

Antioxidant and anti-urolithiatic potential of *Bryophyllum pinnatum* leaf extract against calcium oxalate crystal nucleation: *In vitro* and toxicological evaluation

Sanskruti Mahapatra | Reshma Pradhan | Stuti Dwari | Pradeep K Naik*

Urolithiasis is a common urinary disorder in which kidney stones form as a result of supersaturation of minerals such as calcium and oxalate. The present study evaluated the phytochemical composition, antioxidant capacity and *in vitro* anti-urolithiatic activity of *Bryophyllum pinnatum*. A 70% ethanolic leaf extract was subjected to phytochemical screening, antioxidant assays, *in vitro* calcium oxalate nucleation assay and *in vivo* toxicity evaluation. The extract was rich in phenolics (112.74 ± 4.45 mg GAE/g), flavonoids (119.05 ± 3.83 mg RE/g) and scavenged free radicals in a concentration-dependent manner, with IC_{50} values of 14.62 μ g/mL (DPPH) and 8.67 μ g/mL (ABTS). Inhibition of calcium oxalate nucleation increased with concentration, approximately 70.81% at 0.5 mg/mL, 76.13% at 1 mg/mL, 79.73% at 2 mg/mL and 85.33% at 5 mg/mL. Qualitative microscopic analysis confirmed fewer and less aggregated crystals at the higher concentrations. *In vivo* toxicity study revealed a non-toxic effect of *B. pinnatum* plant extract evidence from the biochemical and histopathology study. These findings collaboratively provide a preliminary evidence of *B. pinnatum* possesses antioxidant and anti-urolithiatic activity consistent with its traditional use in the treatment of kidney stones.

Keywords: *B. pinnatum*, Urolithiasis, Calcium oxalate crystal, Anti-urolithiatic activity, Antioxidant activity, Nucleation assay, Phytochemicals

INTRODUCTION

Urolithiasis, the formation of solid calculi within the kidney, ureter, bladder, or urethra, has emerged as one of the most prevalent urological disorders. It constitutes a major global health burden owing both to its high prevalence and to its strong tendency to recur. An estimated 10.85% of the global population is affected, with regional prevalence ranging from 1% to 13% and a marked male predominance^{1,2}. Stone formation is fundamentally a physicochemical process driven by an imbalance between promoters and inhibitors of crystallization. When urine becomes supersaturated with lithogenic solutes (principally calcium, oxalate, phosphate, and uric acid), calculi develop through the sequential stages

of nucleation, crystal growth, aggregation, and retention within the renal tubules³. Adhesion of these crystals to tubular epithelial cells triggers localized oxidative stress and cellular injury, which in turn promote further crystal deposition and stone enlargement, establishing a self-sustaining cycle that underlies the high recurrence rate of the disease⁴.

Current management of urolithiasis relies largely on extracorporeal shock wave lithotripsy, ureteroscopic stone removal, and pharmacological agents such as thiazide diuretics and citrate supplements. Although effective in the short term, these approaches are limited by procedural complications, high cost, and substantial recurrence rates.

Research Centre of Excellence in Natural Products and Therapeutics, Dept. of Biotechnology & Bioinformatics, Sambalpur University, Jyoti Vihar, Sambalpur-768019, Odisha, India

*Corresponding Author Pradeep K Naik (✉ pknaik1973@gmail.com)

Received | Revised | Accepted



This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>

Such limitations have sustained interest in medicinal plants as safer, more affordable, and often multi-targeted alternatives for the management of stone disease. *Bryophyllum pinnatum*, commonly known as the life plant or miracle leaf, is one such plant, with a long history of traditional use in renal and urinary disorders, including kidney stones⁵. The plant is rich in flavonoids, phenolics, alkaloids, and glycosides, which underlie its reported antioxidant, anti-inflammatory, and nephroprotective activities^{6,7}.

Several earlier reports have examined the anti-urolithiatic potential of *B. pinnatum* in rat models of induced urolithiasis^{8–10}, and its phytochemistry and toxicology have been reviewed elsewhere¹¹. Most of this work, however, has evaluated antioxidant or anti-urolithiatic activity in isolation. Few studies have combined *in vitro* antioxidant profiling, a concentration-dependent calcium oxalate nucleation assay with qualitative microscopic support, and a systematic acute and sub-acute toxicity evaluation encompassing haematological, biochemical, and histopathological endpoints within a single, internally consistent study on the same extract batch. The present study was therefore undertaken to characterize the phytochemical composition and antioxidant potential of a 70% ethanolic leaf extract of *B. pinnatum*, to determine its inhibitory effect on calcium oxalate crystal formation, and to assess its safety *in vivo*. By linking antioxidant capacity to nucleation-inhibitory activity and pairing both with a safety assessment in the same animal model, this integrated design provides a more complete pharmacological and toxicological profile of the extract than has been reported to date, and serves to scientifically validate its traditional use and evaluate its potential as a natural therapeutic agent for the prevention and management of urolithiasis.

