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ANTI-UROLITHIATIC HERBAL FORMULATION: DEVELOPMENT, STANDARDISATION AND EVALUATION

Ujjwal Chaudhary*, Mr. Obed Singh, Dr. Shivanand Patil

Department of Pharmaceutics, Shree Dev Bhoomi Institute of Education Science and Technology, Pounda, Dehradun, Uttarakhand, India

***Corresponding author:** Ujjwal Chaudhary, Department of Pharmaceutics, Shree Dev Bhoomi Institute of Education Science and Technology,Pounda, Dehradun, Uttarakhand, India

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Abstract

Urolithiasis, also known as kidney stones, is a medical condition affecting around 10% of the global population and is more common in men. Treatment strategies include lifestyle and dietary changes to lower risk factors and the possibility of recurrence. Bryophyllumpinnatum, also known as the "miracle leaf" or "life plant," is rich in bioactive substances such as flavonoids, tannins, glycosides, steroids, alkaloids, triterpenes, and phenolic compounds. These compounds contribute to the plant's anti-inflammatory, antibacterial, antioxidant, analgesic, and possibly anti-cancer properties.Conventional therapies for urolithiasis often have drawbacks, such as invasiveness, inadequate therapy, and restricted accessibility. This research focuses on developing an anti-urolithiatic herbal formulation using medicinal plants and aiming to produce a strong, standardized formulation that may improve urolithiasis therapy and improve patient outcomes. The study studied the pharmacological effects of herbal medicine extracts from Bryophyllumpinnatum and Bergenia ciliata, using various sources and instruments.The research established standard calibration curves for Bryophyllumpinnatumin and Bergenia ciliata extracts in 6.8 pH phosphate buffer, conducted anti-urolithiatic activity tests, and established the optimum dissolving efficiency of calcium oxalate crystals in various groups. The herbal capsules were produced and tested for color, shape, size, acceptability criteria, disintegration time, and stability.Over 60 days, the capsules retained their physical qualities and dissolving characteristics, indicating their potential for application in urolithiasis treatment. Overall, these findings demonstrate the viability of employing Bryophyllumpinnatum and Bergenia ciliata extracts in pharmaceutical formulations targeted at treating urolithiasis efficiently.

Keywords: Urolithiasis,Bryophyllumpinnatumin, Bergenia ciliata, Conventional therapies, herbal formulation.

Introduction

Urolithiasis, sometimes referred to as urinary stones or kidney stones, is a medical disorder characterised by the development of calculi inside the urinary system. Kidney stones, also known as renal calculi, may form in the kidneys, ureters, or bladder as a result of the crystallisation of solutes found in urine. Urolithiasis is a common condition that affects about

10% of the global population at some stage in their life [1]. It is more common in males than women. Urolithiasis happens when the levels of stone-forming chemicals such calcium, oxalate, and uric acid go beyond their ability to dissolve, causing them to crystallise and form stones. Dehydration, eating habits, genetic susceptibility, and metabolic abnormalities are all variables that increase the likelihood of developing a certain condition. Urolithiasis may be detected using several imaging techniques such as ultrasonography, X-ray, computed tomography (CT), and intravenous pyelography (IVP) [2]. Additionally, urine analysis can be conducted to identify chemicals that contribute to the formation of stones. Urinary stones come in several kinds, such as calcium oxalate, calcium phosphate, uric acid, struvite, and cystine stones, each with unique causes. Treatment methods vary from conservative approaches including increased fluid intake and dietary adjustments to pharmacological interventions using medicines that modify urine composition. In instances of extreme severity, surgical procedures such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, or percutaneous nephrolithotomy may be necessary. Preventive techniques prioritise modifications in lifestyle and food to diminish risk factors and the likelihood of recurrence [3].

Bryophyllumpinnatum (Lam) is known as the "wonder leaf" or "life plant", and belongs to the Crassulaceae family. The plant is commonly known as air plant and cathedral bells. Native to Madagascar, it is widely naturalized worldwide in tropical and subtropical climates. The plant is morphologically identified by its thick, fleshy and succulent leaves with scalloped edges, which are oppositely grouped and often have small plantlets at the edges. Bryophyllumpinnatum is rich in bioactive chemicals such as flavonoids such as quercetin and kaempferol, tannins, bufadienolides, glycosides such as bryophyllin A and bryophyllin B, steroids, alkaloids, triterpenes and phenolic compounds. These compounds contribute to the plant's various pharmacological actions [4].

Studies have revealed that it has strong anti-inflammatory properties, making it beneficial in treating arthritis. It displays antibacterial action against bacteria and fungi, which is useful for infections. The antioxidant qualities of the plant help neutralize free radicals, lowering oxidative stress. It also offers analgesic effects for pain reduction and possible anti-cancer properties, notably against leukemia and other malignant disorders [5,6]. Some researches have revealed that Bryophyllumpinnatum leaf helps decrease blood sugar levels, has anti-diabetic benefits, and protects the liver from toxins, demonstrating hepatoprotective qualities. Its wound healing capacity is enhanced by its antibacterial and anti-inflammatory actions, and it serves as a diuretic, assisting in the treatment of edema and hypertension. Traditionally, it is used for wound healing by placing crushed fresh leaves on wounds, cuts, and burns. It gives relief from respiratory disorders like asthma, cough and bronchitis, gastrointestinal ailments including dysentery, diarrhoea and stomach ulcers, decreases fever and helps eliminate kidney stones owing to its diuretic properties [7].

