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Research

Assessment of Anti-urolithiatic Activity of *Bryophyllum Pinnatum* Leaves – An *In-vitro* Design

Racha Spandana[*1], Cheelam Madhuri[1], Telsuri Lavanya[1], Akkenapelli Shylaja[1], Suchitra Duddagi[2]

[1]*Siddhartha Institute of Pharmacy, Korremula Road, Narapally, Ghatkesar, Medchal, Hyderabad, Telangana, India.*
[2]*Vision College of Pharmaceutical Sciences and Research, RNS Colony, Boduppal, Hyderabad, Telangana, India.*

*Author for Correspondence: Racha Spandana
Email: spandana.racha65@gmail.com

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Abstract

Urolithiasis in general terminology is known as kidney stone or renal calculi. Urolithiasis is a disease caused by the interaction of epidemiological, biochemical and genetic risk factor. Urolithiasis is caused by many different factors that are related to the development of stone in the urinary tract such as heredity, diet, metabolic abnormality, infection, and slightly by age, gender and climate. *Bryophyllum pinnatum* (family: Crassulaceae) was widely used in traditional medicine. They are found in especially in tropical Africa, India, China, Tropical America. The main objective of the present study is to assess the *in vitro* anti-urolithiatic activity of ethanolic extract of *Bryophyllum pinnatum* leaves. For the *in vitro* study, experimentally calcium phosphate and calcium oxalate stones were prepared and compared with standard drug. Cystone is used as standard drug. The Pharmacological screening was done by Nuleation assay method. *Bryophyllum pinnatum* tonic is rich in phytochemicals such as alkaloids, flavonoids, glycosides, tannins, terpenoids and resins, and has a substantial capacity to dissolve calcium phosphate and calcium oxalate. This study suggests the anti-crystallization activity of *Bryophyllum pinnatum* against CaOx crystallization. One possible method of anti-crystallization activity of *Bryophyllum pinnatum* could be its ability to complex with free calcium and oxalate ions, thus preventing the formation of CaOx complexes. Further *in vivo* and clinical explorations are required to confirm the efficacy of *Bryophyllum pinnatum* as an anti-urolithiatic.

Keywords: *Bryophyllum pinnatum,* anti-urolithiatic activity, nephrolithiasis, cystone.

INTRODUCTION

Medicinal plants are always remained important source of drugs. Some medicinal plants and proprietary composite herbal preparations are reported to be effective in the treatment as well as prevention of recurrence of

renal calculi with minimal side effects. Conventional drugs used in the treatment of urolithiasis are often inadequate. Therefore, it is necessary to search alternative drugs for treatment of urolithiasis and to replace the currently used drugs which are doubtful of itsefficacy and safety [1,2].

Urolithiasis is the third prevalent disorder of the urinary system that is approximately 2–3% in the general population. Urinary calculi, if untreated, may cause serious medical consequences such as extreme obstruction, hydronephrosis, infection, and hemorrhage in the urinary tract system. Surgical operation, lithotripsy, and local calculus disruption using high-power laser are commonly used techniques to remove the calculi. However, these procedures are associated with the risk of acute renal injury leading to decrease in renal function. Moreover, an increase in stone recurrence is also observed. The recurrence rate without preventive treatment is approximately 10% at 1st year, 33% at 5th year, and 50% at 10th year indicating the need to develop suitable alternative therapy [3-7].

Bryophyllum pinnatum plant belongs to family Crassulaceae, commonly used as traditional medicines. *Bryophyllum pinnatum* is derived from greek word Bryo means to sprout and phyllon means leaf. The secondary metabolites which are obtained from different parts of plant such as alkaloids, flavonoids, tannins, glycosides, phenolic compounds, which have therapeutic value. The plant is used in different pharmacological activities such as anti-diabetic anti-hypertensive, anti-leishmanial, anti-microbial, analgesic, anti-cancer. These are also use in bleeding disorder and ulcer and diarrhea [8].

Common names

Zakham-e-hayat, life plant, air or maternity plant, love plant, canterberry bells, cathedral bells, parabija etc.

Geographical distribution

It is perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China, Australia, Asia, New Zealand, Philippines. The plant grows all over India in hot and moist areas, especially in Bengal and Uttarakhand.