MATERIALS AND METHODS

Chemicals and reagents

Calcium chloride and sodium oxalate were used for the calcium oxalate crystallization studies. Folin–Ciocalteu reagent, gallic acid, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide and quercetin were used for the phytochemical estimations. Antioxidant activity was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), with ascorbic acid as the reference standard. All chemicals and reagents were of analytical grade and were procured from standard suppliers (Sigma-Aldrich, SRL and HiMedia).

Plant material and authentication

Fresh leaves of *B. pinnatum* were collected from the medicinal garden of Sambalpur University, Burla, Odisha,

India, and authenticated by a Taxonomist at Regional Plant Resource Centre (RPRC), Bhubaneswar, Odisha, India. The plant was identified as *Bryophyllum pinnatum*, and a voucher specimen (Specimen No. SU/COENPT-0064) was deposited at Sambalpur University for future reference. The leaves were washed thoroughly with distilled water, air-dried at room temperature and pulverized to a coarse powder.

Preparation of the extract

The leaf powder was extracted with 70% ethanol in a continuous Soxhlet extraction system maintained at a constant temperature. Briefly, 20 g of powdered material was weighed, placed in the thimble and extracted with 250 mL of 70% ethanol under controlled heating (40–45°C) for approximately 6 h. The resulting extract was filtered and concentrated in a rotary evaporator to remove excess solvent, and the concentrate was subsequently dried in a hot-air oven to obtain a stable powdered extract¹². The dried extract was stored at 4°C until further use.

Phytochemical analysis

Estimation of total phenolic content

The total phenolic content (TPC) of the extract was determined colorimetrically by the Folin–Ciocalteu method, following the procedure reported earlier¹³. Gallic acid standards (10–100 µg/mL) were prepared from a 0.1 mg/mL stock solution, and the extract was tested at 1000 µg/mL. To each solution, 5 mL of Folin–Ciocalteu reagent (10%) and sodium carbonate (7%) were added, and the volume was made up to 10 mL with methanol. After incubation for 30 min at room temperature, the absorbance was recorded at 765 nm (UV-1900, Shimadzu, Japan) against a methanol blank. All readings were taken in triplicate, and TPC was calculated from the gallic acid calibration curve and expressed as mg gallic acid equivalents per g of dry extract (mg GAE/g).

Estimation of total flavonoid content

The total flavonoid content (TFC) was determined by the aluminium chloride colorimetric method as described previously¹⁴. Quercetin standards (100–1000 µg/mL) were prepared from a 1 mg/mL stock solution, and the extract was tested at 1000 µg/mL. To 1 mL of each solution, 4 mL of distilled water and 0.3 mL of sodium nitrite (5%) were added. After 5 min, 0.3 mL of aluminium chloride (10%) was added, followed shortly by 2 mL of sodium hydroxide (1 M), and the final volume was adjusted to 10 mL. The absorbance was measured at 510 nm (UV-1900, Shimadzu, Japan) in triplicate, and TFC was expressed as mg quercetin equivalents per g of dry extract (mg QE/g).

Determination of antioxidant activity

DPPH radical scavenging assay

The free radical scavenging potential of the extract was evaluated by the DPPH assay with minor modifications¹⁵. The assay is based on the reduction of DPPH, a stable purple-coloured free radical, by an antioxidant or hydrogen-donating compound, which produces a measurable decrease in absorbance. Sample solutions were prepared over a concentration range of 1–100 µg/mL. For each assay, 0.1 mL of sample was added to 3 mL of freshly prepared 0.1 mM DPPH in methanol. The reaction mixtures were vortexed and incubated in the dark at room temperature for 30 min, since DPPH is light-sensitive and prone to photodegradation. The absorbance was then measured at 517 nm using a UV–visible spectrophotometer (UV-1900, Shimadzu, Japan), with DPPH solution without sample as the control. Ascorbic acid was used as the reference standard. All experiments were performed in triplicate, and the percentage inhibition was calculated as:

$$\text{Percentage inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the DPPH solution without sample and A_{sample} is the absorbance of the DPPH solution containing the test extract or standard, both measured at 517 nm. IC_{50} values, defined as the concentration required to scavenge 50% of the DPPH radicals, were derived from the corresponding dose–response curves.

