechin etc tannic acid, albumen, mucilage, glucose, wax, metarbin and mineral salts, saponin, flavonoid, glycosides and polysaccharides, xyloglucan, taraxerol, carotenoids, cryptoxanthin. The aim of the present study is to examine *B. ciliate* rhizomes for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. The total phenolics content of ethyl acetate and methanolic extract was (208,442mg/100mg), followed by flavonoids (433, 446mg/100mg). The present study concluded that the crude extract of *B. ciliate* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. This work also contributes significantly to support the claim about the use of this herb in folk medicines. Further investigation regarding isolation and purification of a number of phytoconstituents from fruits, leaves, stem, flowers and seeds of *B. ciliate* may yield optimal combinations of therapeutic alternates.

Keywords: *Bergenia ciliata* Sternb, Qualitative analysis, Quantitative analysis, TPC, TFC, Folins Ciocalteau

Article Info: Received 31 March 2019; Review Completed 10 May 2019; Accepted 13 May 2019; Available online 15 May 2019

Cite this article as:

Kanth M, Hussain A, K Shrivastva P, Sharma M, Tripath J, Khan MA, Extraction, Qualitative and Quantitative Determination of Secondary Metabolites of *Bergenia Ciliata* (How) Sternb Rhizome, Journal of Drug Delivery and Therapeutics. 2019; 9(3):407-411 **http://dx.doi.org/10.22270/jddt.v9i3.2686**

***Address for Correspondence:**

Mudassir Kanth, Department of Limnology, Barkatullah University Bhopal, India

INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country [1]. Herbal medicine is still the mainstay of about 75%-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in photochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines [2].The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, flavonoids, resins, fatty acids, gums which are capable of

producing definite physiological action on body [3]. *B. ciliata* belong (haw.) Sternb belongs to the family Saxifragaceae which consist of 30 genera and 580 species. *B. ciliata* commonly known as hairy Bergenia is a perennial herb found between the height of 800-3000 m throughout the temperate Himalayas [4,5] from Afghanistan to Southeast Tibet [4]. In Bhutan it is found in Deothang, Phuntsoling, Mongar and Ha districts. In India it is reported from Lushai hills, West Bengal, Arunachal Pradesh, Meghalaya, Himalayas (Kumaon), Kyongnosla, Karponanag, Gangtok in Sikkim, district Almora Uttarakhand [6-10]. In Nepal it occurs in Makanwanpur district [10] Karepalanchwok district [11] and Dolakha district [12]. In Pakistan it is distributed in northern parts mainly FATA region of Khyber Pukhtunkhwa province, Poonch valley, Swat, Abbottabad, Galliyat and Chitral [13-17]. It was long since this plant has been used as medicines for the treatment of different human ailments. In Himalaya region many rural communities use *B. ciliata* for the treatment of various diseases [18]. For century's rhizome of *B. ciliata* has been used for curing pulmonary infections, leucorrhea, piles and for dissolving bladder and kidney stones [19]. In Ayurveda system of medicine it is commonly used as tonic, astringent, antiscorbutic, laxative, spleen enlargement, dysuria and ulcers [20]. Local people of West Bengal use rhizome juice as an anti-tussive for cough and cold [21]. As a medicinal plant *B. cilata* is being widely used against cough, cold, fever, pulmonary infections, heart diseases, ophthalmic, hemorrhoids and stomach disorders [18, 22-24]. *B. ciliata* contains number of important phytochemicals such as

bergenin, gallic acid, (+) – catechin, Paashanolactone, sitoindoside, quercitin, (+) afzelechin etc [25,26]. Presence of tannic acid, albumen, mucilage, glucose, wax, metarbin and mineral salts is also reported [27]. Biological analysis of *B. ciliata* revealed that this plant showed antioxidant, anti-inflammatory, antitussive, antiviral, antiulcer, hypoglycemic and toxicological activities. Rhizome of *B. ciliata* was found to show narrow spectrum of antibacterial activity, leaves and roots show antifungal activity [21, 28]. Though the plant has been reported for many biological activities, no scientific data available to identify the genuine sample. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds of rhizomes of *B. ciliata*.