Bergenia ciliata, widely known as Hairy Bergenia, is a perennial plant famous for its medicinal properties in traditional medicine. Its leaves and rhizomes are rich in diverse bioactive components, including bergenin, catechin, and gallic acid, which contribute to its therapeutic benefits. Pharmacologically, B. ciliata has anti-inflammatory, antioxidant, antibacterial, and hepatoprotective activities. The plant is effective in treating kidney stones, lung disorders, and gastrointestinal difficulties. Its anti-inflammatory abilities are very essential in treating arthritis and wound healing. The many medicinal characteristics of Bergenia ciliata make it a significant plant in pharmacognosy and herbal medicine [8].

A DDS is a formulation or technology that delivers therapeutic chemicals in the body to accomplish a desired therapeutic effect. These systems are intended to optimize medication ADME, ensuring that the drug reaches the target place in the body at the appropriate time and in the proper concentration. Advanced DDS strive to enhance the effectiveness and safety of

therapies by managing the rate, timing, and site of drug release. This subject comprises a number of technologies, including sustained-release formulations, transdermal patches, nanoparticles, and tailored delivery mechanisms, altering the way pharmaceuticals are provided and improving patient outcomes [9].

Conventional techniques for treating urolithiasis, or kidney stones, frequently have substantial shortcomings that effect patient outcomes and healthcare efficiency. These treatments, such as open surgery and non-targeted lithotripsy, usually require substantial invasiveness, leading to extended recovery periods and increased risk of complications. Furthermore, they may not successfully target all stone kinds or sizes, leading in inadequate therapy and recurring stone development. Additionally, traditional therapies might be restricted by their accessibility, expense, and the demand for specific equipment and experience. These limitations underline the need for developments in less intrusive, more accurate, and cost-effective treatment techniques for urolithiasis [10].

The rising frequency of urolithiasis, or kidney stones, needs novel research into efficient therapies. Current techniques, including surgery and medication, typically come with considerable side effects and exorbitant costs, underlining the urgent need for better, more inexpensive alternatives [11]. The current study focuses on producing an anti-urolithiatic herbal formulation, exploiting the therapeutic potential of medicinal plants. This unique strategy attempts to harness the natural litholytic and diuretic qualities of selected herbs, delivering a comprehensive, side-effect-free therapy. The project stresses thorough scientific confirmation of these herbal components, seeking to build a powerful, standardized formulation that may transform urolithiasis care and enhance patient outcomes [13].

Method &Material

Chemical used: The Chemicaland excipients used in our investigation were obtained from many trustworthy businesses. Lactose and microcrystalline cellulose were acquired from S. D. Fine Chemicals Ltd., a company located in Mumbai, India. Molychem provided the methanol. Tri's buffer was obtained from Central Drug House (P) Ltd., while talc was procured from S. D. Fine Chemicals Ltd. SRL Pvt. Ltd. supplied potassium permanganate. Both disodium hydrogen phosphate and potassium dihydrogen phosphate were obtained from Central Drug House (P) in New Delhi. In addition, hydrochloric acid (HCl) and sulphuric acid were acquired from Central Drug House (P) Ltd. in New Delhi, India.

Instrument used: Systronic provides the UV Spectrophotometer, while Wensar supplies the electronic balance. The digital pH metre is also from Systronic. Spruce Enterprise Equipment offers various instruments such as the warm plate with magnetic firebrand, disintegration apparatus, dissolution apparatus, water bath, and capsule filling machine. Mlabs supplies the desiccator, and Radical Scientific Equipment provides the incubator. The micropipette is supplied by Recorder & Medicare System, and the FTIR is from Agile.

Plant collection & Authentication: In December 2023, the foliage of Bryophllumpinnatum and the rhizomes of Bergenia ciliata were gathered from the Dehradun region of Uttarakhand, India. The plants that were gathered were verified by the Forest Research Institute (FRI) in Dehradun.

Preparation of Extract: The plant extract was prepared using the soxhlet method, which involved drying the collected leaves of Bryophyllumpinnatum and rhizomes of Bergenia ciliata without direct sunrays. The dried leaves were then crushed into a well fine powder, which was stored in a closed container. The powdered material was weighed, prepared, and weighed into a thimble. The extraction was carried out in a 70:30 ratio, and the solvent was evaporated. The extract was then filtered and evaporated to dryness using a water bath. The solid extract was then stored in a desiccator for further use. The same procedure was repeated for the powdered plant material of Bergenia ciliata [13].

Pre-formulation studies:

Organoleptic properties: It was determined what the nature, colour, odour, and taste of both extracts were [14].

Solubility studies: The study aimed to determine the soluble nature of herbal medicine extracts in various solvents. 10 mg of the drug sample was liquified in 10 ml of solvents, and the mixture was maintained in an orbital shaker. After reaching equilibrium, the samples were centrifuged for 15 minutes at 3000 rpm, and the supernatant was filtered through Whitman filter paper. The filtrate had been dissolved in the proper solvent and diluted with pH 6.8 and measured using a UV-visible spectrophotometer [15].