ABTS radical scavenging assay

ABTS⁺ radical scavenging activity was determined following a reported method with minor adjustments¹⁶. The radical cation was generated by mixing ABTS (7.4 mM) with potassium persulfate (2.6 mM) and storing the mixture in the dark at 37°C for 12–16 h. The test extract, prepared at concentrations of 5–100 µg/mL, was mixed with 1.90 mL of the ABTS working solution and allowed to react for 2 h, after which the absorbance was recorded at 734 nm. Ascorbic acid was used as the standard, and IC_{50} value was determined from triplicate readings. The percentage inhibition was calculated as:

$$\text{Percentage inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the ABTS working solution without sample and A_{sample} is the absorbance of the ABTS working solution containing the test extract or standard, both measured at 734 nm.

In vitro anti-urolithiatic study

Calcium oxalate nucleation assay

The nucleation assay was performed to assess the inhibitory potential of *B. pinnatum* extract against calcium oxalate crystallization, following reported protocols with minor

modifications¹⁷. Tris buffer (pH 6.5) containing 0.05 mol/L Tris and 0.15 mol/L sodium chloride served as the reaction medium, and working solutions of calcium chloride (4 mmol/L) and sodium oxalate (7.5 mmol/L) were freshly prepared. The extract of different concentrations (0.5–5 mg/mL) were mixed with the calcium chloride solution (1:9 v/v), after which the sodium oxalate solution (900 µL) was added to initiate crystallization. The mixtures were incubated at 37°C to simulate physiological conditions. A parallel reaction without extract served as the control, and distilled water was used as the reference for baseline absorbance. Crystal formation was quantified by recording the absorbance at 620 nm. The inhibitory effect of the extract on nucleation was determined relative to the untreated control. Cystone was included as the standard (positive control). Each experiment was performed independently in triplicate ($n=3$), all the experiments were performed in sterile condition at room temperature, and the data are presented as mean $\pm$ SD. The percentage inhibition was calculated as:

$$\text{Inhibition (\%)} = [(OD_{\text{control}} - OD_{\text{experimental}}) / OD_{\text{control}}] \times 100$$

where OD_{control} is the absorbance (620 nm) of the calcium chloride–sodium oxalate reaction mixture without extract and $OD_{\text{experimental}}$ is the absorbance of the corresponding mixture containing extract or standard at a given concentration.

In vivo toxicity study

The *in vivo* toxicity study was approved by the Institutional Animal Ethics Committee, Department of Biotechnology and Bioinformatics, Sambalpur University, Burla, Odisha (vide approval No. SU/BTBI/IAEC/2026/03). Wistar rats of both sexes, aged 6–8 weeks and weighing 150–200 g were used. Prior to the experiments, the animals were housed in polypropylene cages and acclimatized to laboratory conditions for two weeks at a controlled room temperature of $25 \pm 2^\circ\text{C}$ under a 12 h light/12 h dark cycle. Standard pellet diet and water were available ad libitum throughout the study.

Acute toxicity study

Acute oral toxicity of the ethanolic extract of *B. pinnatum* was evaluated according to OECD Test Guideline No. 423¹⁸. Rats were divided into two groups i.e., control and *B. pinnatum* treated each having 6 no of animals ($n=6$). The overnight fasted animals of the treatment group was treated with *B. pinnatum* extract at a high dose of 2000 mg/kg b.w. and observed for the initial 4, 8, 24 h and then periodically up to 14 days for any signs of toxicity, behavioural changes and mortality.

Sub-acute toxicity study

For the sub-acute toxicity evaluation, rats were randomly

allocated into two groups i.e., control and treated (n=6) in accordance with OECD Test Guideline No. 407¹⁹. The ethanolic extract of *B. pinnatum* was administered orally at 200 mg/kg body weight daily for 28 days to the treatment group of animals. Immediately after every treatment, the animals were observed for any clinical signs of mortality, and changes in behaviour. At the end of the study, the animals were euthanized under mild isoflurane anaesthesia, and blood was collected for biochemical analyses. The vital organs were harvested in 10% neutral buffered formalin and processed for haematoxylin and eosin (H&E) staining to assess the effect of the extract on tissue architecture.

Statistical analysis

All data are expressed as mean $\pm$ standard deviation (SD); *in vitro* assays were performed in triplicate (n = 3) and *in vivo* experiments used six animals per group (n = 6). Comparisons between control and treated groups were made using an unpaired Student's *t*-test in GraphPad Prism (version 8.0, GraphPad Software Inc., San Diego, CA, USA). A *P* value < 0.05 was considered statistically significant.