MATERIALS AND METHODS

Plant materials

The rhizomes of *B. ciliata* were collected from Himalayan region of Jammu and Kashmir, District Udhampur The sample was identified by senior Botanist Dr. Zia-Ul-Hassan, Professor and head of department of Botany, Safia College of Arts and Science, peer gate Bhopal. A herbarium of plants was submitted to the specimen library of Safia College of Arts and Science, peer gate Bhopal and The specimen voucher no. of *B. ciliata* is 119/Bot/Saf/18.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction of plant material

Cold maceration

The rhizomes of *B. ciliata* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. About 1kg of the Powder rhizomes was successive extracted with different organic solvents viz; Pet. ether, ethyl acetate and Methanol and allow to stored for 72 hours in ice cold condition for the extraction of phytochemicals. At the end of the third day extract was filtered using whatmann No. 1 filter paper to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [29].

Qualitative phytochemical analysis of plant extract

The *B. ciliata* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [30, 31]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Test for carbohydrates

Molisch's test: In a test tube containing extract of drug, added two drop of freshly prepared 20% alcoholic solution of α- napthol and mixed concentrated sulphuric acid along the sides of the test tube. If carbohydrate present purple color or reddish violet color produce at the junction between two liquids.

Benedict's test: In a test tube containing extract of drug add benedict's solution, mix well, boiled the mixture vigorously for two minutes and then cooled. Formation of red precipitate due to presence of carbohydrates.

Barfoed's test: The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. Formation of red precipitate of copper oxide was indicated the presence of carbohydrates.

Anthrone test: To the two ml of anthrone test solution, add the extract of drug. A green or blue colour indicated the presence of carbohydrate.

Test for alkaloids

Dragendorff's Test: Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff's reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.

Wagner's test: Acidify the extract of drug with 1.5 % v/v of hydrochloric acid and added a few drop of Wagner's reagent (iodine potassium iodide solution). Formations of reddish brown precipitate indicated the presence of alkaloids.

Mayer's Test: Two ml of extract solution was treated with 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodide solution) formation of dull white precipitate indicated the presence of alkaloid.

Hager's Test: Extract of the drug solution was treated with 3 ml of Hager's reagent (saturated solution of picric acid) formation of yellow precipitate confirmed the presence of alkaloids.

Test for glycosides

Legal's test: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. Pink red colour indicated the presence of glycosides.

Baljet's test: To the drug extract, sodium picrate solution was added, yellow to orange colour was indicated the presence of glycosides.

Borntrager's test: Few ml of dilute sulphuric acid solution, the test solution of extract was added. It was filtered and the filtrate was boiled with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet colour was produced in orange layer confirmed the presence of glycosides.

Keller Kiliani test: Methanolic extract was dissolved in glacial acetic acid containing trace of ferric chloride one ml concentrated sulphuric acid was added carefully by the side of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquid indicated the presence of glycosides.

Test of saponins

1 ml of alcoholic extract was diluted with 20 ml distilled water and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins.

Test for flavonoids

Shinoda test: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

Test for tannins

To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.

To the sample of extract, potassium cyanide was added, deep red colour was confirmed the presence of tannins.

To the sample of extract, potassium dichromate solution was added, yellow precipitate was produced.

Test for protein and amino acid

Biuret's test: To 2 - 3 ml of the extract of drug added in 1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution mix thoroughly, a purplish - violet or pinkish - violet colour produced that indicates the presence of proteins.

Ninhydrin's test: Two drops of freshly prepared 0.2 % ninhydrin reagent was added to the extract and heated to boiling for 1 - 2 min. and allow cooling. A blue colour developed that indicating the presence of proteins, peptides or amino acids.

Xanthoprotein test: To the extract in a test tube, add conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. Added 20 % of sodium hydroxide solution in excess orange colour indicated presence of aromatic amino acid.

Millon's test: The small quantity of extract of the drug dissolved in distilled water added 5 - 6 drop of millon's reagent. A white precipitate was formed which turned red on heating, indicated the presence of proteins.

Lead acetate test: The extract was taken and two ml of 40 % sodium hydroxide solution was added and boiled, glacial acetic acid was added and cooled than added 1 ml of lead acetate solution, gray black precipitate was formed which indicated presence of sulphur containing amino acid.

Test of fats or fixed oils

Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then added 10 % sodium hydroxide solution a clear blue solution was obtain which showed glycerin present in sample.

Using sodium hydrogen sulphate: The extract was taken in test tube added a pinch of sodium hydrogen sulphate pungent odour was formed which showed glycerin present in sample.