Maximum Wavelength of B. pinnatum& B. ciliata: The purpose of the study was to find out how soluble herbal medicine extracts were in different types of solvents. An orbital shaker was used to keep the mixture mixed after 10 mg of the drug sample was dissolved in 10 ml of solvents. The samples were centrifuged at 3000 rpmfor 15 minutes once they had reached equilibrium, and the supernatant was then filtered using Whitman filter paper. A UV-visible spectrophotometer was used to determine the concentration of the drug extracts after the filtrate had been dissolved in the proper solvent and diluted with pH 6.8 buffer [16].

Drug –Drug compatibility studies: Using IR spectroscopy, the drug substance's IR spectra was verified.Notable was the appearance of distinctive peaks connected to certain drug molecule structural features. Drug-drug interactions were investigated using Fourier transform infrared analysis (SHIMADZU). The scanning range for the samples was 400–4000 cm^2[17].

Preparation of standard calibration curve:

Preparation of standard plot of Bryophyllumpinnatumin 6.8 pH phosphate buffer: In a 1000 ml volumetric flask was prepared by dissolving 28.80g of disodium hydrogen phosphate and 11.45g of potassium dihydrogen phosphate in water. 100mg of extract was weighed and placed in a flask with a 6.8 phosphate buffer solution, resulting in a concentration of 1000µg/ml. The stock solution was diluted 10 times with 6.8 pH phosphate buffer solutions, resulting in final concentrations of 10, 20, 30, 40, and 50µg/ml. The absorbances of the samples were measured at 286nm wavelength, and a graph was generated [18].

Preparation of standard plot of Bergenia ciliata in 6.8 pH phosphate buffer: The procedure requires producing a standard plot of Bergenia ciliata in 6.8 pH phosphate buffer. Dissolved disodium hydrogen phosphate and potassium dihydrogen phosphate in water to generate 1000 ml. Transfer 100mg of extract to a 100ml volumetric flask, add a 6.8 phosphate buffer solution, and dilute to reach a concentration of 1000µg/ml stock solution. Dilute further to get final concentrations of 10, 20, 30, 40, and 50µg/ml. The absorbance of the samples was evaluated at 215 nm wavelength, and a graph was drawn to estimate concentration and absorbance [19].

***In –Vitro* Anti-urolithiatic Activity Test by Titrimetric Method:[20]**

Step 1 Stone collection: The kidney stones were acquired from the Synergy Hospital Dehradun, U.K.

Step 2: Preparation of semi-permeable membrane from farm eggs: The semi - permeable membrane of eggs resides in between the exterior calcified shell and the innercontents like albumin & yolk. Apex of eggs was pierced by a glass rod in order to suck out the full content. Empty eggs were rinsed carefully with distilled water and put in a beaker comprising 2 M HCl for an overnight, which induced completed calcification. Further, washed with distilled water and put it in ammonia solution for neutralization of acid tracesin the wet state for a

while and rinsed it with distilled Water. Then membrane was kept in refrigerator at a pH of 7-7.4.

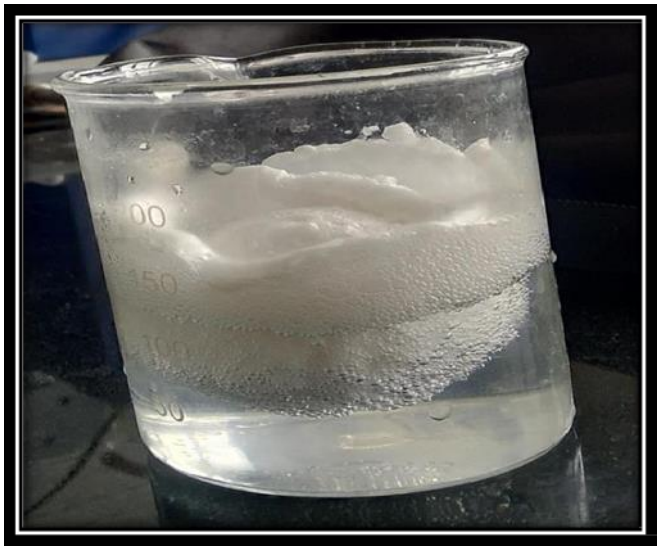

Figure 1 Separation of egg semi permeable membrane

Step 3: To check the best Calcium Oxalate crystal dissolution efficiency of each group: The study aimed to determine the best dissolving efficiency of calcium oxalate crystals in different groups. The dissolving percentage of calcium oxalate was measured by taking 0.10 gm of calcium oxalate crystal and 100,200,300,400 mg of extract, packed together in an egg membrane. The first group was provided as a blank, the second group was a positive control with 0.10 gm of calcium oxalate and varying amounts of standard drug cystone, and the third and fourth groups included 0.10gm of calcium oxalate crystals and hydroalcoholic extracts. The conical flasks were stored in an incubator at 37^{O}C for 4-5 days. The semi permeable membrane contents were collected and titrated with 0.9494 N KMnO4 until a bright pink color end point was reached.