RESULTS

Phytochemical screening of the plant extract

The yield percentage of the hydroethanolic extract of *B. pinnatum* was found to be 10.95%. Quantitative estimation of phytochemicals revealed that the TFC of the 70% ethanolic extract was 119.05 ± 3.83 mg RutinE/g, while the TPC was 112.74 ± 4.45 mg GAE/g. The presence of these compounds suggests strong antioxidant potential of the extract.

Determination of antioxidant activity

DPPH and ABTS radical scavenging assay

In the DPPH assay, the 70% ethanolic extract of *B. pinnatum* showed strong free radical scavenging activity, with an IC_{50} value of $14.62 \mu\text{g/mL}$. Inhibition increased in a concentration-dependent manner, reaching 97.88% at $100 \mu\text{g/mL}$. In the ABTS assay, the extract was even more effective, with an IC_{50} value of $8.67 \mu\text{g/mL}$, comparable to that of the standard, ascorbic acid (Fig. 1). Together, these findings indicate that the extract has potent antioxidant activity, likely due to its high phenolic and flavonoid content. By reducing oxidative stress, this antioxidant capacity may contribute to the anti-urolithiatic effect of the extract.

In vitro anti-urolithiasis study

Nucleation assay

The nucleation assay showed that the 70% ethanolic extract of *B. pinnatum* exhibited the concentration dependent

inhibitory activity (Fig. 2A,B). Inhibition of calcium oxalate nucleation increased in a concentration-dependent manner estimated in different sample concentration. The quantitative estimation revealed that the inhibition at 0.5 mg/mL, 1 mg/mL, 2 mg/mL and 5 mg/mL was 70.81%, 76.13%, 79.73% and 85.33%, respectively. On microscopic examination, crystal in the extract treated mixture appeared qualitatively fewer and less aggregated than the control as the concentration (5mg/ml) increases. Together, these findings indicate that *B. pinnatum* exerts a dose-dependent anti-crystallization effect and effectively inhibits the initial nucleation stage of calcium oxalate stone formation.

In vivo toxicity study

Acute toxicity study

The acute oral toxicity study of the 70% ethanolic extract of *B. pinnatum* revealed no mortality or visible signs of toxicity to the experimental animals. The animals remained normal in behaviour and physical activity, indicating that the extract is safe up to 2000 mg/kg body weight.

Sub-acute toxicity study

In the sub-acute toxicity study, the extract was administered orally at 200 mg/kg body weight for 28 days. No mortality, abnormal clinical signs, or noticeable behavioural changes were observed during the study period, suggesting that the extract is safe for repeated administration at the tested dose. Body weight increased steadily in both groups, with no progressive weight loss and no significant difference has been observed between the control and treated groups (Fig. 3). Similarly, the biochemical parameters of both the groups showed within the normal range (Table 1).

Histopathological study of vital organ

Histopathological examination of vital organs after administration of the plant extract revealed no significant abnormalities compared with the control group. Brain sections displayed normal architecture with intact neurons and glial cells. Cardiac muscle fibres were well organised, with no evidence of necrosis or inflammatory infiltration. Kidney sections showed preserved glomerular and tubular structures without degeneration, necrosis, or inflammation, and the liver retained normal hepatic lobular architecture with intact hepatocytes and no congestion or cellular damage (Fig. 4). The absence of histopathological alterations in the brain, heart, kidney, lungs, and liver indicates that sub-acute administration of *B. pinnatum* extract did not produce neurotoxic, cardiotoxic, nephrotoxic, or hepatotoxic effects at the tested dose.

DISCUSSION

Despite advances in the surgical and pharmacological management of urolithiasis, recurrence rates remain