Saponification: Four ml of 2 % sodium carbonate solution was taken and the extract was added. Shaked vigorously and boiled. A clean soapy solution was formed cooled and added few drops of conc. HCl and observed that fatty separate out and float up.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from rhizomes of *B. ciliata* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total phenol determination

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Concentration of (20-100 µg/ml) of gallic acid was prepared in methanol. Concentration of 100 µg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2 ml of a 10 fold dilute folin Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and it was then Incubated at room temperature for 30 mins with intermittent shaking and the absorbance were taken at 765 nm against using methanol as blank. Total phenolic content was calculated by the standard regression curve of Gallic acid and the results were expressed as gallic acid equivalent (mg/gm) [32].

Total flavonoids determination

Different concentration of rutin (20 to 100µg/ml) was prepared in methanol. Test sample of near about same polarity (100µg/ml) were prepared. An aliquot 0.5ml of diluted sample was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% $NaNO_2$ solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 5min, and then 2 ml of 4% NaOH solution was added to the mixture. The final volume was adjusted to 5ml with distilled water and allowed to stand for another 15 mins. Absorbance was determined at 510 nm against water as blank. Total Flavonoid content was calculated by the Standard regression curve of Rutin/ Quercetin [33].

RESULTS AND DISCUSSIONS

The crude extracts so obtained after cold maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the rhizomes of the plants using petroleum ether, ethyl acetate and methanol as solvents are depicted in the Table 1.

Table 1 Results of percentage yield of fruits extracts

Plant Name	Percentage yield (%)		
	Pet. ether	Ethyl acetate	Methanol
Bergenia Ciliata	2.5	3.6	5.6

The results of qualitative phytochemical analysis of the crude powder of rhizomes of *B. ciliata* are shown in Table 2. Methanolic extracts of sample of *B. ciliata* showed the presence of alkaloids, terpenoids, flavonoids, phenols, tannins, carbohydrate, glycosides and saponins, Ethyl acetate extracts show the presence of carbohydrates, alkaloids, saponins, flavonoids treterpenoids, steroids, tannin and phenolics but in petroleum ether extracts all phytoconstituents was absents.

Table 2 Phytochemical evaluation of *Bergenia Ciliata* rhizomes

Tests	Pet ether	Ethyl acetate	Methanol
Carbohydrates			
Molish	- ve	+ ve	+ ve
Fehlings	+ ve	+ ve	+ ve
Benedit's	+ ve	+ ve	+ ve
Protien & amino acids			
Biurets	- ve	- ve	- ve
Ninhydrin	- ve	- ve	- ve
Glycosides			
Borntrager	- ve	- ve	+ ve
Killer killani	- ve	- ve	+ ve
Alkaloids			
Mayers	+ ve	+ ve	+ ve
Hagers	- ve	+ ve	+ ve
Wagners	- ve	+ ve	+ ve
Saponins			
Froth	- ve	+ ve	+ ve
Flavonoids			
Lead acetate	- ve	+ ve	+ ve
Alkaline reagent test	- ve	+ ve	+ ve
Treterpenoids & Steroids			
Salwoski	+ ve	+ ve	+ ve
Liebermann	- ve	+ ve	+ ve
Tannin & Phenolics			
Ferric chloride	- ve	+ ve	+ ve
Lead acetate	- ve	+ ve	+ ve
Gelatine	- ve	+ ve	+ve

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of methanolic and ethyl acetate extract of *B. ciliata* rhizomes showed the content values of 442 and 208 mg/gm respectively. But petroleum ether extracts of *B. ciliata* rhizomes have no phenolic content. The total flavonoids content of the extracts was expressed as percentage of rutin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of methanolic and ethyl acetate extract of *B. ciliata* rhizomes showed the content values of 446 and 433mg/gm respectively. The above results showed that ethyl acetate extract contain less phenolic and flavonoids content than the alcoholic extract. It may due to the solubility of principle contents presence be higher in case of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents. Results are provided in (Table 3 and Fig. 1, 2).

