

PHYTOCHEMICAL STUDY AND ANTIURROLITHIATIC ACTIVITY OF BRYOPHYLLUM PINNATUM (LAM.) STEM EXTRACT

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Abstract- *Bryophyllum pinnatum*, commonly known as *Pattharcatta*, is used traditionally in ethnomedicinal practices and treatment of kidney stone and urinary insufficiency. The present study evaluated the effect of *Bryophyllum pinnatum* on Ethylene Glycol induced renal calculi in rats. Renal calculi were induced in rats by administrating ethylene glycol 0.75% in drinking water and co-treated orally with standard drug, Cystone (750mg/kg), or aqueous, Ethyl acetate, hydroalcoholic extract in doses of 200mg/kg for 28 days. Treatment with extract attenuated the EG-induced decrease in body weight and elevation in urine excretion. Results were comparable to standard drug, cystone. Results indicates that EG administration caused renal calculi formation which is prevented by treatment with extract. The observed antilithiatic effect may be attributed to the presence of high content of phenolics, flavonoids and saponin in the extract.

Keywords- *Bryophyllum pinnatum*, antiurolithiatic, kidney, ethylene glycol, flavonoids, cystone.

INTRODUCTION

Urolithiasis, also referred to as renal stone or urinary calculi, is a condition in which the urinary system develops hard, non-metallic mineral calcifications. Urinary stones affect a high percentage of people, accounting for 12% of the global population, and have a recurrence rate of 70%–80% in men and 47%–60% in women [1]. Animals can get urolithiasis, just like humans, and males are shown to be more vulnerable than females because of their longer urethras [2]. Numerous multifactorial aetiopathogenesis, including bacterial infection, anatomical factors, and dietary practises, contribute to the formation of the urolith. High dietary calcium intake, insufficient hydration for bodily metabolism, gout, hyperparathyroidism, and obesity, among other factors.

There is currently no proven method of preventing urolithiasis. Therefore, a medical strategy must be developed to stop the production of stones again and again (5, 6). Since ancient times, local plants have been used as potential sources of medicine. The search for a medical cure for renal calculi is still ongoing, despite the fact that many plants have been studied for their potential antiurolithiatic effects. India, China, Australia, New Zealand, the Philippines, and other countries grow *Bryophyllum pinnatum* (Lam.), sometimes known as life plant or air plant. (7)

It is employed as an anticancer, antinociceptive, antidiabetic, anti-inflammatory, anti-fungal (8), anti-ulcer, anti-hypertensive (9), antimicrobial (10), hepatoprotective, and antihelminthic agent in conventional medicine. Numerous investigations have shown that *Bryophyllum pinnatum* (Lam.) has antioxidant and nephroprotective properties (11). There isn't enough scientific proof to support its usefulness in treating renal calculi, despite the fact that it is frequently used as a folk remedy in India. The goal of the current investigation was to confirm the therapeutic effectiveness of the hydroalcoholic extract of *Bryophyllum pinnatum* (Lam.) stem on calcium oxalate urolithiasis in rats with renal calculi caused by ethylene glycol. (12)

2. MATERIALS AND METHODS

2.1 Authentication and collection of plant material:

The stem of *Bryophyllum pinnatum* (Lam.) was procured in the neighborhood of Loni B.K., Ahmednagar district, Maharashtra, India, and was authorized by Dr. A. S. Wabale, Research guide, Department of Botany and Research Centre, PVP College of Arts, Science, and Commerce, Pravaranagar, Maharashtra, via letter number PVPC/Bot/2022-23/68 dated November 21, 2022, as shown in fig1.

2.2 Chemicals and apparatus:

A metabolic cage, UV spectrometer, centrifuge, and other apparatus were utilized in the investigation. Ethylene glycol was purchased from Research-Lab Fine Chem Industries., Mumbai, India. All other chemicals and reagents were analytical grade and obtained from licenced chemical suppliers.