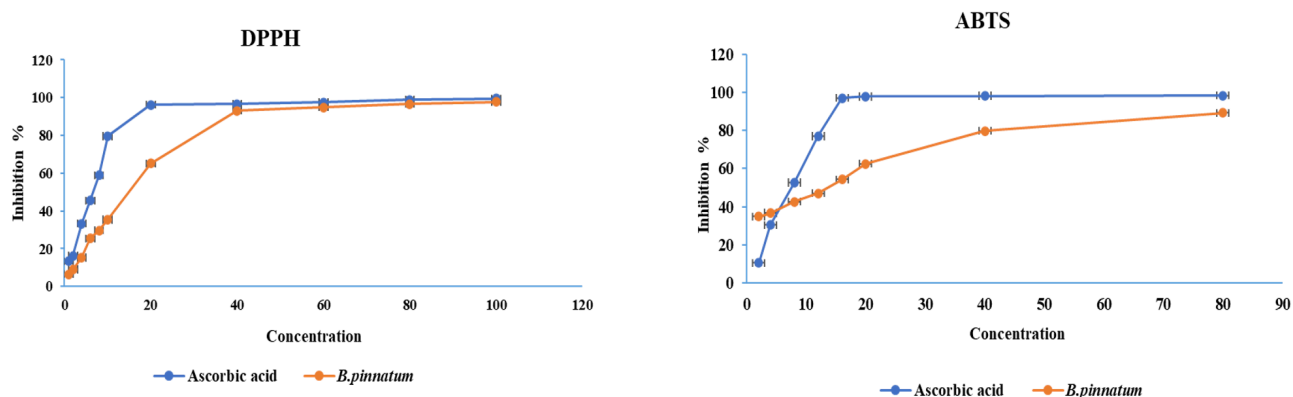


Figure 1. DPPH and ABTS radical scavenging activity of 70% ethanolic extracts of plant *B. pinnatum* in comparison with ascorbic acid

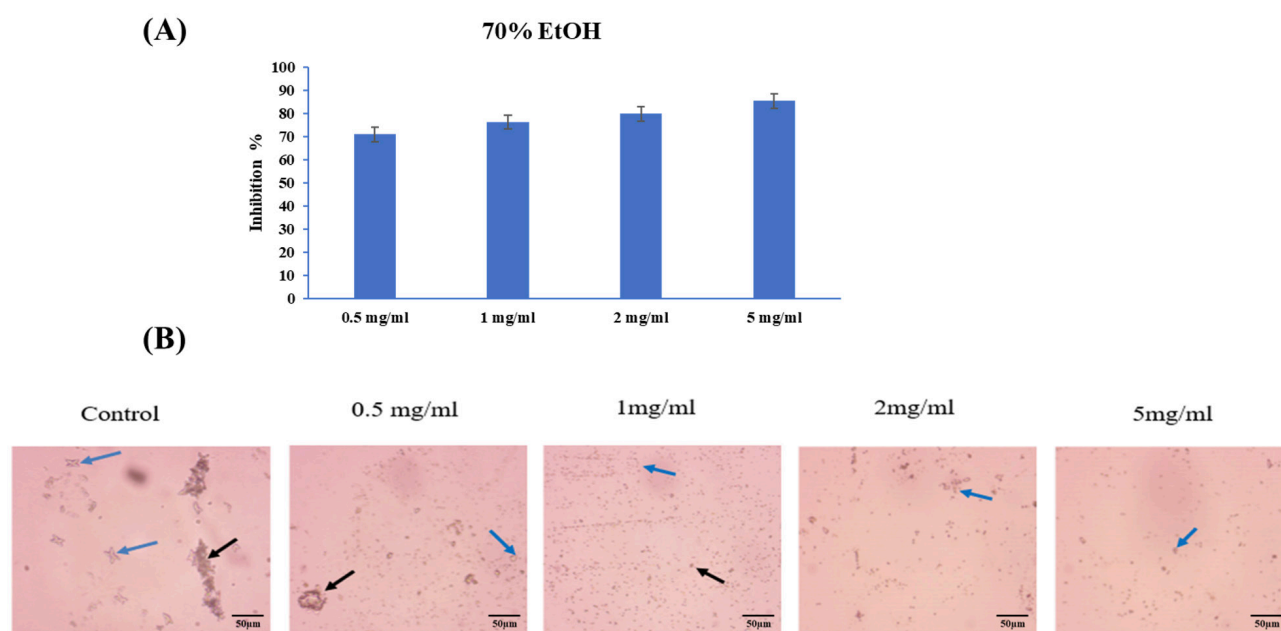


Figure 2. (A) Showed effect of different concentrations (0.5, 1, 2, and 5 mg/mL) of 70% ethanolic extract of *B. pinnatum* on calcium oxalate crystal nucleation, showing a concentration-dependent increase in percentage inhibition. (B) show the microscopic evaluation of calcium oxalate crystal inhibition (nucleation assay) by *B. pinnatum* extract at different concentrations (0.5, 1, 2, and 5 mg/mL) compared to control. The blue arrow indicates individual crystals, while the black arrow indicates the aggregated crystal structure

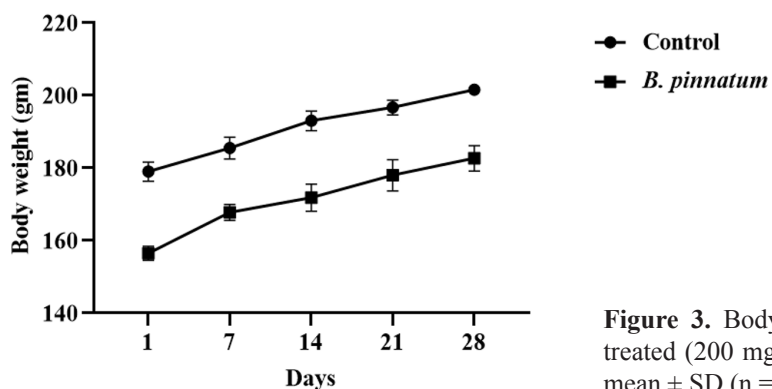


Figure 3. Body weight changes in control and *B. pinnatum*-treated (200 mg/kg, 28 days) Wistar rats. Data are presented as mean $\pm$ SD (n = 6)

