Research Article

*Invitro* Urolithiatic Activity Of *Bryophyllum Pinnatum* Against Experimentally Designed Calcium Oxalate And Calcium Phosphate Stones

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**ARTICLE INFO**

**ABSTRACT**

*Bryophyllum pinnatum* commonly known as panphuti belonging to family *Crassulaceae* has been investigated for its anti-urolithiatic activity by constructing an in-vitro model. Various crude extracts of *Bryophyllum pinnatum* were evaluated for their potential to dissolve experimentally prepared kidney stones of calcium oxalate, calcium phosphate and the results were compared with cystone as a standard polyherbal drug. The detailed phytochemical screening was done to identify active phytoconstituents and photo-micrography was carried out to understand dissolution of stones by extracts. Chloroform ,alcoholic and acetone fractions of plant leaves showed highest dissolution of Calcium oxalate stones as compare to others (68, 52, 43 % resp). while Ethyl acetate, pet ether, acetone fractions of plant leaves were more effective in dissolving calcium Phosphate (81, 33, 25% resp.) compared to reference drug Cystone.

**INTRODUCTION**

*Bryophyllum pinnatum* (Family- Crassulaceae) is a plant commonly found in India, China and has been reported to decrease renal calculi. Renal stone is a multi-factorial disorder resulting from the combined effect of epidemiological, biochemical and genetic risk factors. Calcium containing stones, especially calcium oxalate monohydrate, calcium phosphate dihydrate are the most commonly occurring ones to an extent of 70-90 %. The present treatments of kidney stone involves lithotripsy and surgery. These are much painful, expensive, often exposes Socio-economic burden and even cause risk to patients life. The recurrence rate is very high. The present study was aimed to evaluate in vitro urolithiatic potential of *B. pinnatum*.
Urinary tract and renal stone ailments have affected human beings since ancient time. Ancient Vedic literature describes stones as Ashmari. It occurs both in men and women but the risk is generally higher in adult men and is becoming more common in young women[1]. It is a major problem and an important cause of renal failure in India. This is an entity which has high morbidity and low mortality but having serious consequences and significant socio-economic impact. Renal stone is a multi-factorial disorder resulting from the combined effect of epidemiological, chemical, biochemical and genetic risk factors. Calcium-containing stones, especially calcium oxalate and calcium phosphate are the most commonly occurring ones to an extent of 80-90%. Medical conditions that are associated with increased risk of kidney stone formation include hyperparathyroidism, hyperthyroidism, gout, cystic fibrosis[2]. Symptoms for urolithiasis include abnormal urine colour, blood in urine, fever, nausea and vomiting[3]. The modern treatments of renal stone include lithotripsy, shock wave therapy, surgical techniques, local calculus disruption using high-power laser and modern medicines. These are much painful, expensive and have many side effects even post therapy the recurrence rate of renal calculi is very high. So there is no suitable medical therapy is available for such stone disorder, it is very important to show an interest in indigenous system of medicine and traditional herbal remedies which are regarded as quite safe with no side effects. Furthermore it should be cost effective, readily accessible and quiet affordable.

Kidney stones and urinary disorders are most painful conditions and have been affected people for several centuries. A large population of India suffers from urinary tract and Kidney stones, formed due to deposition of Calcium, Phosphate and oxalates. These stones may persist for indefinite time, lead to secondary complications causing serious consequences to patients life. It is very painful and proper cure is needed to get rid of the problem[4]. Depending on where they are located, kidney stones are known as urinary calculi, urinary tract stone disease, renal calculi, nephrolithiasis, ureterolithiasis and urolithiasis. Appropriate and timely treatment of kidney stones can prevent severe complications such as partial or complete failure kidney functions. Stone in the urinary tract are common disorders because urine is a chemical solution that contains a large number of chemical substances. These substances can crystallize easily and then grow in size to form stones[5]. Though treatment of kidney stone is revolutionized by the development of non-invasive methods of stone disruption but the patients always try to refrain from surgical procedures. Moreover, it also carries the factors like high cost, availability, side effects etc: the recurrence rate is approximately 50-80% [6].

As no suitable medical therapy is available for such stone disorders, it is imperative to search for some new or less known medicinal plants, which are potential source for new bioactive compounds of therapeutic value. In the traditional systems of medicine including Ayurveda, most of the remedies were taken from plants and they were proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects[7]. As per the indigenous system of medicine, the leaves of Bryophyllum pinnatum were reported to be useful in the treatment of a wide range of ailments including urinary stones [8,9]. However, so far no scientific study has been reported regarding the in vitro anti-ureolithiatic activity of various extracts of B. pinnatum leaves
against Calcium stones. The present study was carried out to evaluate the in vitro anti-urolithiatic activity of B. pinnatum leaves extracts against Calcium oxalate and Calcium phosphate stones.

