Use of bergenin as an analytical marker for standardization of the polyherbal formulation containing *Saxifraga ligulata*

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ABSTRACT

Background: Bergenin is the principle constituent of the well-known medicinal plant *Saxifraga ligulata*. Bergenin has anti-inflammatory, antipyretic, antiviral, immunostimulant, antihyperglycemic, and antioxidant properties. In this study, the presence of bergenin in *Saxifraga ligulata* and the formulations was identified using high performance thin layer chromatograpy fingerprinting technique. **Objective:** To develop a novel quantitative method for the estimation of bergenin using high performance liquid chromatography. **Materials and Methods:** The compound was separated, characterised and quantified using authentic reference standard. The method was validated per ICH guidelines for the parameters of accuracy, precision, linearity, limit of detection, limit of quantification and robustness. **Results:** The method was found to be accurate, linear (r2 = 0.998) and precise (%RSD <2%). The limits of detection (0.001%) and quantification (0.002%) were found to be suitable for detection and quantification of bergenin in commercial formulations. **Conclusion:** The developed methods are suitable for the quality control applications of Saxifraga ligulata containing formulations.

Key words: Bergenin, dosage forms, high-performance liquid chromatography, high-performance thin layer chromatography, *Saxifraga ligulata*

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INTRODUCTION

The plant *Saxifraga ligulata*, well-known as "Pashanbheda" in the indigenous system of medicine has a number of therapeutic benefits. *S. ligulata* has been widely recognized for its role in dissolving kidney stones.[1-3] It is also effective in the treatment of fever, eye ailments, dysentery and diarrhea, piles, inflammation, and chronic ulcers. Currently available data on Pashanbheda are limited and further research is needed to identify other key constituents with therapeutic properties.[4,5] Extensive survey in and around Kumaon region of Himalaya revealed that this plant is distributed in mixed vegetation on the rocky slopes in moist and shady habitats; predominantly on the northern and western slopes.[6-8] The root, rhizome, and whole plant of *S. ligulata* are used in the therapy for kidney and bladder stones and urinary problems.[9-12]

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Rhizome is the main part or source of drug. In this bitter, has a useful effect in cough and cold.[13] Flowers are consumed in the form of pickles. With honey, whole plant of *S. ligulata* is applied to gums during teething in infants to allay irritation.[14] *S. ligulata* has been reported to exhibit various pharmacological activities and thus has several traditional uses. It is used as an antidiabetic, antipyretic,[15,16] and as a tonic.[17] Ethnobotanical study conducted in the upper Siran Valley of Pakistan shows that *S. ligulata* (but pewa) is used as diuretic,[18] and hepatoprotective. Alcoholic extract of *S. ligulata* showed anticancer, antiprotozoal, diuretic, antiscorbutic, antilithiatic,[19-21] litholytic, and anti-inflammatory activities in a dose-dependent manner in rats.[22] *S. ligulata* is used in the formulation of herbal composition. Its composition is useful for caring the skin around the eyes.[23,24] The whole plant of *S. ligulata* showed activities like calcium oxalate monohydrate growth inhibition, decreased calcium phosphate nucleation, calcium oxalate inhibition, diuretic, hypermagneseuric, and antioxidant effect in *in vitro* and *in vivo* models.[25]

Bergenin is a trihydroxy benzoic acid glycoside with an IUPAC nomenclature as (2 R,3S,4S,4aR,10bS) -3,4,8,10

-tetrahydroxy -2 -(hydroxymethyl) -9 -methoxy -3,4,4a, 10b -tetrahydro -2H -pyrano[3,2 -c] isochromen -6 -one. -tetrahydroxy -2 -(hydroxymethyl) -9 -methoxy -3,4,4a, 10b -tetrahydro -2H -pyrano[3,2 -c] isochromen -6 -one. Various methods have been published for quantitative estimation of bergenin in S. ligulata. Several protocols employing high -performance liquid chromatography (HPLC) have been reported for estimation of bergenin in S. ligulata. However, the methods described hitherto have several limitations like extensive sample preparation.[26,27] In this study, an accurate, simple, and reproducible HPLC method was developed and validated for the determination of bergenin in S. ligulata herb extract and dosage form.