Table 5 Estimation of total phenolics and total flavonoids content in *Bergenia Ciliata* rhizomes

S. No	*Bergenia Ciliata* Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Petroleum ether	-	-
2.	Methanol	442	446
3.	Ethyl acetate	208	433

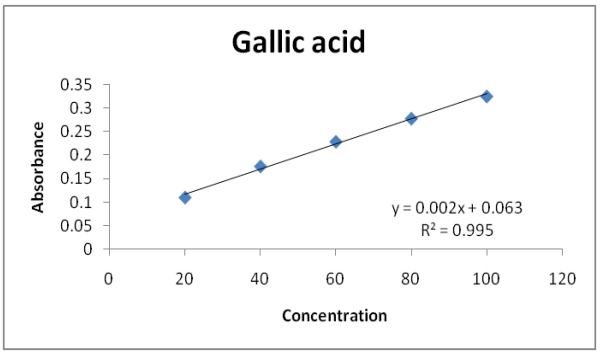

Fig. 1 Graph of estimation of total phenolic content

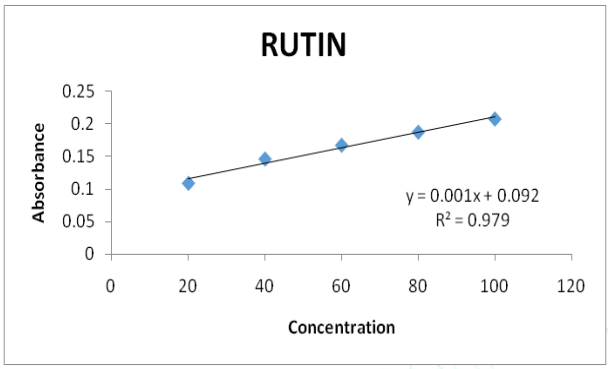

Fig. 2 Graph of estimation of total flavonoid content

CONCLUSION

The present study concluded that this medicinal plant viz. *Bergenia Ciliata* is a promising source of various activities and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of the existing data is not enough to suggest a reasonable mode of action for antioxidant effects. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant activity and to explore the existence of synergism if any, among the compounds.

