ANTIUROLITHIATIC ACTIVITY OF *BERGENIA CILIATA* LEAVES

[1]Vivek V. Byahatti*, [1]Vasantakumar Pai K, [2]Amjad K, [1]Marina G. D'Souza

[1]Dept. of PG studies and Research in Industrial Chemistry
Kuvempu University, Shankaragatta- 577451
Shimoga dist. Karnataka ,India
vivekbyahatti68@gmail.com/vivekbyahatti68@yahoo.com

[2]Dept. of Pharmacognosy
KLESs College of Pharmacy
Hubli-580031, Karnataka

Summary

Urolithiasis or kidney stone formation is a complex process that results from series of several physicochemical events including super-saturation, nucleation, growth, aggregation and retention within the kidneys. Among the treatments include extracorporeal shock wave lithotripsy (ESWL) and drug treatment. Even this ESWL treatment may cause acute renal injury, decrease in renal function and increase in stone recurrence. In addition, persistent residual stone fragments and possibility of infection after ESWL represent a serious problem in the treatment of stones. Data from *in-vitro, in-vivo* and clinical trials reveal that phytotherapeutic agents could be useful as either alternative or an adjunct therapy in the management of urolithiasis. Medicinal plants / natural products are more acceptable to the body because they promote the repair mechanism in natural way. Various plant species of the genus *Bergenia,* have been reported to posses antiurolithiatic property. *Bergenia ligulata* is one the ingredient of reputed herbal formulation Cystone for the treatment of kidney stones. In this study alcohol, butanol, ethyl acetate extracts and isolated phenolic compound from the Ayurvedic and Unani herb, *Bergenia ciliata* leaves (Saxifragaceae) were evaluated for their potential to dissolve experimentally prepared kidney stones-calcium oxalate and calcium phosphate, by an *in-vitro* model. Phenolic compound P1, isolated from the ethyl acetate fraction of the leaves, demonstrated highest dissolution of both stones when compared to test extracts at 10 mg concentration. However, it was more effective in dissolving calcium phosphate stones (67.74 %) than oxalate (36.95%). Reference standard-formulation Cystone was found to be more effective (48.48%) when compared to compound P1.

Key words: Antiurolithiatic, *Bergenia ciliata*, kidney stones, calcium oxalate, calcium phosphate

Introduction

Kidney stones, one of the painful disorders of urinary tract, are ancient one and still remain a common problem world-wide. The pathogenesis of urinary calculi continues to be more or less an enigma. The incidence of urolithiasis is more in men (recurrence rate 70-80%) than women (47-60%). The most common type of kidney stones contains calcium, in combination with either oxalate or phosphate. These chemicals are part of a person's normal diet and make up important parts of the body, such as bones and muscles. Cystinuria and hyperoxluria are two other rare, inherited metabolic disorders that often cause kidney stones. Many remedial measures have been employed during ages to treat this condition.

Most of them were from plants and prove to be useful. However, the rational behind their use is not well established except for a few plants and reported to be effective with no side effects.

Bergenia ciliata (Haw) sternb. (Saxifragaceae), is a perennial herb with stout underground rhizomes and large hairy leaves, often called as MEGASEAS, found in temperate Himalayas between altitudes 900-3000 mts. It is commonly known as Pashana bheda/ silabheda and is endowed with phytoconstituents flavonoids, tannins, sterols etc. This Ayurvedic and Unani herb has been documented earlier for its therapeutics in treating kidney stones, ulcers, spleen enlargement, dysentery, fever, cough, as an astringent, antisorbutic, laxative.

The rhizomes of the herb have been reported to possess antiurolithiatic, anti-inflammatory, antioxidant, antibacterial properties[1-7]. This study has been undertaken to evaluate *Bergenia ciliata* leaves for their possible potential to dissolve experimental urinary stones by an *in-vitro* model and to isolate the chemical entity (s) responsible for the activity.