Table 1. Blood biochemical parameters of the control and *B. pinnatum*-treated groups

Parameter	Normal control	<i>B. pinnatum</i>	P-value	Range
GLU (mg/dl)	131.24±4.02	117.11±5.78	0.0012**	74.0-143.0
ALB(g/dl)	2.65±0.28	3.01±0.92	0.4683 ns	2.3-4.0
UREA (mg/dl)	34.27±1.52	29.29±5.78	0.0927 ns	15.0-58.0
CREA (mg/dl)	1.24±0.05	1.37±0.43	0.5103 ns	0.5-1.8
CHOL (mg/dl)	140.5±16.63	157.86±25.94	0.2364 ns	109.0-202.0
TG (mg/dl)	134.16±11.76	90.53±41.8	0.0485 *	40.0-165.0
ALT(U/L)	80.56±3.41	84.53±16.79	0.6158 ns	10.00-125.00
AST(U/L)	21.45±2.84	32.49±4.01	0.0005***	0-50.0
TP(g/dl)	6.32±0.49	5.54±0.56	0.0432 *	5.2-8.2
DBIL (mg/dl)	0.42±0.07	0.40±0.13	0.7478 ns	0-0.50
HDL (mg/dl)	63.66±2.11	55.01±10.2	0.0934 ns	35.0-88.0
GGT(U/L)	2.12±0.24	5.13±1.89	0.0056 **	0-10.0

Statistical comparison by unpaired Student's *t*-test, performed in GraphPad Prism (version 8.0). **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns = not significant vs. control

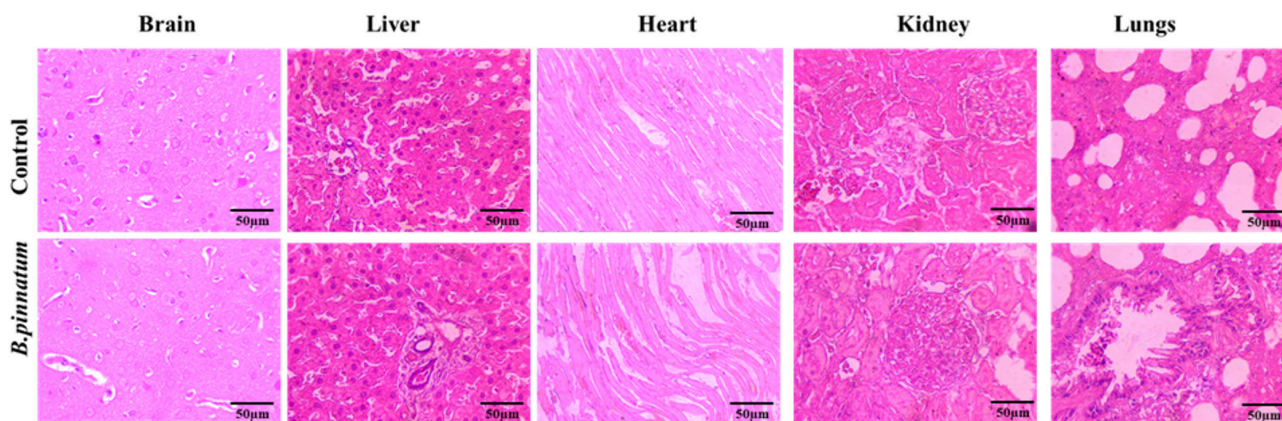


Figure 4. Brain, liver, heart, kidney, and lungs histology of rats administered with crude of *B. pinnatum* at a daily dose of 200mg/kg body weight for 28 days revealed no significant changes compared to normal groups

high, and no agent in routine clinical use directly targets nucleation, the earliest stage of calcium oxalate crystallization. This gap continues to drive interest in plant-derived agents such as *B. pinnatum*, which has a long history of ethnomedicinal use for renal and urinary complaints^{5,8,20}. Once urine becomes supersaturated with calcium and oxalate, stone formation proceeds through nucleation, crystal growth, aggregation, and retention on the renal epithelium^{2,3}. Agents that interfere with nucleation are of particular interest because they act before crystals large enough to injure the epithelium can form. The present study was designed on this rationale and combined phytochemical, antioxidant, nucleation-inhibition, and toxicological evaluations of a single *B. pinnatum* extract.