### MATERIAL AND METHODS

**Collection of Plant material and preparation of extracts**
The leaves of *B.pinnatum* were collected from the local area of place Sangola (District Solapur) of Maharashtra state in the month of July 2015 and shade dried. The plant was authenticated by botanists. The dried leaves were coarsely powdered (passed through sieve no. 40). The 5g. of powder was extracted by using 100 ml of various organic solvents such as acetone, ethyl acetate, petroleum ether, chloroform, ethyl alcohol and distilled water. The crude extract obtained was evaporated at 45°C, then dried and stored in airtight container (yield 29.82 %, w/w).[^10]

**Chemicals**
The solvents used for extraction were acetone, ethyl acetate, petroleum ether, chloroform, ethyl alcohol purchased from Merck Ltd., Mumbai, India.

**Phytochemical Analysis**
The extracts were screened for the presence of active phytochemical constituents by employing standard procedures.  
[^11],[^12]

1) **Test for Phenols**
   To 1 ml of extract 1% ferric chloride solution was added. Appearance of green colour indicated the presence of phenols.

2) **Test for Flavonoids**
   To 1 ml of alcoholic extract 3-5 drops of 2% lead acetate solution was added. Development of orange or yellow colour indicated the presence of flavonoids.

3) **Test for Alkaloids**
   *Hagers Test* - one drop of Hagers reagent was added into 1 ml of extract, yellow precipitate is formed.

4) **Test for Triterpenoid Saponins**
   *Froth formation test* - 1 ml of extract was shaken with water in a test tube, froth developed

**Evaluation of Anti-urolithiatic Activity for B. pinnatum**[^13],[^14]

**Step-1 Preparation of experimental kidney stones (Calcium oxalate stones) by homogenous precipitation:**
Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Sodium oxalate (AR) in 10ml of 2N H2SO4 were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Equimolar solution of Calcium chloride dehydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10ml of (2N H2SO4), was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium phosphate. Both precipitates freed from traces of sulphuric acid by Ammonia solution. Washed with distilled water and dried at 60°C for 4 hours. Calcium phosphate (AR) was purchased from Merck and used directly for directly for the study.

**Step-2 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 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with asharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. Then egg membrane washed thoroughly with distilled water, and placed in it ammonia solution, in the moistened condition for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

**Step-3: Estimation of Calcium oxalate/phosphate by Titrimetry**
Weighed exactly 1mg of the calcium oxalate and 10mg of the extract/compound/ standard and packed it together in semi permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100ml 0.1 M TRIS buffer. One group served as negative control (contained only 1mg of calcium oxalate). Placed the conical flask of all groups in
an incubator, preheated to 37 0C 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 sulphuric acid and titrated with 0.9494 N KMnO₄ till a light pink colour end point obtained. 1ml of 0.9494 N KMnO₄ equivalent to 0.1898mg of Calcium. The amount of undissolved calcium oxalate/phosphate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate/phosphate actually test substance(s) could dissolve.

### RESULTS AND DISCUSSION

The detailed phytochemical analysis revealed that Chloroform and alcoholic, ethyl acetate, pet ether extracts are found to be rich in saponins, flavonoids, terpenoids, alkaloids and phenols. 

Table 1. Phytochemical analysis of *B.pinnatum* extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>Saponin</th>
<th>Flavonoid</th>
<th>Terpenoid</th>
<th>Alkaloid</th>
<th>Phenol</th>
<th>Terpenoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.ether</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EtOH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EtOAc</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetone</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) = present, (-) = absent

Detailed phytochemical analysis indicated the presence of phenolic compounds, flavonoids, phenols, terpenoids and Saponin in different extracts of *Bryophyllum pinnatum*. On the basis of this fraction we performed *in vitro* anti-Urolithiatic Activity by comparing different extracts of *Bryophyllum pinnatum* with standard cystone against Calcium oxalate and Calcium phosphate stones. The Table. 2 represents percentage dissolution of Calcium oxalate crystals by *B. pinnatum* extracts in comparison with Cystone.