Materials and Methods

Plant material- *Bergenia ciliata* leaves were collected in the month of July/August from the local forests of Chota Shimla and authenticated by Dolphin Institute of Biomedical and Natural Sciences, Dehradun (Uttaranchal state) India and the voucher specimen of the same (No. Vb-Bc-02) has been deposited in the Pharmacognosy research laboratory of KLESs College of Pharmacy, Hubli, for future reference.

Extraction and isolation- Shade dried leaves pulverized, & about 100 gms of powder was extracted with ethanol (95%) in a soxhlet. Another 100 gms of the crude drug powder was extracted successively with pet. ether (40-60), chloroform, n-butanol, ethyl acetate and finally with alcohol. All extracts were concentrated in a rotary flash evaporator and the residue was dried in a desiccator over sodium sulphite [8].

All the prepared extracts were subjected to qualitative chemical tests to detect the presence of different class of Phytoconstituents. TLC studies were done to substantiate the presence of constituents detected in chemical tests and to know how many compounds are present in each extract [9]. The ethyl acetate fraction was column chromatographed over silicagel for column chromatography and eluted with ethyl acetate: glacial acetic acid: formic acid: dist. water (126.8:5:5:12.2). Fractions showing the same number of compounds & same R_f values, were combined (based on TLC studies) [4,5] **,** concentrated & evaporated to dryness and named as P1, P2 & P3. These separated compounds subjected to physical, chemical & spectral (UV, FT-IR & FT-NMR) studies. The compound P1, which was obtained in relatively high quantity (565 mgs) and responded more positively for identity parameters, was taken-up for pharmacological evaluation.

Acute Toxicity Studies : Albino mice of either sex weighing between 20-30 gm were used. The animals were fasted over night prior to the experimental procedure. The Up & Down or 'Staircase' method was adopted [10], and accordingly doses of alcohol, butanol, ethyl acetate, successive alcohol & aqueous extracts were calculated.

Procedure- Two mice were injected with particular dose, and observed for a period of 24 hr for any mortality. The subsequent doses are then increased by a factor of 1.5, if the dose was tolerated or decreased by a factor of 0.7 if it was lethal.

Evaluation for Antilithiatic Activity (*In vitro*)[6,11,14,15]: Alcoholic extract, n-butanol fraction, ethyl acetate fraction and isolated phenolic compound P1, were evaluated for antilithiatic activity by *in-vitro* model using prepared calcium oxalate and calcium phosphate stones. Formulation Cystone was used as a reference standard.

i) Preparation of experimental kidney stones (Calcium oxalate & Calcium phosphate stones) by homogenous precipitation [11] - Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Sodium oxalate (AR) in 10 ml of 2N H_2SO_4 were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate.

Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10 ml of (2N H_2SO_4), was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium phosphate. Both precipipitates freed from traces of sulfuric acid by ammonia solution .Washed with distill water and dried at 60^0 C for 4 hours.

ii) Preparation of semi-permeable membrane from farm eggs- The semi - permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2 M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. Washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while & then rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Figure-I *In-vitro* experimental model setup to evaluate antiurolithiatic activity

iii)Estimation of Calcium oxalate by Titrimetry [13]- Weighed exactly 1 mg of the calcium oxalate and 10 mg of the extract/compound/standard and packed it together in semi permeable membrane by suturing as shown in Model design Figure-I. This was allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer. One group served as negative control (contained only 1 mg of calcium oxalate)**.** Placed the conical flask of all groups in a incubator, pre heated to 37^0 C for 2 hours, for about 7-8 hours. Removed the contents of semi-permeable membrane from each group into a test tube. Added 2 ml of 1 N sulfuric acid and titrated with 0.9494 N $KMnO_4$ till a light pink color end point obtained.1ml of 0.9494 N $KMnO_4$ equivalent to 0.1898 mg of Calcium.

iv) Estimation of Calcium phosphate by colorimetry [6, 12]-- Up to removal of contents from semi-permeable membrane same as above. Removed the contents of semi permeable membrane from each group into a test tube. Added 2 ml of 1 N sulfuric acid, 2.5 ml of Molybdic-sulphuric acid reagent, 1 ml of Reducing solution and made up the volume to 10 ml using distill water. Standard dilutions of calcium phosphate were prepared, (200, 400, 600, 800 and 1000 µg/ml) containing 2.5 ml of Molybdic-sulphuric acid reagent, 1 ml of Reducing solution and made up the volume to 10 ml using distilled water respectively. Measured the optical density of standard dilutions and for the groups under study in colorimeter at 600-750 nm. The undissolved calcium phosphate was determined from the standard calibration curve by extrapolation.