Antiurolithiatic Activity Test by Titrimetry: The dissolving percentage of calcium oxalate was determined by taking varied ratio of both extracts i.e. 1:1, 1:2, 1:3, and 1:4 and 0.10 gm of stone, packed it together in semipermeable membrane of egg. This was allowed tosuspend in a conical flask containing 100 ml of 0.1M Tri's buffer. The conical flasks of all groups were housed in an incubator warmed to 37oC for 4-5 days.The contents of semipermeable membranes from each group were removed into separate test tubes. 2 ml of 1N Sulphuric acid was added to each test tube and titrated with 0.9494 N KMnO4 until a bright pink hue end point was attained[20].

Formulation of Herbal Capsule[21]

Selection of Excipients: Apart from the active ingredients, diluents (filler), binder, disintegrating agent, lubricant, and preservatives are required excipients for the manufacturing of capsules. The choice of excipients was done bearing in mind the current Food and Drugs Administration (FDA) restrictions.

Preparation of Granules: Dry granulation technique was used to make granules of the extracts: Calculated quantity of crude extract, Micro crystalline cellulose, were weighed and blended well. After drying this substance moved to screen no.6 to make homogeneous granules and lactose and talc were added.

Table 1 Formulation Batch F1 F2 and F3

Ingredients	**F1(Mg)**	**F2(Mg)**	**F3(Mg)**
***Bryophyllumpinnatm*extract**	320	320	320
***BergeniaCiliata*extract**	80	80	80

MicrocrystallineCellulose	50	50	50
Lactose	25	5	35
Talc	24	4	14
Sod.Methyl paraben	0.5	0.5	0.5
Sodiumbenzoate	0.5	0.5	0.5

Pre-filling parameters

Angle of Repose: Angle of repose was determined by using funnel method. The accurately weighed blend was taken in a funnel. The height of the funnel was repositioned such that its tip just touched the top of the blend head/heap. The mixture of medicine excipient was free to pass through the funnel and reach the surface. The powder cone's diameter was measured and angle of repose was calculated using the equation,

Tan θ = h/r

Where, h = Height of pile, r = Radius of pile

Relationship between angle of repose and powder flow was determined as per pharmacopoeia standards, mentioned in table [22].

Compressibility index: It is also one of the simple methods to evaluate flow property of powder by comparing the bulk density and tapped density. A useful empirical guide is given by the Carr's compressibility [23].

Carr's index= TD-BD/TDX100

Hausner's ratio: It provides an indication of the degree of densification which could result from vibration of the feed hopper [24].

Hausner's ratio = Tapped density/ Bulk density

LowerHausner'sratio=Betterflowability,HigherHausner'sratio=Poorflow Ability

Table 2 Flow properties and corresponding Angle of Repose, CI, Hausner's ratio

Flowproperty	**Angleofrepose**	**Compressibilityindex**	**Hausner'sratio**
Excellent	25-30	<10	1.00-1.11
Good	31-35	11-15	1.12-1.18
Fair	36-40	16-20	1.19-1.25
Passable	41-45	21-25	1.26-1.34
Poor	46-55	26-31	1.35-1.45

Capsule Filling: Capsule filling was done by hand filling method. The formulated granules were filled in "0" size capsules to an average net content weight of 520 mg. The capsules were then deducted, transferred into polybags, and labelled. The samples were evaluated as per the testing requirements. From the trials, samples were taken for accelerated stability studies as per the testing requirements [25].

Evaluation of Capsules[26]

Description: Colour, shape and size of formulated capsules were determined

Uniformity of weight: Twenty distinct units were chosen at random, and after their contents were weighed, the average weight of the units was determined. The percentage that separates the average weight from any 2 of the different weights cannot be more than that indicated in the table.

Table 3 Acceptance Criteria I.P Limit

Dosageform	**Averageweight**	**%Deviation**

Capsules	Lessthan300mg	10
	300mgor more	7.5

Disintegration Time: Disintegrating apparatus was used to conduct the disintegration test. Six Vassals held one capsule each, and the equipment was kept at 37 ± 0.50°C with the immersion liquid (phosphate buffer, pH 6.8). It was recorded how long it took for the capsules to completely dissolve. When there are no more particles above the gauge after they have easily gone through a 10# mesh screen, the capsules are considered dissolved.

In-vitro Dissolution Studies: The USP type 2 dissolving equipment was used to conduct 1-hour dissolution investigations of the formulation in 900 ml of phosphate buffer (pH 6.8) from 3 to 24 hours at 100 rpm while keeping the temperature at 37 ± 0.5°C. Over the course of a day, five millilitre samples of each were gathered. The removed sample was quickly swapped out for an equivalent amount of brand-new buffer. The collected samples underwent spectrophotometric analysis using a UV-Vis spectrophotometer (Systronics Double beam) at wavelengths measured for B.pinnatum and B.ciliata. The result was a cumulative percent drug release calculation. Plotting the cumulative percentage of medication release against time in minutes was done.

Stability Study as Per ICH Guideline: According to ICH requirements, a two-month stability study was conducted at 40°C±2°C and 75% relative humidity±5%. We assessed the formulation's in vitro drug release, weight homogeneity, and disintegration time.