Phytochemical analysis showed that the extract was rich in phenolics (TPC:112.74±4.45 mg GAE/g) and flavonoids (TFC:119.05±3.83 mg RE/g). The extract also exhibited

concentration-dependent radical-scavenging activity, with IC₅₀ values of 14.62 µg/mL and 8.67 µg/mL in the DPPH and ABTS assays, respectively. These findings agree with earlier reports attributing the antioxidant activity of *B. pinnatum* to its phenolic and flavonoid content^{6,11}. Because oxidative stress promotes renal epithelial injury and facilitates crystal adhesion and retention, it is plausible that the antioxidant activity of the extract contributes, at least in part, to its anti-urolithiatic potential by limiting such damage.

The calcium oxalate nucleation assay provided the most direct measure of anti-urolithiatic potential in this study. The extract inhibited nucleation in a concentration-dependent manner, estimated quantitatively as 70.81% at 0.5 mg/mL, 76.13% at 1 mg/mL, 79.73% at 2 mg/mL and 85.33% at 5 mg/mL. A similar concentration-dependent pattern and comparable maximal inhibition have been reported for *B. pinnatum* in earlier *in vitro* studies of calcium oxalate crystallization²¹. Anti-urolithiatic effect

has been reported for *B. pinnatum* in earlier *in vivo* studies of ethylene glycol-induced renal calculi in rats⁵. Likewise, alcoholic and hydro-alcoholic extracts of *B. pinnatum* lowered urinary oxalate, improved renal function markers, and prevented renal calculi formation in ethylene glycol-induced urolithiasis in rats⁸, and a hydro-alcoholic extract was effective in sodium oxalate-induced urolithiasis⁹. The present nucleation data are therefore consistent with, rather than a first demonstration of, the anti-urolithiatic effect of *B. pinnatum*. The contribution of this work lies instead in profiling a specific 70% ethanolic extract across a range of concentrations using both quantitative and qualitative microscopic endpoints, together with a matched toxicity assessment.

The safety of the 70% ethanolic extract was evaluated through acute and sub-acute oral toxicity studies. In the acute study, a single dose of 2000mg/kg b.w. caused no mortality or signs of toxicity, and the animals maintained normal behaviour and physical activity throughout the observation period. Similarly, repeated administration of 200mg/kg for 28 days produced no signs of toxicity or behavioural changes. Biochemical analysis showed that glucose, urea, creatinine, ALT, ALB, GGT, bilirubin, or the other parameters evaluated parameters remained within their references range in both the control and treated groups. These findings were supported by histopathological examination, which showed preserved architecture of the heart, brain, liver, lungs, and kidney, with no evidence of necrosis, degeneration, or other pathological changes. Thus, under the experimental conditions, the extract produced no organ-specific toxicity and was well tolerated during repeated administration.

CONCLUSIONS

The present study provides preliminary evidence that the 70% ethanolic leaf extract of *B. pinnatum* possesses notable antioxidant activity and concentration-dependent inhibitory activity against calcium oxalate nucleation, together with a favourable acute and sub-acute toxicity profile. By evaluating antioxidant, anti-urolithiatic, and safety properties in a single extract, this study extends previous reports on *B. pinnatum*, which have largely examined these properties separately, and lends scientific support to its traditional use in urinary disorders. However, these findings remain preliminary. The nucleation assay does not model crystal growth or renal retention *in vivo*, and the active constituents have not yet been identified. Further studies using *in vivo* urolithiasis models, together with mechanistic investigations, are needed before therapeutic efficacy can be established.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest.

AUTHORS' CONTRIBUTION STATEMENT

Sanskruti Mahapatra: Methodology, investigation, data analysis, writing – original draft, Reshma Pradhan: Conceptualization, and writing-review and editing, Stuti Dwari: Methodology, investigation, Pradeep K Naik: Conceptualization, and writing-review and editing.

DECLARATION OF GENERATIVE AI USE

Generative AI was not used in the research or preparation of manuscripts.