The amount of undissolved calcium oxalate/phosphate is then subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate/phosphate actually the test substance (s) could dissolve.

Results

Qualitative chemical tests indicated the presence of phenolic compounds, steroids, glycosides, proteins & amino acids and carbohydrates in different extracts of *Bergenia ciliata* leaf. Isolated compound P1 responded positively for ferric chloride test (green color) & shinoda test (yellow color), and showed a single black spot (R_f 0.92) in ethyl acetate: GAA: FA: dist. water (126.8:5:5:12.2) solvent system when sprayed with 5% alcoholic ferric chloride reagent.

Acute toxicity studies of alcohol and butanol extracts of *Bergenia ciliata* leaves showed that, up to 5000 mg/kg body wt. they are practically non-toxic. Hence,$1/10^{th}$ of which i.e., 500 mg/kg b.wt. was considered as effective dose. However, the ethyl acetate fraction at 2000 mg/kg b.wt., produced lethal effect and was found to be toxic.

The crude phenolic compound P1 at 10 mg concentration produced highest dissolution of both calcium oxalate & phosphate stones in comparison to alcohol, butanol & ethyl acetate extracts. Cystone was found to be more effective when compared to compound P1.

Table-I

Dissolution of calcium oxalate stones by test extracts/phenolic compound P-1 and Cystone

Group	Vol. of stand. $KMnO_4$	Wt. of Calcium Estimated	Wt. of Calcium Reduced	% Dissolution
Control*	4.6 ml	0.8730 mg	---	---
Standard* (Cystone)	2.6 ml	0.4934 mg	0.3796 mg	48.48
Alcohol extract*	3.8 ml	0.7212 mg	0.1518 mg	17.38
Butanol extract*	4.0 ml	0.7592 mg	0.1138 mg	13.03
Ethyl acetate extract*	3.2 ml	0.6073 mg	0.2657 mg	30.43
Phenolic Comp. P1*	2.9 ml	0.5504 mg	0.3226 mg	36.95