Results

Pre-formulation studies:

Organoleptic properties: Organoleptic properties of extracts were done. The results are given in table 4.

Table 4Organoleptic properties of extracts

Nameof plantextract	**Nature**	**Colour**	**Odour**	**Taste**
Bryophyllumpinnatum	Gummy	DarkBrown	Characteristic	Acrid
Bergeniaciliata	Amorphous	Rust orange	Noodour	Bitter

Solubility studies*of B. pinnatum& B. ciliata:*PhosphateBuffer>Methanol>Distilledwater>0.1 N HCL. Maximum solubility was found to be in phosphate buffer pH 6.8

Table 5Solubility of B. pinnatum& B .ciliata in different Solutions

Solution	**Solubility (B.P) (µg/ml)**	**Solubility (B.C) (µg/ml)**
Distilled Water	465.08	477.25
0.1N HCl	408.33	436.63
Methanol	521.25	535.01
PhosphateBuffer	550.03	552.2

Determination of Maximum Wavelength of B. pinnatum& B. ciliata: Maximum Wavelength ofB. pinnatum and B. ciliata was found to be 286 nm& 215 nm respectively. Thispeak was used as a marker for quantification in all dissolution studies of both extract preparations. The results are shown in table no 6.

Table 6Absorbance of B. pinnatum& B. ciliata at different Wavelength

Wavelength (B.C)	Absorbance (B.C)	Wavelength (B.P)	Absorbance (B.P)
211	0.527	282	0.296
212	0.567	283	0.357
213	0.609	284	0.436
214	0.653	285	0.529
215	0.707	286	0.635
216	0.638	287	0.530

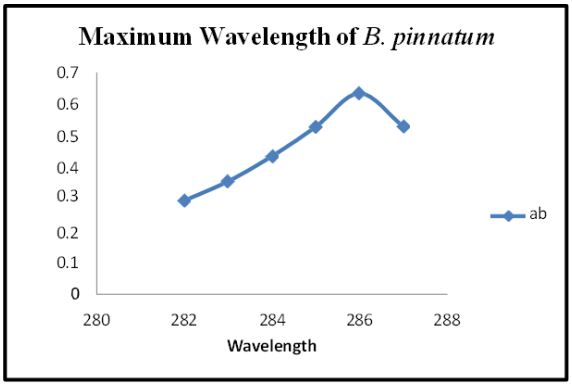

Figure 2Graph of B. pinnatum showing Maximum Wavelength at 286 nm

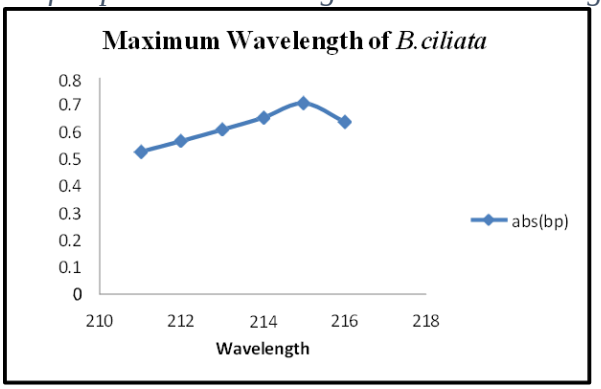

Figure 3Graph of B. pinnatum showing Maximum Wavelength at 215 nm

Fourier Transfer Infrared (FTIR) studies:

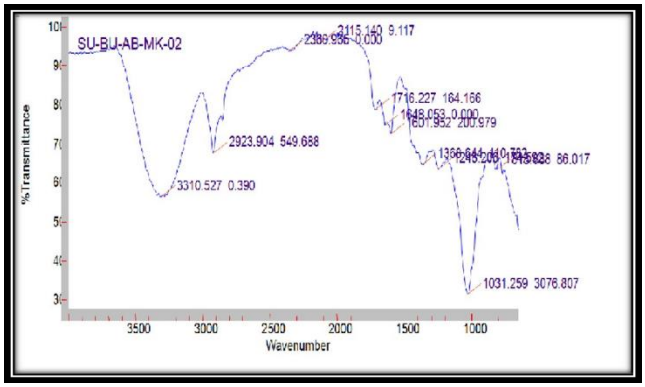

Figure 4FTIR spectra of BryophyllumPinnatum

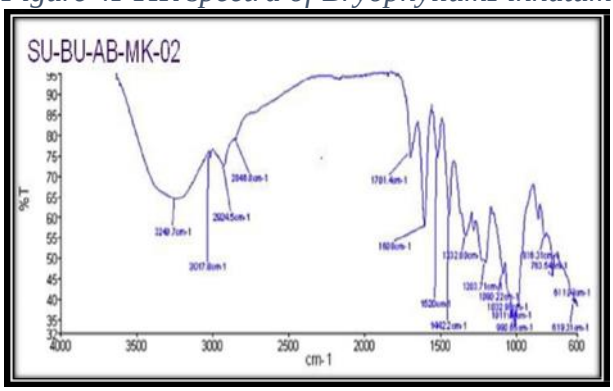

Figure 5FTIR spectra of Bergenia ciliata

Table 7FTIR spectral data of BryophyllumPinnatum

Vibrationfrequencycm-1	**MajorPeakcm-1**	**Molecularvibration**
3310	3600-3200	StretchingOH
2923	3600-2500	StretchingO-HAcid
2115	2260-2100	StretchingC=O
1716	1745-1715	C=OKetone
1648	1700-1500	StretchingC=O Amide
1368	1400-1300	StretchingNO2
1031	1400-1000	C-F