REFERENCES

1. Ahmed L, Mohammed Z, Mohammed F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998; 62(2):183-193.
2. Arora DS, Kaur J. Antimicrobial activity of spices. Int J Antimicrobial Agents 1999; 12:257-262.
3. Joshi B, Lekhak S, Sharma A. Antibacterial property of different medicinal plants: Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana. Kathmandu Univ J Sci Eng Technol 2009; 5: 143-150.
4. K. Ruby, R. Chauhan, S. Sharma, J. Dwivedi, Polypharmacological activities of Bergenia species, Int. J. Pharm. Pharm. Sci. 2012; 1:100–109.
5. P. Pokhrel, R.R. Parajuli, A.K. Tiwari, J. Banerjee, A short glimpse on promising pharmacological effects of Begenia ciliata, JOAPR 2014; 2(1):1–6.
6. A.J. Grierson, D.G. Long, Flora of Bhutan, including a record of plants from Sikkim, R. Bot. Gard. 1 (parts 1 and 2) (1983).
7. R. Hafidh, A. Abdulamir, F. Jahanshiri, F. Abas, F. Abu Bakar, Z. Sekawi, Asia is the mine of natural antiviral products for public health, Open Complement Med. J. 2009; 1:58–68.
8. R. Chauhan, K. Ruby, J. Dwivedi, Bergenia ciliata mine of medicinal properties: a review, Int. J. Pharm. Sci. Rev. Res. 2012; 15 (2):20–23.
9. A. Kumar, M. Mitra, B. Adhikari, G. Rawat, Depleting indigenous knowledge of medicinal plants in cold-arid region of Nanda Devi Biosphere Reserve, Western Himalaya, Med. Aromat. Plants 2015; 4 (195):2167-0412.10001.
10. M. Hasan, P. Gatto, P. Jha, Traditional uses of wild medicinal plants and their management practices in Nepal—a study in Makawanpur district, Int. J. Med. Aromat. Plants 2013; 3:102–112.
11. R. Gyawali, Phytochemical screening and anti-microbial properties of medicinal plants of Dhunkharka community, Kavrepalanchowk, Nepal, Int. J. Pharm. Biol. Arch. 2011; 2 (6).
12. P.M. Shrestha, S.S. Dhillion, Medicinal plant diversity and use in the highlands of Dolakha district, Nepal, J. Ethnopharmacol. 2003; 86(1):81–96.
13. M. Hamayun, S.A. Khan, E.Y. Sohn, I.-J. Lee, Folk medicinal knowledge and conservation status of some economically valued medicinal plants of district Swat, Pakistan, Lyonia 2006; 11(2):101–113.
14. S.A. Gilani, R.A. Qureshi, S.J. Gilani, Indigenous uses of some important ethnomedicinal herbs of Ayubia National Park, Abbottabad, Pakistan, Ethnobot. Leafl. 2006; 1:32.
15. W. Hussain, J. Hussain, R. Ali, S. Hussain, M.A. Khan, I. Khan, W.A. Lopes, I.A. Nascimento, Phytomedicinal studies of Kurram agency in the federally administered tribal areas (FATA) of Pakistan, J. Appl. Pharm. Sci. 2012; 2(10):081–085.
16. M. Khan, M.A. Khan, G. Mujtaba, M. Hussain, Ethnobotanical study about medicinal plants of Poonch valley Azad Kashmir, J. Anim. Plant Sci. 2012; 22:493–500.
17. N. Khan, A.M. Abbasi, G. Dastagir, A. Nazir, G.M. Shah, M.M. Shah, M.H. Shah, Ethnobotanical and antimicrobial study of some selected medicinal plants used in Khyber Pakhtunkhwa (KPK) as a potential source to cure infectious diseases, BMC Complement. Altern. Med. 2014; 14 (1):122.
18. S. Chowdhary, D. Verma, H. Kumar, Biodiversity and traditional knowledge of Bergenia spp in Kumaun Himalaya, Sci. New York N.Y. 2009; 2(6):105–108.
19. L. Asolkar, K. Kakkar, O. Charke, Second Supplement to Glossary of Indian Medicinal Plants with Active Principles. Part-I (A–K) (1965–1981), Publications and Information Directorate (CSIR), New Delhi, 1992.
20. M.S. Bagul, M. Ravishankara, H. Padh, M. Rajani, Phytochemical evaluation and free radical scavenging properties of rhizome of Bergenia ciliata (Haw.) Sternb. forma ligulata Yeo, J. Nat. Remedies 2003; 3(1):83–89.
21. S. Sinha, T. Murugesan, K. Maiti, J. Gayen, B. Pal, M. Pal, B. Saha, Antibacterial activity of Bergenia ciliata rhizome, Fitoterapia 2001; 72 (5):550–552.
22. L. Rai, P. Prasad, E. Sharma, Conservation threats to some important medicinal plants of the Sikkim Himalaya, Biol. Conserv. 2000; 93(1):27–33.
23. K. Biswas, Common Medicinal Plants of Darjeeling and the Sikkim Himalayas, (1956).
24. S. Kapur, Ethnomedico Plants of Kangra Valley (Himachal Pradesh), (1993).
25. V. Kumar, D. Tyagi, Review on phytochemical, ethnomedical and biological studies of medically useful genus Bergenia, Int. J. Curr. Microbiol. App. Sci. 2013; 2 (5):328–334.
26. R. Dharmender, T. Madhavi, A. Reena, A. Sheetal, Simultaneous Quantification of Bergenin, (+)-Catechin, Gallicin and Gallic acid; and quantification of (-Sitosterol using HPTLC from Bergenia ciliata (Haw.) Sternb. Forma ligulata Yeo (Pasanbheda). Pharm Anal Acta 1: 104. 10.4172/2153-2435.1000104, OMICS Publishing Group Pharm Anal Acta ISSN, 2010.
27. R. Chauhan, K. Ruby, J. Dwivedi, 6. Golden herbs used in piles treatment: a concise report, Int. J. Drug Dev. Res. 2012; 4 (4):50–68.
28. I.A. Mazhar-ul-Islam, K. Usmanghani, A.A. Shahab-ud-Din, Antifungal activity evaluation of bergenia ciliata, Pak. J. Pharmacol. 2002; 19 (2):1–6.
29. Sangeetha, J., & Vijayalakshmi, K. Determination of bioactive components of ethyl acetate fraction of Punica granatum rind extract. *Int J Pharm Sci Drug Res*, 2011; *3*(2):116-122.
30. Khandelwal KR, Practical pharmacognosy technique and experiments. 23rd Ed. Nirali Prakashan; 2005.
31. Kokate CK. Practical pharmacognosy. 4th Ed. Vallabh Prakashan; 1994.
32. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissue using Folin-Ciocalteu reagent. Nature Protocol, 2007; 2(4): 875-877
33. Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 1999, *64*, 555-559.