REFERENCES

1. Vera-Ponce, V.J., Sanchez-Tamay, N.M., Ballena-Cacedo, J., Zuzunaga-Montoya, F.E., De Carrillo, C.I.G. and Poemape Mestanza, R.L. (2025). Global prevalence of urolithiasis: a meta-analysis accounting for methodological heterogeneity. *Front. Urol.* 5: 1705953.
2. Stamatelou, K. and Goldfarb, D.S. (2023). Epidemiology of kidney stones. *Healthcare.* 11(3): 424.
3. Tamborino, F., Cicchetti, R., Mascitti, M., Litterio, G., Orsini, A., Ferretti, S., Basconi, M., De Palma, A., Ferro, M., Marchioni, M. and Schips, L. (2024). Pathophysiology and main molecular mechanisms of urinary stone formation and recurrence. *Int. J. Mol. Sci.* 25(5): 3075.
4. Bai, J., Lu, T., Zhao, Z., Wei, F., He, K., He, X., Jing, Y., Wang, F., Qin, W., Xu, Z. and Yang, L. (2025). The role of inflammation and ROS in CaOx kidney stones. *Eur. J. Med. Res.* 30(1): 1224.
5. Shukla, A.B., Mandavia, D.R., Barvaliya, M.J., Baxi, S.N. and Tripathi, C.R. (2014). Evaluation of anti-urolithiatic effect of aqueous extract of *Bryophyllum pinnatum* (Lam.) leaves using ethylene glycol-induced renal calculi. *Avicenna J. Phytomed.* 4(3): 151-159.
6. Isibor, J. (2026). Phytochemistry and bioactivity studies of *Bryophyllum pinnatum*: a review. *Discover Chem.* 3(1): 33.
7. Sharma, G., Jangra, A., Sihag, S., Chaturvedi, S., Yadav, S. and Chhokar, V. (2024). *Bryophyllum pinnatum* (Lam.) Oken: unravelling therapeutic potential and navigating toxicity. *Physiol. Mol. Biol. Plants.* 30(9): 1413.
8. Yadav, M., Gulkari, V. and Wanjari, M. (2016). *Bryophyllum pinnatum* leaf extracts prevent formation of renal calculi in lithiatic rats. *Anc. Sci. Life.* 36(2): 90.
9. Pandhare, R.B., Shende, R.R., Avhad, M.S., Deshmukh, V.K., Mohite, P.B., Sangameswaran, B. and Daude, R.B. (2021). Anti-urolithiatic activity of *Bryophyllum pinnatum* Lam. hydroalcoholic extract in sodium oxalate-induced urolithiasis in rats. *J. Tradit. Complement. Med.* 11(6): 545–551.
10. Shinde, S.K., Chaware, V.J. and Redasani, V.K. (2024). Anti-urolithiatic effect of ethanolic extract of *Bryophyllum pinnatum* (Lam) against chemicals induced renal calculi in rats. *Asian J. Res. Chem.* 17(4): 223–229.
11. Fernandes, J.M., Cunha, L.M., Azevedo, E.P., Lourenço, E.M.G., Fernandes-Pedrosa, M.F. and Zucolotto, S.M. (2019). *Kalanchoe laciniata* and *Bryophyllum pinnatum*: an updated review about ethnopharmacology, phytochemistry, pharmacology and toxicology. *Rev. Bras. Farmacogn.* 29(4): 529–558.

12. **Alara, O.R., Abdurahman, N.H., Ukaegbu, C.I. and Kabbashi, N.A. (2019).** Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. *J. Taibah Univ. Sci.* 13(1): 414–422.
13. **Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M. (1999).** Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* 299: 152–178.
14. **Shraim, A.M., Ahmed, T.A., Rahman, M.M. and Hijji, Y.M. (2021).** Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT.* 150: 111932.
15. **Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R.P. and Chang, C.M. (2022).** Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules.* 27(4): 1326.
16. **Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999).** Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 26(9–10): 1231–1237.
17. **Saha, S. and Verma, R.J. (2013).** Inhibition of calcium oxalate crystallisation *in vitro* by an extract of *Bergenia ciliata*. *Arab J. Urol.* 11(2): 187.
18. **OECD (2002).** Test No. 423: acute oral toxicity - acute toxic class method. OECD Guidelines for the Testing of Chemicals, Section 4: 1–14.
19. **OECD (2025).** Test No. 407: repeated dose 28-day oral toxicity study in rodents. OECD Guidelines for the Testing of Chemicals, Section 4.
20. **Nirumand, M.C., Hajialyani, M., Rahimi, R., Farzaei, M.H., Zingue, S., Nabavi, S.M. and Bishayee, A. (2018).** Dietary plants for the prevention and management of kidney stones: preclinical and clinical evidence and molecular mechanisms. *Int. J. Mol. Sci.* 19(3): 765.
21. **Agarwal, K. and Varma, R. (2014).** *In vitro* calcium oxalate crystallization inhibition by *Achyranthes aspera* L. and *Bryophyllum pinnatum* Lam. *British Journal of Pharmaceutical Research.* 5(2): 146–152.