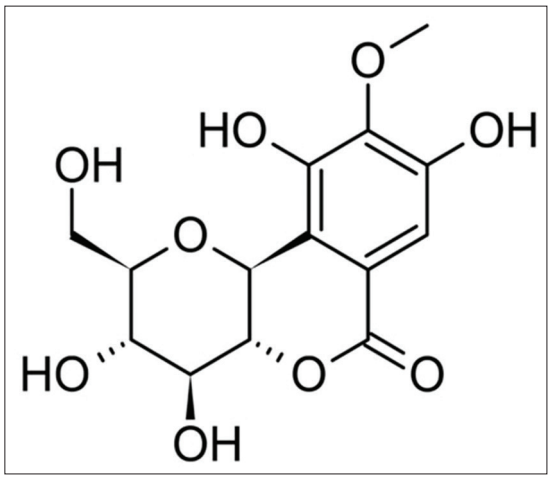

Figure 1: Bergenin structure

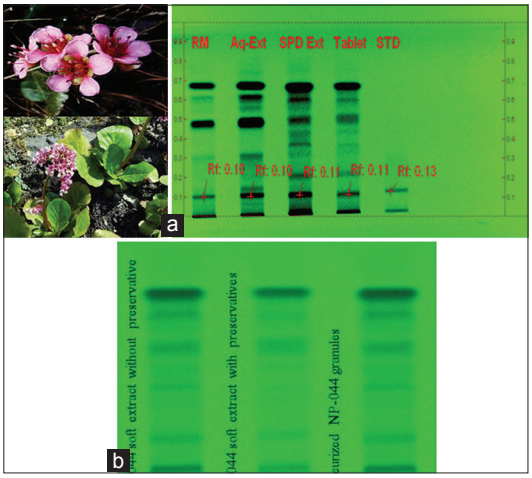

Figure 1 (a,b): Identification of bergenin by high performance thin layer chromatography (HPTLC) method HPTLC chromatogram of Saxifraga ligulata sample viewed under UV 254 nm. (a) Raw material of Saxifraga ligulata root, Aqueous extract of Saxifraga ligulata root, NP-044 SPD extract, NP-044 SPD extract, NP-044/ERNP tablets, STD berginin (0.1 mg/ml). (b) Formulation 1: NP-044 soft extract without preservative, Formulation 2: NP-044 soft extract with preservatives, Formulation 3: Auraclaved (pasteurized) NP-044 granules

MATERIALS AND METHODS

Materials

Whole plant of *S. ligulata* was authenticated by Dr. Kannan, Botanist, The Himalaya Drug Company, (Bangalore, India). Extracts and formulations were prepared by the Department of Formulation Development, The Himalaya Drug Company.

Standard bergenin (total purity 98%) was procured from ChromaDex. Methanol and acetonitrile were of HPLC grade was procured from Rankem. Milli-Q water generated by the Waters System Model ZMQX5V0001 was used during the analysis. Methanol was used as a solvent for the preparation of standards and samples, acetonitrile (solvent-A) and purified water (solvent-B) (75:25, v/v) were used as mobile phase for HPLC analysis. All solutions used for HPLC analysis were filtered through 0.45 µm membrane filter using Millipore type filtration unit. A C18 ODS LUNA-phenomenex (250 mm × 4.60 mm) 5 µ column was used as the stationary phase.

Precoated silica gel 60 F_{254} plates with a thickness of 0.2 mm were used (E-Merck, Mumbai) for thin layer chromatographic assay. Toluene: Ethyl acetate: Formic acid in a ratio of 6:6:1 was used as the mobile phase.

Instrumentation

A CAMAG Linomat V instrument was used for a sample application; reprostar IV was used for photo-documentation.

A Shimadzu Prominence HPLC system, comprising quaternary pumps, photodiode array (PDA) auto-injector was used for HPLC analysis. Liquid chromatography (LC) solution software (Shimadzu corporation, JAPAN) was used for integration and calibration.

Sample preparation

High-performance liquid chromatography

About 100 mg of finely powdered *S. ligulata* plant and dosage forms powder and 50 mg of *S. ligulata* extracts were weighed separately in a 100 ml beaker. About 30 ml of methanol was added and sonicated for about 2–3 min and allowed to settle. The extracts were decanted separately into 50 ml volumetric flasks. The residues were again extracted twice in a similar fashion with 10 ml of methanol each time. The extracts were combined with their respective volumetric flasks. The volume was made up with methanol and filtered through 0.45 µm syringe filter and used for further analysis.

High-performance thin layer chromatography fingerprinting

One-gram sample was dissolved with methanol and made up to 100 ml with the same solvent and used for

high-performance thin layer chromatography (HPTLC) analysis. Standard bergenin at 1 mg/ml concentration in methanol was used.