Table 8FTIR spectral data of Bergenia ciliata

Vibrationfrequencycm-1	**MajorPeakcm-1**	**Molecularvibration**
3240	3600-3200	StretchingOH
3017	3100-3000	Stretching=C-H
2924	2950-2840	Stretching-C-H
1520	1700-1500	C=Camide
1442	1465-1440	CH3Bending
1332	1400-1300	StretchingNO2
1011	1400-1000	C-F

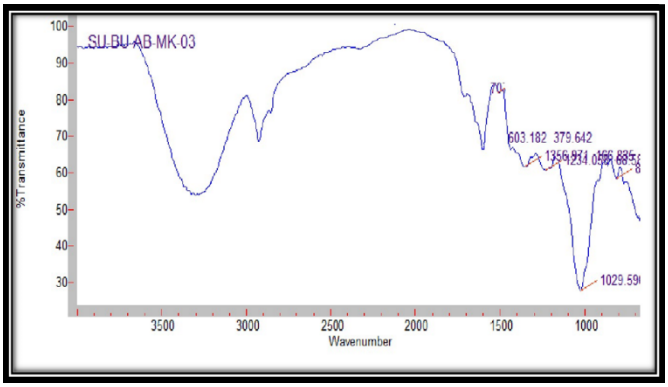

Figure 6FTIR of plant extracts (Bergenia ciliata& B. pinnatum)

FTIR studies confirmed Drug-Drug compatibility studies. From the FTIR graph there was none of the extra peak found. Thus, FTIR studies showed that there was no drug-drug and drug-excipient interaction.

Standard Calibration Curve: -

Table 9Standard plot of B. pinnatum in Phosphate Buffer pH 6.8

Concentration(µg/ml)	**Absorbance**
10	0.121
20	0.180
30	0.262
40	0.321
50	0.383

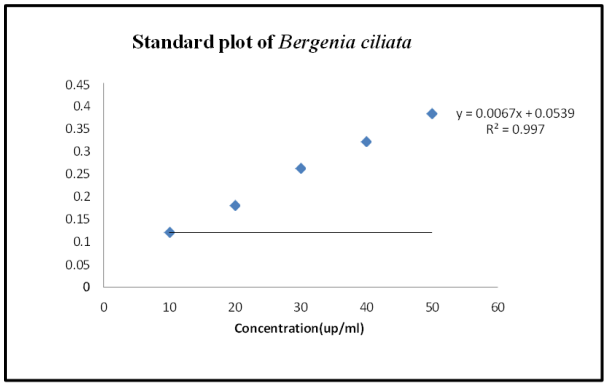

Figure 7Standard plot graph of B.P extract in phosphate buffer pH 6.8

The standard plot of the extract was formed in 6.8pH phosphate buffer at λ max of 286nmand R2 value found to be 0.997.

Table 10Standard plot of B. ciliata Phosphate Buffer

Concentration(µg/ml)	**Absorbance**
10	0.086
20	0.114
30	0.156
40	0.206
50	0.274

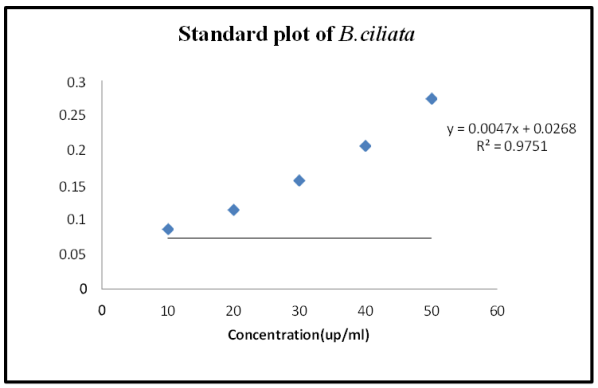

Figure 8Standard plot graph of B.C extract in phosphate buffer pH 6.8

The standard plot of the extract was formed in 6.8pH phosphate buffer at λ max of 215nmand R2value found to be 0.975.