*** Correspond to 10 mg**

Histogram No.1

Percentage dissolution of calcium oxalate by various groups

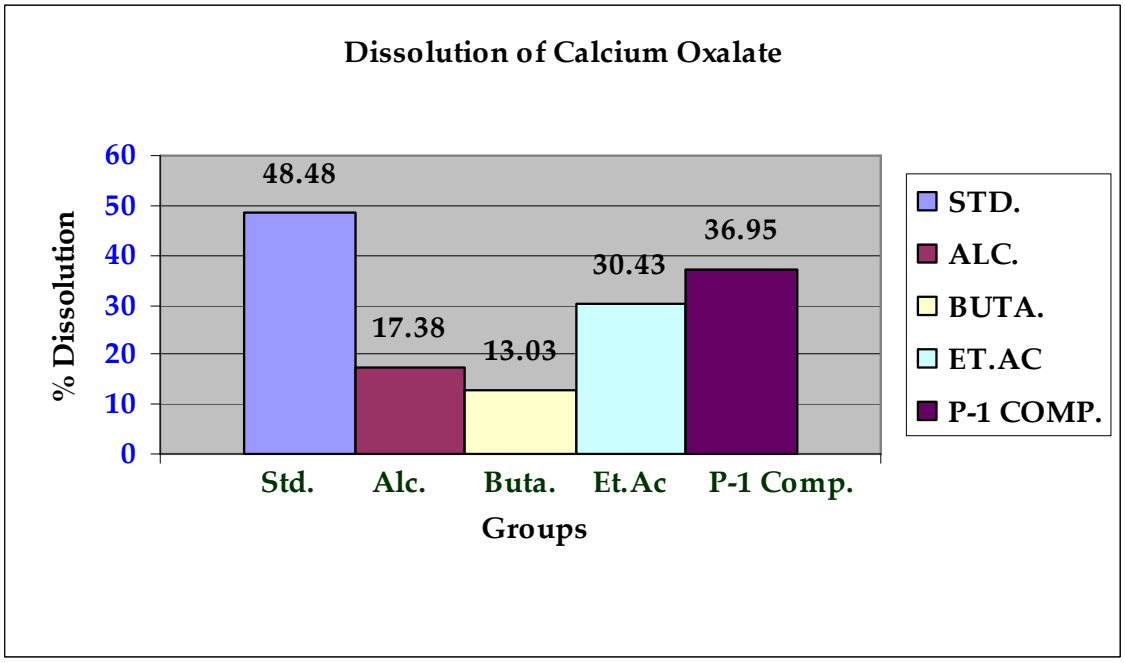


Table-II

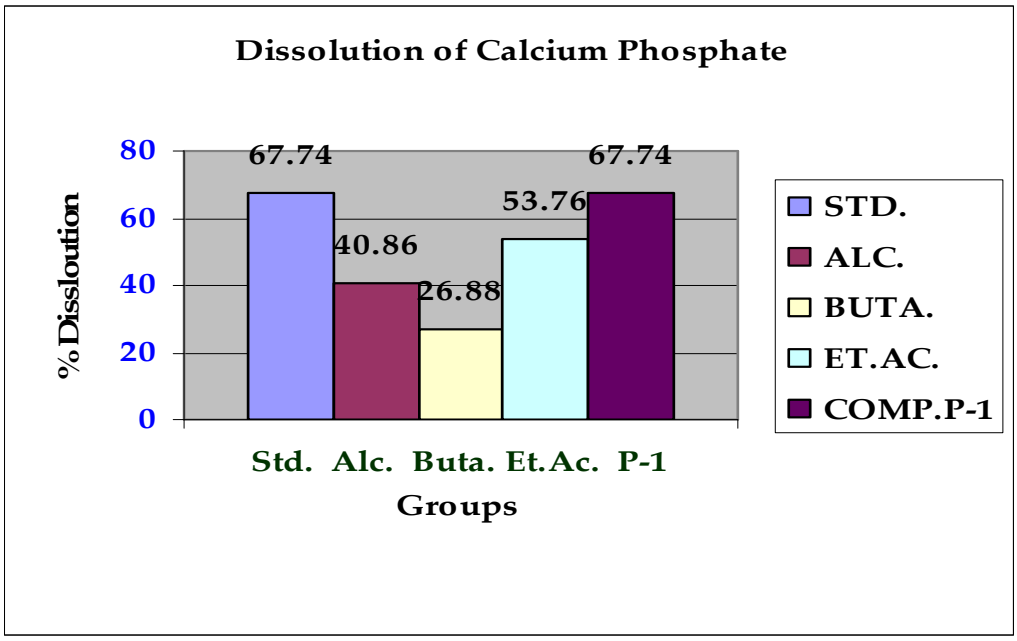
Dissolution of calcium phosphate stones by test extracts/phenolic compound P-1 and Cystone

Group	Wt .of Calcium Estimated	Wt. of Calcium Reduced	%Dissolution
Control	0.8730 mg	---	---
Standard (Cystone)*	0.4934 mg	0.3796 mg	67.74
Alcohol extract*	0.7212 mg	0.1518 mg	40.86
Butanol extract*	0.7592 mg	0.1138 mg	26.88
Ethyl acetate extract*	0.6073 mg	0.2657 mg	53.76
Compound P1*	0.5504 mg	0.3226 mg	67.74