High-performance thin layer chromatography fingerprinting

High-performance thin layer chromatography

10 µl of sample and standard were spotted on CAMAG Linomat V of 12 mm bandwidth on a precoated silica gel 60 F_{254} plate of 0.2 mm thickness. The plate was developed in the solvent system of toluene: Ethyl acetate: Formic acid in a ratio of 6:6:1. Identification of bergenin was captured at 254 nm. The Rf value was identified at 0.13 with dark blackish brown spot [Figure 1a and b].

High-performance liquid chromatography

The HPLC system (Shimadzu prominence quaternary pumps), PDA auto-injector and C18 ODS LUNA-phenomenex (250 mm × 4.60 mm) 5 µ column was used at 40°C temperature. Binary elution was carried out with acetonitrile: Purified water, 75:25, v/v at a flow rate of 0.8 ml/min, detection was at 275 nm using PDA. LC solution software was used for integration and calibration. Evaluation was via peak areas with linear regression.

Estimation of bergenin

To estimate the bergenin content in *S. ligulata* plant, tablets and extract samples, 20 µl aliquots of the sample were injected into HPLC. The HPLC analysis was continued for 30 min. The content of bergenin in dosage form was calculated by linear regression and mean percentages were calculated from triplicate experiments. The percentage of bergenin was calculated using 0.1 mg/ml of bergenin standard curve. The values were tabulated as shown in Table 1. The chromatograms are shown in Figure 2a-d.

Calibration curve of standard and sample bergenin

Bergenin standard solution was prepared in methanol at a concentration of 1 mg/ml (stock solution). Working standard solutions were prepared by diluting standard stock solution with methanol in the concentration range 20–100 µg/ml. 20 µl from each working standard solution was injected in triplicates. Calibration curve was generated by linear regression based on peak areas.

0.2 mg/ml–4 mg/ml of bergenin tablet solution was prepared in methanol. 20 µl from each solution was injected in triplicates. Calibration curve was generated by linear regression based on concentration and peak areas.

Validation

The developed HPLC method was validated as per the principles of International Conference on Harmonization guidelines.

Accuracy

Accuracy was determined by spiking known concentrations of bergenin in the formulation sample at the rate of 10%, 20%, and 30% over the observed concentration. The experiment was done in triplicates. The results were expressed as mean recovery ± % relative standard deviation (RSD).

Precision

Interday and intraday precision of bergenin estimation were determined using two different instruments on two different days. The estimations were performed in triplicates. The results were expressed as mean assay ±%RSD.

Linearity

In order to check if the method response follows a linear regression on a 5-point calibration curve was constructed for the sample and standard. Since the polyherbal formulations available in the market may show wide range of bergenin content due to different proportions of *S. ligulata*, we have constructed the linearity curve in wide range from 10% to 200% of the expected concentrations of the current formulation.

Limit of detection

Limit of detection was calculated using the standard error of the slope method and 3.3 times of such error was considered as a limit of detection.

Table 1: Intraday and interday precision of bergenin

Analysis on day 1			Analysis on day 2		
Repeatability (mg/ml)	Bergenin (%)	Mean ± %RSD	Intermediate precision (mg/ml)	Bergenin (%)	Mean ± %RSD
Sample 1	1.6978	-	Sample 1	1.6826	-
Sample 1	1.6928	-	Sample 1	1.6258	-
Sample 1	1.6842	1.6916±0.186	Sample 1	1.6325	1.647±0.851
Sample 2	1.7016	-	Sample 2	1.5884	-
Sample 2	1.6622	-	Sample 2	1.5314	-
Sample 2	1.6487	1.6708±0.749	Sample 2	1.5797	1.567±0.862
Sample 3	1.7455	-	Sample 3	1.5391	-
Sample 3	1.6537	-	Sample 3	1.5494	-
Sample 3	1.6408	1.6620±0.723	Sample 3	1.5229	1.537±0.378

RSD: Relative standard deviation

Limit of quantification

Limit of quantification was calculated using the standard error of the slope and 10 times of such error was considered as a limit of quantification.

RESULTS AND DISCUSSION

The chemical composition of herbal extracts are very complex, evaluating the quality of these extracts can only be assured by the use of validated analytical methods for identification and quantification of the active ingredients. The HPLC methods for the quantitative estimation of bergenin were validated with regard to their precision, accuracy and linearity.