In-Vitro anti-urolithiatic assay

Table 11Dissolution of Calcium oxalate stone by extract &cystone

Group	Concentration	Vol. of standard KMnO4	Wt. of calcium estimated	Wt. of calcium Reduced	% Dissolution
Control	-	4.7	0.8920	-	-
Standard (Cystone)	100	3.5	0.6643	0.2277	25.52
	200	3.2	0.6073	0.2847	31.91
	300	2.9	0.5504	0.3416	38.29
	400	2.7	0.5124	0.3796	42.55
Bergeniaciliate (extract)	100	4.5	0.8541	0.0379	4.24
	200	4.3	0.8161	0.0759	8.50
	300	4.1	0.7781	0.1139	12.76
	400	4	0.7592	0.1328	14.88
Bryophyllumpinnatu(extract)	100	4.4	0.8351	0.0637	6.37
	200	4.2	0.7971	0.0949	10.63
	300	4.1	0.7781	0.1139	12.76
	400	4	0.7402	0.1518	17.01

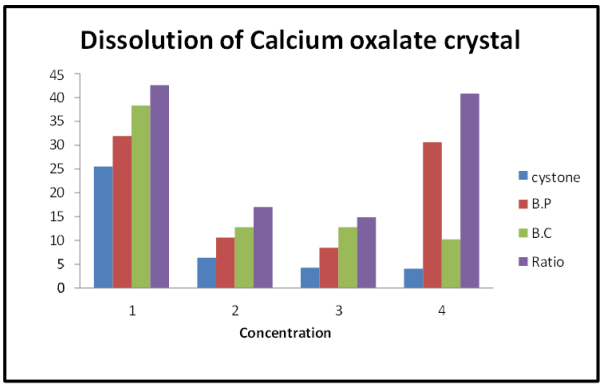

Figure 9Dissolution of calcium oxalate crystal by extracts &cystone

From the investigation, it was found that the B.pinnatum& B .ciliata extract exhibited maximal dissolving at 400 mg. It was compared with standard Cystone 400 mg. From this result, we concluded that we may utilise B. pinnatum& B .ciliata extract in urolithiasis therapy.

Table 12In-Vitro dissolution of stones in different ratio of extract in combination

Ratio BC:BP	**Wt.Ofratio (mg)**	**Wt.ofstone (g)**	**Wt. of stone reduced**	**Vol. of standard KMnO4 (ml)**	**Wt. of calcium estimated**	**Wt. of calcium Reduced**	**%Dissolution**
Control	-	0.10	-	4.9	0.93002	-	-
1:1	200:200	0.10	0.09	4.7	0.89206	0.03796	4.08
1:2	133.33:266.66	0.10	0.07	3.4	0.64532	0.2847	30.61
1:3	100:300	0.10	0.09	4.4	0.8351	0.09492	10.20
1:4	80:320	0.10	0.07	2.9	0.5504	0.3796	40.81

Figure 10Dissolution of calcium oxalate crystal of different groups before incubation

Figure 11Dissolution of calcium oxalate crystal of different groups after incubation

Evaluation of Pre-compression parameters:

Table 13Evaluation of physical properties of powder blend of all formulations

Formulations	Angleof repose (θ)	Bulkdensity (gm/ml)	Tappeddensity (gm/ml)	Carr'sIndex (%)	Hausner's Ratio
F1	30.96	0.45	0.52	13.46	1.15
F2	27.47	0.41	0.54	15.07	1.31
F3	32.2	0.46	0.50	8.00	1.08

Angle of repose: Angle of repose was determined by fixed funnel method. Angle of repose was found to be in range of 27.47-32.2 for different powder blend batches, which indicates good powder flow.

Bulk and Tapped density: Pre-compression blend was evaluated for bulk and tapped densityby using tapped density apparatus. Bulk and tapped density was found to be 0.41-0.46gm/ml and 0.50-0.54 gm/ml respectively. This shows good repacking ability of powder blend.

Carr's index and Hausner's ratio: Carr's index and Hausners ratio was calculated from bulk and tapped density. The carr's index of the ingredients was found to be in the range of 8-15.07% and hausner's ratio in the range of 1.08- 1.31. These findings proves that the composition of ingredients for compression possess good compression property and good flow property.

Figure 12Formulated capsules of plant extracts

Evaluation of capsules

Description: The polyherbal capsules were evaluated for organoleptic characters which include colour, odour, taste and nature.

Table 14Description of capsules

Parameters	Observations
Colour	Cap-orange, Body-Light grey
Size	0
Shape	Cylindrical
Taste	Tasteless

Uniformity weight of the capsule: The maximum weight variation of the capsules was 498.3±0.57 which falls within the acceptable weight variation range of + 7.5%, hence the capsules of all batch passed the weight variation test.

Table 15Uniformity weight of different formulation

Formulation	Uniformityofweight
F1	496.3±0.57
F2	497.3±0.57
F3	498.3±0.57

Disintegration Test- The capsule passed the test as no residue of drug was remained. The disintegration time for F3 formulation was found 15 min 25 second.

Table 16Disintegration time of different formulation

Formulation	Disintegrationtime(min)
F1	16 min 45 sec
F2	17 min 15 sec
F3	15 min 25 sec

The capsule passed the test as no residue of drug was remained. The disintegration time forF3 formulation was found 15 min 25 sec.

In-vitro Dissolution Studies: In-vitro dissolution studies were carried out in phosphate buffer6.8 pH.

