Short Research Article

In-vitro calcium oxalate crystallization inhibition by *Achyranthes aspera* L. and *Bryophyllum pinnatum* Lam.
ABSTRACT

**Introduction:** Urolithiasis has plagued human kind since antiquity and in the present era, its frequency is increasing with maximum occurrence rate of calcium oxalate stones. Although dramatic changes in the methods of its treatment has undertaken, still need exists for search of antilithic drugs from the nature. Thus, the inhibition of in-vitro calcium-oxalate crystal formation by Achyranthes aspera L. and Bryophyllum pinnatum Lam. was investigated.

**Method:** Leaf extracts of both the plants were screened by using nucleation assay. Different concentrations of both the extracts were screened.

**Result:** In the nucleation assay the % inhibition for calcium oxalate crystal formation was found to be directly proportional to the increase in concentration of the plant extracts. Achyranthes aspera showed maximum % inhibition of 60.06 ± 0.19 % at 1000 mg/ml while Bryophyllum pinnatum showed maximum % inhibition of 49.93 ± 0.07 % at the same concentration.

**Conclusion:** The present in-vitro study provides evidence that Achyranthes aspera is a potent anti-urolithiatic agent; however these in-vitro results should be confirmed in-vivo.

*Keywords:* Calcium oxalate stones; in vitro; nucleation assay; optical density; anti-urolithiatic agent; Achyranthes aspera L.; Bryophyllum pinnatum Lam.

1. INTRODUCTION

Urolithiasis is a urinary disorder, known to the mankind since ancient times. In India ancient sanskrit documents have reference of stone formation. It is considered as the third most common affliction of the urinary tract [1]. In India, calcium oxalate remains the most predominant constituent of urolithiasis [2]. Though technological advancements have made dramatic improvement in methods for treating urolithiasis, still some of the drawbacks of these methods exists which includes their being too costly and re-occurrence of stone formation along with number of other side effects [3]. Hence, search for new antilithic drugs from natural sources has assumed greater importance as herbal drugs are cost effective as well as confer least side effects.

Achyranthes aspera L. (Amaranthaceae) commonly known as chinchita or aandhi jhadha, [del/is a member of the Amaranthaceae family, it] is a herb that grows as weed in [the] rainy season. Traditionally it is used in the treatment of oedema, dropsy, piles, boils, skin disorders, asthma and cough. Leaves are emetic, hydrophobic, and carminative, resolve swelling, digestive and expel phlegm. The crushed leaves are rubbed on aching back to cure strained back. Paste of fresh leaves is used for allaying pain from bite of wasps. The plant shows many pharmacological activities like spermicidal, anti-allergic, cardiovascular, antiparasitic, hypoglycaemic and analgesic [4]. Leaf extracts are reported to possess hypoglycemic, thyroid stimulating, antiperoxidative properties [5], analgesic [6], antimicrobial [7], anticancer [8] and antipyretic activity [9] etc.
**A. aspera** has been documented for its therapeutic effects in treating urinary complaints and antilithic activity. The ethanolic extract of its leaves, were tested against ethylene glycol induced lithiasis in rats [10]. Its root paste is employed in urinary trouble in various parts of India [11]. Earlier reports suggested that the methanolic extract of **A. aspera** whole plant possessed nephroprotective activity against lead acetate induced nephrotoxicity in rats [12]. The aqueous extract of its roots were found to inhibit nucleation and growth of calcium oxalate crystallization. In addition, **A. aspera** provided protective effect against oxalate induced renal tubular epithelial cell injury in-vitro and it has been reported to be an active component of various drug formulations for treating kidney stone [13]. It is worth mentioning that the aqueous extract of its roots was also found to prevent ethylene glycol induced urolithiasis in rats as well as it reduced the growth of calcium oxalate stones [14]. In an ethnobotanical study its whole plant has been reported to be used in case of kidney stones [15]. Some other species of *Achyranthes* has been reported to inhibit crystallization of calcium oxalate in synthetic urine [16].