Triplicates of three different concentrations of bergenin tablet on the same day (intraday) and different days (inter day) were carried out, integrated and expressed as % RSD. The results noted in Table 1, reveal no significant intraday and interday variations. The % RSD for intraday and interday analysis was found to be in the range, bergenin 0.10–0.58, which is <2%. The accuracy of the method was determined from recovery studies. The preanalyzed sample was spiked with three different concentrations of bergenin, 10%, 20%, and 30%, and the mixtures were analyzed by the proposed method. The bergenin recoveries were in the range of 92.51–95.72 as reported in Table 2. The linearity of the sample curve was evaluated by injecting five concentrations ranging from 10% to 200% that is, 0.2, 1, 2, 3, 4 mg/ml solutions [Table 3]. Peak area and concentrations were subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficient. Linearity was obtained over a concentration range of 10–200% of the sample. The

Table 2: Recovery study of bergenin

Amount of bergenin added (%)	Amount of bergenin assay (%)	% Recovery	Mean recovery ± %RSD
Unspiked	1.5878	-	-
Unspiked	1.5775	-	-
Unspiked	1.5912	-	-
Spiking 10	1.9116	107.95	-
Spiking 10	1.9031	108.52	-
Spiking 10	1.9203	109.70	108.7226±0.8195
Spiking 20	2.2399	108.68	-
Spiking 20	2.2323	109.12	-
Spiking 20	2.2544	110.53	109.4443±0.8814
Spiking 30	2.5139	102.90	-
Spiking 30	2.5062	103.19	-
Spiking 30	2.5077	101.83	102.6402±0.6958

RSD: Relative standard deviation

Table 3: Standard and sample linearities of bergenin

Standard linearity of bergenin		Sample linearity of bergenin	
Standard concentration (mg/ml)	Standard area under curve (cumulative)	Sample concentration %	Sample area under curve
0.02	1639,975	10	658,499
0.04	3225,979	50	3036,757
0.06	4881,040	100	6120,062
0.08	6679,351	150	8718,084
0.1	7974,016	200	12177,281

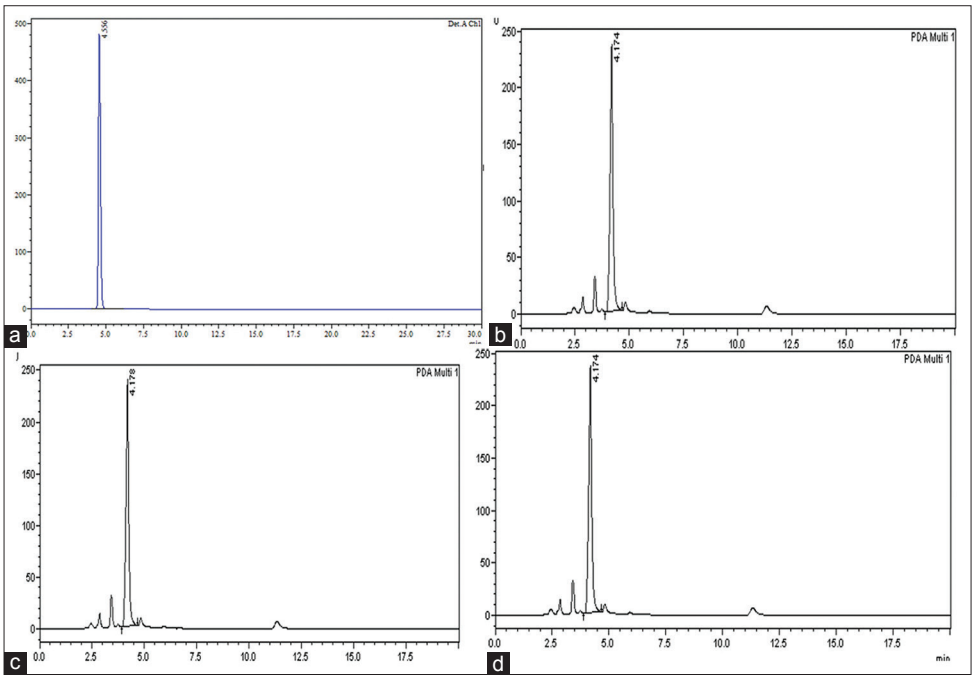

Figure 2: (a) Bergenin Standard Chromatogram (b) Sample Saxifraga ligulata plant Chromatogram (c) Sample Chromatogram (d) Sample Saxifraga ligulata Extract Chromatogram