Table 17% CDR of different formulations of capsule at different time interval

Time(min)	%CDRof F1		%CDRof F2		%CDRof F3	
	B.C	B.P	B.C	B.P	B.C	B.P
5	17.22	21.03	16.11	20.5	18.67	22.19
10	23.01	31.55	22.32	30.27	24.75	32.85
15	28.21	35.03	27.45	34.23	29.7	36.14
20	30.85	40.75	31.25	39.08	33.07	41.1
25	37.06	43.15	36.33	42.52	38.47	44.7
30	41.22	48.11	40.36	46.33	42.52	49.95
35	44.33	51.24	44.1	50.1	46.57	52.8
40	49.02	56.32	49.65	54.59	51.3	57.9
45	53.11	62.22	52.87	60.35	54.67	63.3
50	56.22	64.57	56.07	62.3	58.27	65.7
55	60.95	66.14	59.65	65.03	61.87	67.8
60	65.21	69.41	65.74	68.74	67.95	70.5
65	72.06	70.1	71.22	69.62	73.12	71.7
70	76.03	73.18	76.26	72.02	78.97	74.1
75	77.22	75.66	78.3	74.65	80.77	76.2
80	84.36	77.05	83.33	76.24	85.27	78.75
85	87.20	79.85	86.47	78.67	88.65	80.25

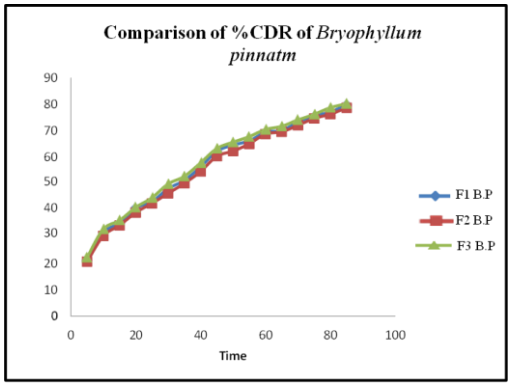

Figure 13Comparison of %CDR of different formulations of Bryophyllumpinnatum

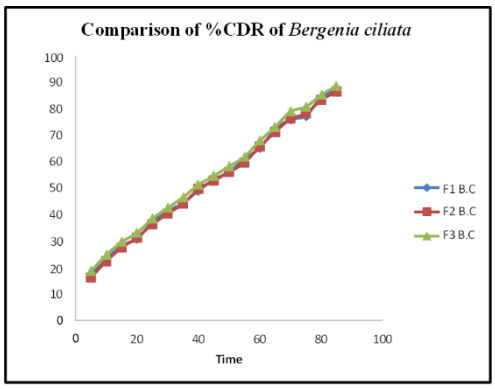

Figure 14Comparison of %CDR of different formulations of Bergenia ciliata

Comparative study of different (F1-F3) is shown in the plot. The in-vitro release offormulation F3 was found to be maximum.

Stability Study:

Table 18Stability study result of Formulation (F3) after 60 Days

Parameter		**Initial study**	**30 days**	**60 days**
Nature		Powder	NC	NC
Colour		Cap-orange, Body-Grey	NC	NC
Taste		Characteristic	NC	NC
Odour		NC	NC	NC
Uniformityof weight		498.3±0.57	NC	NC
Disintegration time		15 min 25 sec	15 min	15 min
In-vitro drug release	**B.P**	80.25	80.6	80.3
	B.C	88.65	88.65	88.65

Conclusion

In conclusion, the pre-formulation experiments done on extracts of Bryophyllumpinnatum and Bergenia ciliata give complete insights into their physical, chemical, and pharmacological characteristics, crucial for developing successful therapeutic medicines.

Organoleptic studies indicated different features of each extract, with Bryophyllumpinnatum being sticky, dark brown, acrid in taste, and having a particular odor, whereas Bergenia ciliata seemed amorphous, rust orange in color, bitter in flavour, and odorless.

Solubility experiments indicated that both extracts displayed maximal solubility in phosphate buffer pH 6.8, critical for their bioavailability and formulation design. Spectrophotometric study indicated the maximum wavelengths for quantification: 286 nm for Bryophyllumpinnatum and 215 nm for Bergenia ciliata, assisting in standardization and quality control.

Fourier Transform Infrared (FTIR) spectroscopy demonstrated the lack of drug-drug and drug-excipient interactions, verifying the chemical stability and compatibility of the extracts. Standard calibration curves in phosphate buffer pH 6.8 further verified their quantitative analytical procedures, exhibiting strong linearity (R2 values of 0.997 for Bryophyllumpinnatum and 0.975 for Bergenia ciliata).

In vitro anti-urolithiatic experiments indicated considerable dissolving of calcium oxalate stones by both extracts, similar to the standard medication Cystone, suggesting their potential therapeutic utility in urolithiasis therapy. Formulation experiments indicated excellent physical qualities of the powder blends and capsules, with uniformity of weight and suitable disintegration durations, crucial for dose consistency and optimal drug administration.

Moreover, in vitro dissolution experiments in phosphate buffer 6.8 pH demonstrated sustained release patterns for both extracts, with formulation F3 displaying the largest cumulative drug release percentages over time. Stability experiments over 60 days demonstrated that the formulation maintained its physical and chemical integrity, with minor changes in nature, color, taste, odor, and drug release profiles, demonstrating its potential for long-term preservation and usage.

In summary, the extensive pre-formulation experiments underline the pharmacological potential of Bryophyllumpinnatum and Bergenia ciliata extracts, encouraging their development as effective therapeutic agents for urolithiasis and perhaps other medical uses.

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