**A. aspera** (roots and leaves) were screened for triterpenoids content [17]. Similarly, the presence of alkaloids, flavonoids, glycosides, saponins, tannins and proteins has been reported [18]. The aqueous extract of its leaves has been reported to show the presence of alkaloids, flavonoids and resins [19-20]. Similar work has been reported on petroleum ether extract [21] and ethanolic and aqueous extract of its leaves [22]. Phytochemicals in its dried leaf powder has been studied [23]. Similarly, phytochemicals in methanolic extract of its leaves was reported [24].

**Bryophyllum pinnatum** Lam. (Crassulaceae) commonly known as patharchatta, is a member of the Crassulaceae family. It is a perennial plant which is grown for ornamental and medicinal purposes in different parts of India. It is traditionally used for the treatment of: abdominal discomfort, boils, bruises, cholera, cuts, diabetes, diarrhea, dysentery, flatulence, headaches, kidney stones, indigestion, insect bites, scabies, sores, urinary insufficiency and wounds. Leaf extract is taken in empty stomach for treating bladder stones while leaf juice is used in cough, dysentery, leucoderma and in case of bleeding [25].

In a study alcoholic extract of leaf of **B. pinnatum** showed promising antiurolithiatic activity [26]. **B. pinnatum** (fresh leaf juice) along with some *Piper nigrum* powder is taken twice a day for 15 days to expel the stones [27-28] while its aqueous leaf extract was found to have nephroprotective effect against gentamicin induced toxicity in rats [29]. In a review, the anti-urolithiatic activity of hydroalcoholic extract [30] and ethanolic extract [26] of its leaves in rats has been reported. Other ethnobotanical studies have also shown its use in urinary disorders like kidney stone (1 cup of leaf juice is taken daily) [31].

**B. pinnatum** was found to be rich in alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroids, bufadienolides and lipids [32]. Its leaves were found to contain proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids [33]. The preliminary phytochemical studies with the help of thin layer chromatography, revealed the presence of alkaloid in chloroform, acetone, and methanolic extract, while flavonoids were found in its chloroform, acetone, and ethanolic extracts respectively [34]. Similarly, work on aqueous extract of its leaves was
also reported [33, 35, 36, 37]. Phytochemical screening of fresh and dried leaf was reported [38-39].

Several phytochemicals like flavonoids, triterpenes, saponins, tannins, alkaloids, glycoside derivatives, proteins, tannins and steroids are reported to be responsible for antiurolithiatic effect by either inhibiting the formation of calcium oxalate crystals, preventing their attachment to renal cells or by their calcium channel blocking activity [14, 40-55].

The present study was undertaken to investigate the antilithic potential of aqueous extracts of leaves of *A. aspera* and *B. pinnatum* by inhibiting calcium oxalate crystallization in *in-vitro* nucleation assay.

2. MATERIAL AND METHODS

2.1 Chemicals

All chemicals used were of high purity grade and were purchased from Sumeet Enterprises Pvt. Ltd, Bhopal. Calcium chloride and sodium oxalate were obtained from Burgoyne Reagents.

2.2 Plant collection and Identification

The leaves of *A. aspera* and *B. pinnatum* were collected from Kolar road, Bhopal, Madhya Pradesh, India, during the month of September 2011. The plant material was identified by the herbarium of Botanical survey of India (Allahabad) where voucher specimens were kept for record: numbers 1310-130.01-629 and 1131-60.01-263 respectively [56].

2.3 Extraction

Fresh leaves of both the plants were collected, washed with tap water followed by distilled water after which they were shade dried at room temperature. Aqueous extracts were prepared by boiling the fully dried and powered leaves (100 g) with distilled water. The dried extracts were filled in glass viols and kept in refrigerator until used for further analysis.