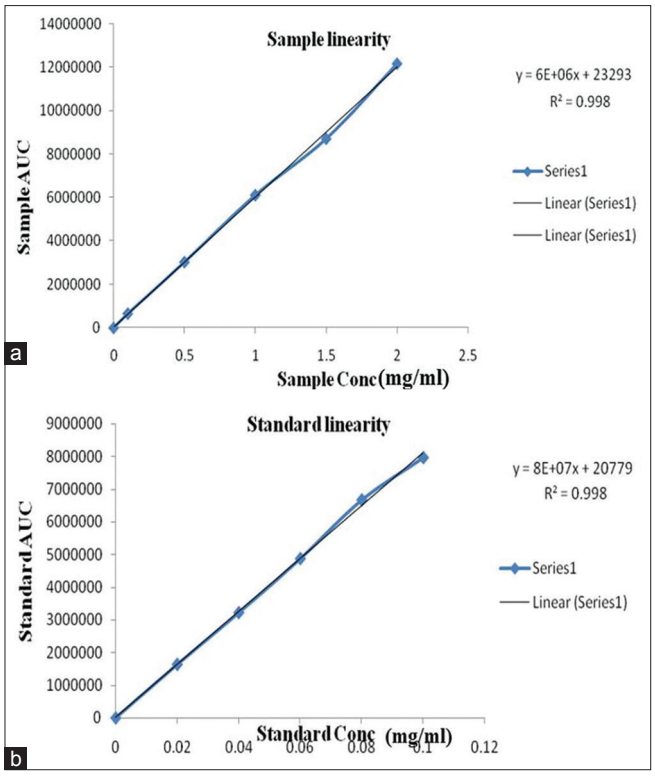

Figure 3: (a) Sample linearity (b) Standard linearity

Table 4: LOD and LOQ

Signal to noise	Percentage	Average	LOD (% ×3.3)	LOQ (% ×10)
Run-1	0.0002	-	-	-
Run-2	0.0002	-	-	-
Run-3	0.0003	0.00019	0.001	0.002

LOD: Limit of detection; LOQ: Limit of quantification

Table 5: Estimation of bergenin

Sample	HPLC method	
	Estimated amount of bergenin (%)	Mean ± %RSD
NP-044 formulation 1-Run 1	1.5112	-
NP-044 formulation 1-Run 2	1.5034	-
NP-044 formulation 1-Run 3	1.4982	1.5042±0.1524
NP-044 formulation 2-Run 1	1.3548	-
NP-044 formulation 2-Run 2	1.3364	-
NP-044 formulation 2-Run 3	1.3664	1.3525±0.3684
NP-044 formulation 3-Run 1	1.3680	-
NP-044 formulation 3-Run 2	1.3691	-
NP-044 formulation 3-Run 3	1.3608	1.3659±0.1093

Formulation 1: NP-044 soft extract without preservative; Formulation 2: NP-044 soft extract with preservatives; Formulation 3: Auraclaved (pasteurized) NP-044 granules; RSD: Relative standard deviation; HPLC: High-performance liquid chromatography

linearity of standard and sample calibration graphs is shown in Figure 3a and b. The results are mentioned in Table 4. In the theoretical plates, asymmetry observed were within the limits of USP.[28] Reproducibility of bergenin content in three different formulations is provided [Table 5]. This optimized RP-HPLC method serves as the best quality control tool for qualitative and quantitative evaluation of bergenin in polyherbal formulations containing *S. ligulata*.

Application of high-performance liquid chromatography methods

The HPLC method developed serves as a very precise and robust tool for quantitative determination of bergenin. This method can be adopted to assess the quality of the drugs which can add more value to herbal research.

CONCLUSION

The developed HPLC and HPTLC methods can be utilized for the qualitative and quantitative estimation of bergenin in *S. ligulata* herb samples, extracts and dosage forms. The methods developed are simple, sensitive, and statistically validated for linearity, accuracy, and precision.

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REFERENCES

1. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: CSIR; 1956.
2. Yaginuma A, Murata K, Matsuda H. β-glucan and *Saxifraga ligulata* as cosmetics ingredient. Fragr J 2003;31:114-9.
3. Panda H. Medicinal Plant Cultivation and Their Uses. Delhi: National Institute of Industrial Research; 2002.
4. Dush B, Kashyap L. Herbal plants in kidney stone. In: Materia Medica of Ayurveda. New Delhi: Concept Publishing Co.; 1979. p. 89.
5. Panday G. Medicinal Plants of Himalaya. New Delhi: Sri Satguru Publications; 1995.
6. Plant Database. © Plant Database Ltd.; 2010-2014. Available from: http://www.plantdatabase.co.uk/Bergenia_cordifolia. [Last accessed on 2014 Mar].
7. Badon AK. Bulletin of Botanical Survey of India 1993;32:103-115.
8. Patel AM, Savadi RV. Pharmacognostic and phytochemical evaluation of *Bergenia ciliate* rhizome. Int J Pharm Rev Res 2014;4:52-5.
9. Chauhan RR, Sharma S, Dwivdei J. Polypharmacological activities of *Bergenia* species. Int J Pharm Sci Rev Res 2012;13:100-10.
10. Harsoliya MS, Pathan JK, Khan N, Bhatt D, Patel UM. Effect