Plant profile

Bryophyllum pinnatum also known as miracle leaf, katakataka, life plant and Pashan Bheda Patharchur, is a succulent perennial herb, which grows up to 1-2 m in height. The plant grows in the tropical, sub-tropical and warm temperature climatic zone. The stems are thick, flexible and short and the flowers are pink, white or purple. It forms a cymose panicle. Dried carrot like tuberous root is the most important part of patharchur. This is a branched aromatic herb. The nodes are often hairy in some species. It is grown in the garden for ornamental purposes [9].



Fig 1: *Bryophyllum pinnatum* plant

The main aim of the present research work is to screen the *in vitro* anti-urolithiatic activity of ethanolic extract of *Bryophyllum pinnatum* leaves.

MATERIALS AND METHODS

Plant selection, collection, and authentication

The leaves of *Bryophyllum pinnatum* plant were collected from Central Institute of Medicinal and Aromatic plants (CIMAP), Hyderabad, India. The plant material was identified and authenticated by Prof. Dr. Jnanesha AC, Hyderabad, Telangana, India. The voucher specimen is available on the herbarium file of our department.

Chemicals and reagents

Ethanol, calcium chloride, sodium oxalate, Tris HCL, sodium chloride. All the chemicals were purchased from local suppliers.

Instruments

Soxhlet apparatus, UV-Visible spectrophotometer, hot air oven, analytical balance, mixer, digital pH meter.

Preparation of extract [10-12]

The leaves of the *Bryophyllum pinnatum* were shade dried at room temperature then grinded in the mixer grinder. The fine powder of the plant was evaluated through sieve no. 40 and stored in air tight container for further study. The extraction was carried out through maceration procedure. About 50 g of powder were soaked separately in ethanol in a stopper container for 5 days with frequent agitation. The extract was dried in the rotary evaporator and stored. The remaining drug was extracted through hot percolation method using Soxhlet apparatus.

Pharmacological screening [13-15]

Nuleation assay

The inhibitory effect of the ethanolic extract of *Bryophyllum pinnatum* on initiation of CaOx (calcium oxalate) crystallization. The solutions of calcium chloride (5mmol/lit) and sodium oxalates (7.5mmol/lit) were prepared in the buffer solution containing 0.05mol/lit Tris HCL, and 0.15mol/lit sodium chloride at pH range of 6.5. The dilutions of the leaves extract were prepared at different concentrations (100 - 1000 µg/ml) along with distil water. One milliliter of each drug concentration was mixed with 3ml of $CaCl_2$ and 3ml of the $Na_2C_2O_3$ solution. Final mixtures were incubated for 30 min at 37 ^{0}C. The optical density of the mixtures was measured at 620 nm wavelength using UV-Visible spectrophotometer. The percentage inhibition was calculated and compared to that of the standard drug cystone.

RESULTS

Crystallization in nucleation phase

The addition of $Na_2C_2O_4$ solution to the reaction mixture consisting of $CaCl_2$ resulted in the formation of numerous CaOx crystals. Presence of *Bryophyllum pinnatum* (1000ug/ml) in the reaction mixture produced a percent inhibition in the nucleation of 78±1.55% which was significantly higher than that produced by the cystone.

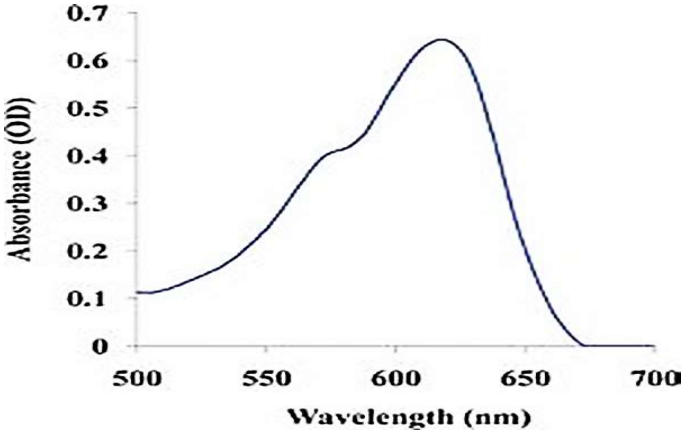


Fig 2: Spectrum of *Bryophyllum pinnatum*

Table 1: Linearity data

Concentration (µg/ml)	Absorbance
100	0.132
200	0.251
300	0.366
400	0.495
500	0.627