Table. 2 % Dissolution of Calcium oxalate

<table>
<thead>
<tr>
<th>Extract</th>
<th>B. pinnatum extracts</th>
<th>Cystone extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aq. Extract</td>
<td>12.00%</td>
<td>24%</td>
</tr>
<tr>
<td>EtOAc extract</td>
<td>16%</td>
<td>20%</td>
</tr>
<tr>
<td>P.Ether extract</td>
<td>23%</td>
<td>55%</td>
</tr>
<tr>
<td>Acetone extract</td>
<td>43%</td>
<td>27%</td>
</tr>
<tr>
<td>EtOH extract</td>
<td>52%</td>
<td>18%</td>
</tr>
<tr>
<td>CHCl₃ extract</td>
<td>68%</td>
<td>10%</td>
</tr>
</tbody>
</table>

Fig. 2 represents graphical representation of percentage dissolution of Calcium oxalate crystals by *B. pinnatum* extracts in comparison with Cystone.
Fig. 2: % Graphical representation of dissolution of Calcium oxalate

The graphical representation shows % Dissolution of calcium oxalate by in vitro Anti-Urolithiatic Activity of extracted fractions of Bryophyllum pinnatum. drug. Chloroform, alcoholic and acetone extracts at 10 mg concentration produced higher dissolution of calcium oxalate as compared to other fraction and the Standard. Standard shows higher dissolution as compared to others for aqueous, ethyl acetate and petroleum ether extracts.

Table. 3 represents percentage dissolution of Calcium phosphate crystals by B. pinnatum extracts in comparison with Cystone.

<table>
<thead>
<tr>
<th>Extract</th>
<th>B. pinnatum extracts</th>
<th>Cystone extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aq. Extract</td>
<td>17%</td>
<td>22%</td>
</tr>
<tr>
<td>EtOAc extract</td>
<td>81%</td>
<td>56%</td>
</tr>
<tr>
<td>P. Ether extract</td>
<td>33%</td>
<td>29%</td>
</tr>
<tr>
<td>Acetone extract</td>
<td>25%</td>
<td>5%</td>
</tr>
<tr>
<td>EtOH extract</td>
<td>28%</td>
<td>32%</td>
</tr>
<tr>
<td>CHCl₃ extract</td>
<td>20%</td>
<td>20%</td>
</tr>
</tbody>
</table>

Fig. 3 % Graphical representation of dissolution of Calcium phosphate

Fig. 3 represents graphical representation of percentage dissolution of Calcium oxalate crystals by B. pinnatum extracts in comparison with Cystone. The graphical representation shows % Dissolution of calcium phosphate by in vitro Anti-
Urolithic Activity of extracted fractions of *Bryophyllum pinnatum*. drug. Ethyl acetate, petroleum ether and acetone extracts at 10 mg concentration produced higher dissolution of calcium oxalate as compared to other fraction and the Standard. Standard shows higher dissolution as compared to others for aqueous, alcoholic and chloroform extracts.

**Photomicrography Study:**

The photomicrographical study for dissolution of CaOx crystals was conducted with the help of Labline 10x 10 compound microscope using Olympus Digital Camera. The images of the slides were recorded at first day i.e. before treating CaOx crystals with extracts(Image-a), at the third day when dissolution was in progress(Image-b) and finally at the seventh day where crystals were reduced in size(Image-c).

![Image-a](image1.png)  
*Image-a*  
CaOx crystals (Without extracts)(Day 1)

![Image-b](image2.png)  
*Image-b*  
CaOx crystals –dissolution in progress (Day 3)

![Image-c](image3.png)  
*Image-c*  
CaOx crystals –reduced in size (Day 7)

This study evaluates that antiurolithic activity of different extracts of *Bryophyllum pinnatum* leaves and isolated compound. 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 Chloroform, alcoholic and acetone fractions of plant leaves fraction show highest dissolution of calcium oxalate in comparison to other fractions and standard drug cystone(68, 52, 43 % resp.) while Ethyl acetate, pet ether, acetone fractions of plant leaves were found to be more effective in dissolving calcium Phosphate(81, 33, 25 % resp.). This study has given primary evidence for *Bryophyllum pinnatum* as plant which possess lithotriptic property. The present in vitro study has given lead data, and shown that various active components present in these extracts are quite promising for further work in this regard. medicinal plants / natural products are more useful for body because they promote the repair mechanism in natural way. The plant based therapeutics are easily available, cost effective with no or minimum side effects and hence rural and remote area residing people trust it as primary health care system. Further strong scientific explorations and experimental proofs are needed to support these preliminary findings. In future, there is a strong need to carry out number of in vitro, in vivo and clinical trials to examine efficacy and safety of plant based therapeutics. An additional work can be carried out to isolate, purify and characterize active compounds and to identify their possible mechanism of action.

**DISCUSSION**

The detailed phytochemical analysis revealed that the crude extracts of Bryophyllum pinnatum leaves are rich in bioactive compounds like terpenoids, alkaloids, flavonoids, phenols and saponins. Evaluation of anti-urolithic activity has been performed for *Bryophyllum pinnatum* using *in vitro* antiurolithic model for calculating % dissolution of kidney stone and results were compared with standard drug. Many extracts are found to be the most effective in urolithic action. Data from our preliminary findings of *in vitro* evaluation revealed that phyotherapeutic agents from *B. pinnatum* could be useful as either alternative or an adjunct therapy in the management of urolithiasis.
ACKNOWLEDGEMENT

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