of ethanolic extracts of *Bergenia ligulata, Nigella sativa* and combination on calcium oxalate urolithiasis in rats. Int Drug Formul Res 2011;2:268-80.

11. A manual on participatory inventory and management of Pakhenbed (*Bergenia ciliata syn. Bergenia ligulata*) based on results of case studies from six CFs of Ramechhap district. Nepal Swiss Community Forestry Project (NSCFP); 2006. April 07, Ref. No. 24/062/63.
12. Pant S, Samant SS. Ethanobotanical observations in the Mornaula Reserve Forest of Kumoun, West Himalaya, India. Ethnobotanical Lealf 2010;14:193-217.
13. Samant SS, Butola SJ, Sharma A. Assessment of diversity, distribution, conservation status and preparation of management plan for medicinal plants in the catchment area of Parbati Hydroelectric project Stage – III in Northwestern Himalaya. J Mt Sci 2007;4:34-56.
14. Pant S, Samant SS, Arya SC. Diversity and indigenous household remedies of the inhabitants surrounding Mornaula reserve forest in west Himalaya. Indian J Tradit Knowledge 2009;8:606-10.
15. Samal PK, Dhyani PP, Dollo M. Indigenous medicinal practices of bhotia tribal community in Indian central Himalaya. Indian J Tradit Knowledge 2010;1:140-4.
16. Sharma HK, Chhangte L, Dolui AK. Traditional medicinal plants in Mizoram, India. Fitoterapia 2001;72:146-61.
17. Ballabh B, Chaurasia OP, Ahmed Z, Singh SB. Traditional medicinal plants of cold desert Ladakh-used against kidney and urinary disorders. J Ethnopharmacol 2008;118:331-9.
18. Negi CS, Nautiyal S, Dasila L, Rao KS, Maikhuri RK. Ethnomedicinal plant uses in a small tribal community in a part of central Himalaya, India. J Hum Ecol 2010;14:23-31.
19. Saijyo J, Suzuki Y, Okuno Y, Yamaki H, Suzuki T, Miyazawa M. Alpha-glucosidase inhibitor from Bergenia ligulata. J Oleo Sci 2008;57:431-5.
20. Singh N, Juyal V, Gupta AK, Gahlot M. Evaluation of ethanolic extract of root of *Saxifraga ligulata* for hepatoprotective, diuretic and antipyretic activites. J Pharm Res 2009;2:958-60.
21. Singh N, Gupta AK, Juyal V. A review on *Saxifraga ligulata* wall. Int J Chem Anal Sci 2010;1:71-3.
22. Chowdhary S, Kumar H, Verma DL. Biodiversity and traditional knowledge of *Bergenia spp*. Sci J 2009;2:105-8.
23. Havagira YR, Chitme SA, Jain SK, Sabharwal M. Herbal treatment for urinary stones. Int Pharm Sci Res 2010;1:58-60.
24. Shah GM, Khan MA. Check list of medicinal plants of Siran valley Mansehra, Pakistan. Ethnobotanical Lealf 2006;10:63-71.
25. Aggarwal BB, Prasad S, Reuter S, Kannappan R, Yadev VR, Park B, *et al.* Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases: "Reverse pharmacology" and "bedside to bench" approach. Curr Drug Targets 2011;12:1595-653.
26. Umashankar D, Reddy C, Chawla AS, Deepak M, Singh D, Handa SS. High pressure liquid chromatographic determination of bergenin from different parts of Paashaanbhed (*Saxifraga ligulata* Yeo). Phytochem Anal 1999;10:44-7.
27. Singh DP, Srivastava SK, Govindarajan R, Rawat AKS. Pharmacological and analytical aspects of bergenin. Pac J Trop Dis 2012;2:163-7.
28. 1225 VALIDATION OF COMPENDIAL PROCEDURES Pharmacopeial Forum: USP32-NF27 Page 733 United States Pharmacopoeia. Vol. 34. p. 794.

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