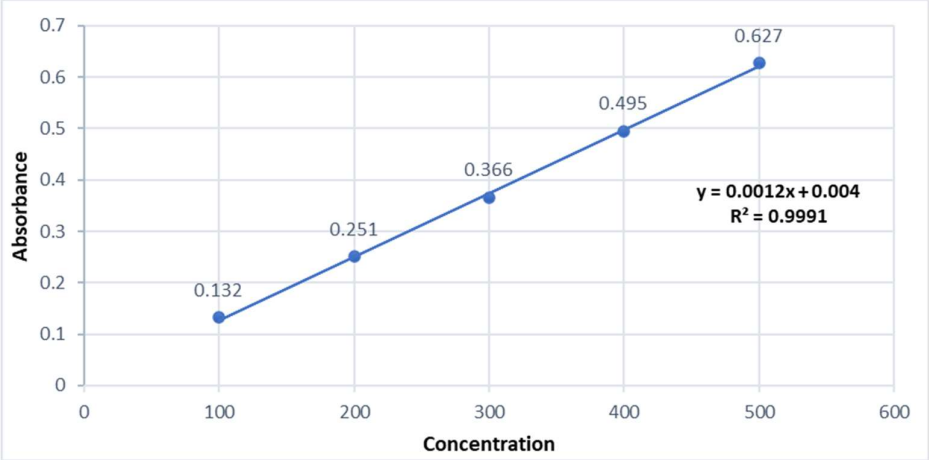


Fig 3: Linearity

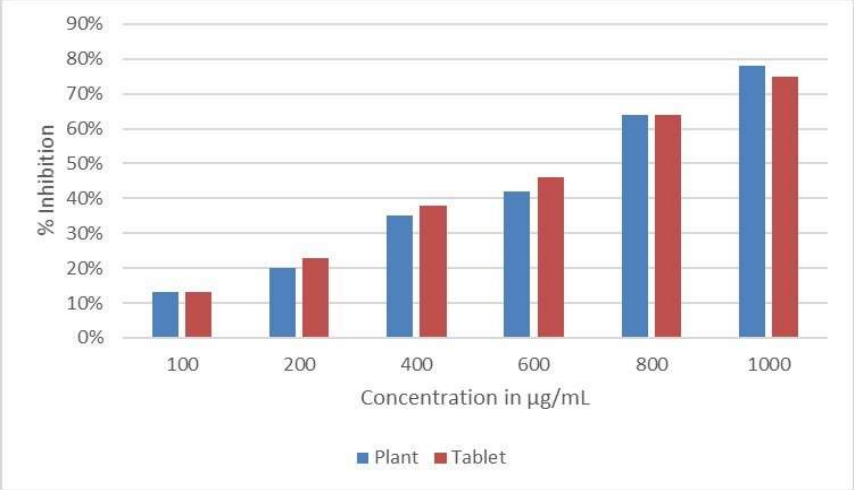


Fig 4: Effect of *Bryophyllum pinnatum* on CaOx crystal formation in nucleation phase

Crystallization in nucleation assay

Bryophyllum pinnatum produced a significant reduction in aggregation of performed CaOx crystals. Percent reduction in aggregation produced by *Bryophyllum pinnatum* was found to be 47.1±2.07 comparable to that of cystone at 1000ug/ml concentration.