2.4 Nucleation assay

The classical model for the study of oxalate crystallization as given by Atmani *et al.*, (2000) was chosen because of its simplicity and satisfactory reproducibility [57]. This model includes the study of crystallization without inhibitor (control) and with it (plant extracts), in order to assess the inhibiting capacity of the plant extracts used. Solutions of crystal forming salts i.e. calcium chloride and sodium oxalate were prepared at the final concentrations of 5 mmol/L and 7.5 mmol/L respectively in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. The solution of calcium chloride (950 ml) was mixed with 100 ml of herb extracts at different concentrations (100 mg/ml to 1000 mg/ml). Crystallization was started by adding 950 ml of sodium oxalate solution. In case of control no inhibitor substance was added to the crystal forming solutions. The temperature was maintained at 37 °C. The optical density of the solution was monitored at 620 nm using spectrophotometer (Systronics digital spectrophotometer 166) after 30 minutes of reaction. The rate of nucleation was estimated by comparing the induction
time in the presence of the extract with that of control. Data was represented in percentage inhibition. The growth of crystals was expected due to the following reaction:

\[
\text{CaCl}_2 + \text{Na}_2\text{C}_2\text{O}_4 \rightarrow \text{CaC}_2\text{O}_4 + 2\text{NaCl}
\]

2.5 Statistical Analysis
Data were presented as mean ± standard deviation (S.D), and values were considered significant at \(P<0.05\).

3. RESULTS

In the present study out of the two aqueous extracts, the extract of \textit{A. aspera} showed maximum % inhibition of 60.06 ± 0.19 % at 1000 mg/ml and minimum of 37.74 ± 0.46 % was obtained at 100 mg/ml. On the other side, the extract of \textit{B. pinnatum} showed maximum % inhibition of 49.93 ± 0.07 at 1000 mg/ml and minimum of 29.96 ± 0.04 % at 100 mg/ml (Fig.1). The control (without any inhibitor) was found to show no inhibition. These results clearly demonstrated that \textit{A. aspera} extract is a better antilithic agent as compared to \textit{B. pinnatum}. The % inhibition was found to be directly proportional to the increase in concentration of extract. Thus these results were found to be in accordance with the use of both these plants for treating lithiasis in the traditional medicine.

![Figure 1](image_url)  
**Fig. 1.** \textit{In vitro} antiurolithiatic activity of plant extracts in nucleation assay. Note: AALAE, \textit{Achyranthes aspera} leaf aqueous extract; BPLAE, \textit{Bryophyllum pinnatum} leaf aqueous extract.

4. DISCUSSION
Incubating the metastable solutions of calcium chloride and sodium oxalate resulted in the formation of calcium oxalate crystals. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. Optical density of the solutions was monitored at 620 nm after 30 minutes. The turbidity of solution in the presence of plant extracts was lower than in the control, showing that oxalate crystallization was less in the presence of extracts. Data represents that % inhibition for CaOx crystal formation was directly proportional to the increase in concentration of the plant extracts.

Although the aqueous extract of roots of *A. aspera* was reported to inhibit nucleation and growth of calcium oxalate crystallization [13], however, it is the first report on *in vitro* evaluation of antilithic potential [57] of the aqueous extract of its leaves. *A. aspera* and *B. pinnatum* are reported to have alkaloids, flavonoids, glycosides, saponins, tannins, proteins and phenolic compounds which are reported to play significant role in inhibiting calcium oxalate crystallization. The phytochemical constituents isolated from these plants could be responsible for their observed antilithic potential. Based on the results of present study, detailed studies are warranted on the extracts as well as individual chemical constituents of these plants and their effect both *in vivo* and *in vitro* experimental models.

4. CONCLUSION

The *in-vitro* results revealed that the plant extract has potent antiurolithiatic ability, however, these *in-vitro* results should be confirmed *in-vivo*. As reported earlier the presence of some of the phytochemicals may be considered responsible for this inhibitory action. Thus, phytochemicals in these extracts will be analysed in future studies.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

The authors hereby declare that they have no competing interest or any other conflict of interest. The study reported is only for academic interest.

REFERENCES