* **Correspond to 10 mg**

Histogram No. 2

Percentage dissolution of calcium phosphate by various groups



Discussion

This study evaluates antiurolithiatic activity of different extracts of *Bergenia ciliata* leaves and crude phenolic compound isolated from the same. The study of the urinary chemistry with respect to the stone-forming minerals will provide a good indication of the risk of stone formation. From the study results it is observed that isolated crude phenolic compound P1 produced highest dissolution of both calcium oxalate & phosphate stones in comparison to alcoholic extract, butanol & ethyl acetate fractions. However, it was more effective in dissolving calcium phosphate stone than oxalate. The dissolution capacity of phenolic compound P1 can be further enhanced by purification. Further, it is observed that isolated compound P1 is more potent in dissolving stones than ethyl acetate extract, from which it has been isolated. This study has given primary evidence in favour of *Bergenia ciliata* why it is commonly known as shailagarbhaja / pashaanabhedha, meaning plants grow between rocks, appearing to break them or they possess lithotriptic property. This i*n vitro* study has given lead data, and shown that compound P1is quite promising for further work in this regard.

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References

1. Anonymous. The Wealth of India - A dictionary of Indian raw materials & industrial products, revised edition. New Delhi : Publication & Information Directorate, CSIR; 1988 : Vol-2 B: 119-120.
2. Kirtikar K.R and Basu B.D Indian Medicinal Plants. Dehradun : International book distributors; 2006: 993-994.
3. Anonymous. Indian herbal pharmacopoeia. Revised new edn. Mumbai : Indian drug manufacturer's association ; 2002 : 79-87.
4. Warrier PK, Nambiar VPK, Raman Kutty C. Indian medicinal plants. A compendium of 500 species. Madras : Orient Longman Ltd.; 1994: Vol- I : 95-97
5. Rakesh K Sharma, Rajesh Arora. Herbal Drugs. A twenty first century perspective. New Delhi: Jaypee Brothers; 2006: 221-228
6. Garimella T.S., Jolly C.I., Narayanan S. Invitro studies on antilithiatic activity of seeds of *Dolichos biflorus* Linn. and rhizomes of *Bergenia ligulata* Wall. Phytother Res**.** (England) 2001; 15(4): 351-5.
7. Sinha S., Murugesan T., Pal M., Saha B.P. Evaluation of anti-tussive activity of Bergenia *ciliata* Sternb. rhizome extract in mice**.** Phytomedicine. 2001; 8 (4):298-301.
8. Harborne J.B. Phytochemical Methods- A guide to modern techniques of plant analysis, 3 rd edition. New Delhi : Springer (India) private limited ; 1998 : 5-32.
9. Wagner H. Bladet S., Zgainski E.M. Plant Drug Analysis - A TLC Atlas; 1st edition. New York : Springer Verlag Berlin Heidelberg ; 1996 : 195-214.
10. Ghosh M.N. Fundamentals of Experimental Pharmacology , 2nd edition. Culcutta : Scientific Book Agency ; 1998 : 174-179.
11. Saso l, Valentini G, Leone MG, Grippa E. Development of an *Invitro* assay for the screening of substances capable of dissolving calcium oxalate crystals. Urology International. 1998; 61(4): 210.
12. Sakakura T, Fujita K, Yasui T, Sasaki and et al. Calcium phosphate stones produced by Madin -Darby canine kidney (MDCK) cells inoculated in nude mice. Urology Research. 1999 ; 27(93): 200-5.
13. http://www.olympiades-de-chimie.org/pdf/30tp.pdf
14. Christianaa A.J.M, .Priyamole M, muthumani P and Sthyanarayana S. Antilithiatic study of Chicorium intybus linn. against glycolic acid induced stone formation. Adv. Pharmacol. Toxicol. 2004 ; 5(1) : 33-6.
15. Khan ZA, Assiri AM, AL-Afghani HM, Maghrabi TM. Inhibition of oxalate nephrolithiasis with Ammi visnaga (Al-Khillah). Int. Urol. Nephorol. 2001; 33(4) : 605-8.