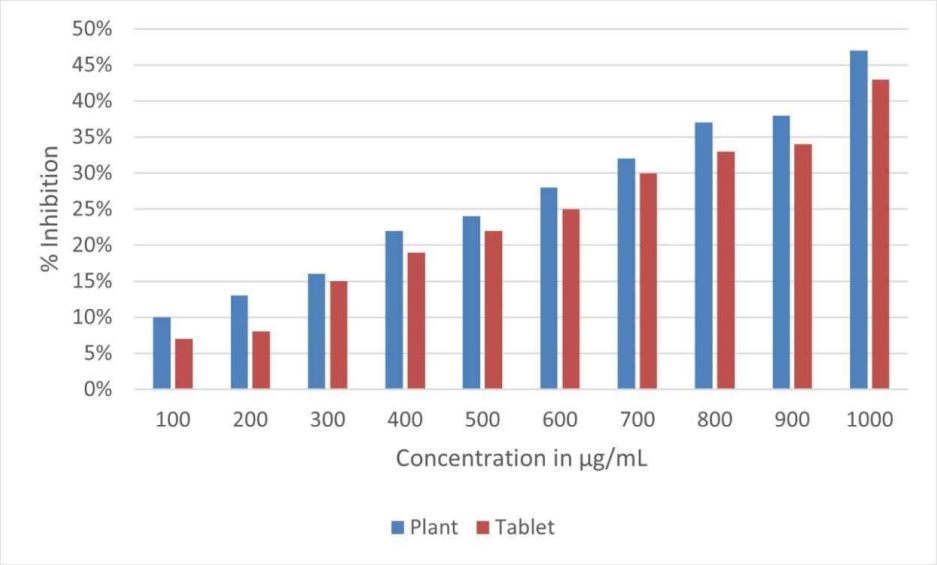


Fig 5: Effect of *Bryophyllum pinnatum* on CaOx crystal in aggregation phase

DISCUSSION

Formation of kidney stones is the complex process and involves a series of biological events that are most likely triggered by genetic susceptibility together with dietary factors and life styles changes. CaOx urolithiasis is the most prevalent type of all kidney stone disease. The key points involved in its pathological biomineralization include crystal nucleation, growth and aggregation. Present study was designed to address these key points involved in CaOx stone formation as a means to investigate the efficacy of *Bryophyllum pinnatum* as an anti-urolithiatic.

Nucleation is the necessary in the pathogenesis of CaOx urolithiasis. Nucleation basically marks a kinetically driven event of phase change wherein dissolved substances in a concentrated solution spontaneously crystallize. Phase change and formation of CaOx crystals was observed while carrying the nucleation assay. inhibition in the nucleation of CaOx crystals was observed in the presence of *Bryophyllum pinnatum* which was even better than in the presence of cystone.

This suggests the anti-crystallization activity of *Bryophyllum pinnatum* against CaOx crystallization. One possible method of anti-crystallization activity of *Bryophyllum pinnatum* could be its ability to complex with free calcium and oxalate ions, thus preventing the formation of CaOx complexes. Growth of CaOx crystals marks the event of deposition of crystal forming ions present in the concentrated solution on preformed CaOx crystal lattice. This event of growth of CaOx crystals was also seen in the present study. *Bryophyllum pinnatum* exhibited growth inhibitory activity which was confirmed from the crystals of reduced size produced in the presence of *Bryophyllum pinnatum*.

Aggregation of the crystals marks the process where number of crystals in the solution come together and adhere forming large crystal agglomerates. Aggregation is the key determinant of crystal retention as large crystal agglomerates are the ones that produce renal tubular blockage which promotes stone formation. *Bryophyllum pinnatum* showed significant inhibitory effect on CaOx crystal aggregation.

CONCLUSION

Plants are essential part of life on earth, they play a vital important role in the lives of animals and also in humans. Plants provide food, raw materials for medicine and various other requirements for the very existence of life from the origin of human beings. Even the current conventional medicine is using a lot of plant derived chemicals as therapeutic agents. The overuse of synthetic drugs results in higher incidence of adverse drug reactions has motivated humans to return to nature for safe remedies. Herbs and herbal drugs have created interest among the people by its clinically proven effects. Therefore, there is a compelling need for detailed scientific validation of all traditional medicinal plant drugs in order to establish their efficacy and safety in modern science.

From the reported evidences along with above findings, it is declared that *B. pinnatum* leaf extract may reduce the availability of calcium in renal tubules to form CaOx crystals by virtue of their Ca^{2+} chelating ability and may eventually prevent aggregation of already formed CaOx crystals and subsequent stone formation.

Bryophyllum Pinnatum showed prominent inhibition of all the phases of CaOx stone formation viz. nucleation, growth and aggregation. In addition, the plant extract also reduced the weight of CaOx kidney stone in *in vitro* studies. However, detail findings are needed to elucidate the long-term benefit/side-effects of this extracts of *B. pinnatum*. More interdisciplinary research is needed to develop new plant-derived high-quality natural products to treat and prevent the formation of kidney stones.

Competing interest statement
None.

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