Animals:

For this investigation, male Wistar albino rats (8 weeks old, 150–200 g body weight) were employed. They were kept in a controlled environment with a temperature of 27–20°C and 12 h of light/dark cycles, with the light turning on at 7:00 h. They were housed in polypropylene cages with six animals per cage. The animals had a regular food. The institutional animal ethical committee of CPCSEA, Government of India, authorized the experimental protocol used in this work, and it was carried out in compliance with OECD recommendations.

Drug administration:

Through an oral feeding tube made of stainless steel, the reference medication and the test sample were both given orally. To each isolated chemical, water and weight-based dimethyl form amide were added in order to prepare test dosages.

2.3 Extraction of plant material:

Under running water, the stem of *Bryophyllum pinnatum* (Lam.) was extensively cleaned. After being thoroughly cleaned, the stems were cut and air dried under cover for a week. The dried stems were ground into a coarse powder and then utilized in the extraction procedure. The extraction procedure employed the successive extraction approach. The sample was ground up and extracted using aqueous, ethyl acetate, and hydro alcoholic solvents (70%-30% each) at 40° to 45° C over the course of three hours. The resulting extract was evaporated at 45°C, dried, and kept in an airtight container for further research. (13) Extraction yield showed in (Table 1)

2.4 Preliminary phytochemical screening:

The classes of phytoconstituents found in the stem of *Bryophyllum pinnatum* (Lam.) that also have therapeutic properties are revealed by preliminary phytochemical screening. Aqueous, ethyl acetate, and hydro alcoholic extracts of the stem of *Bryophyllum pinnatum* (Lam) were subjected to phytochemical screening chemical tests to identify the chemical contents. The tests also looked for the presence of secondary metabolites such as alkaloids. Protein, steroids, tannins, phenol, glycosides, saponins, flavonoids, phenol, and carbohydrates. (Table 2) (Fig. a, b, c)

A) Test for alkaloid:

a) Dragendroffs test:

Add a few drops of Dragendorff's reagent to 2-3 ml of filtrate. Alkaloids are present when orange-brown precipitate forms.

B) Test for Carbohydrates:

a) Molisch's Test (General Test):

Add a few drops of alpha-naphthol solution dissolved in alcohol to 2-3 ml of extract, shake, and then add concentrated H₂SO₄ from the test tube's sides. The presence of carbohydrates is shown by the formation of a violet ring at the intersection of two liquids.

b) Fehling's Test:

Boil for a minute after combining 1 ml each of Fehling's solutions A and B. Test solution is added in an equal amount. For 5–10 minutes, heat in a pot of boiling water. Yellow precipitate that forms first and later brick red precipitate show that there is reducing sugar present.

C) Test for Glycosides:

a) Killer Kililani Test:

Add glacial acetic acid, one drop of 59% FeCl₃, and conc. HSO₄ to two milliliters of extract. The presence of glycosides is indicated by the formation of a reddish-brown color at the intersection of the two liquid layers and by the upper layer's appearance of bluish green.

D) Test for Saponin:

a) Foam Test:

Shake the dry powder or medication extract with water ferociously. The presence of saponin is indicated by persistent, stable foam.

E) Test for Phenol:

a) 5% FeCl₃ Solution:

Add a few drops of 3% ammonia solution to 2-3 ml of the extract to produce a strong blue-black hue that shows the presence of phenol.

F) Test for Flavonoids:

a) Sulphuric acid test:

The presence of flavonoids is shown by the addition of sulphuric acid to the extract, which causes flavones and flavonols to dissolve into it and produce a bright yellow solution.

G) Test for Protein:

a) Biuret Test (General Test):

Add 4% NaOH and a few drops of 1% CuSO₄ solution to 3 ml of T.S. The colour violet or pink occurs.

(b) Millon's test (for proteins):

Combine 5 ml of Millon's reagent with 3 ml of T.S. ppt in white. Warm PowerPoint slides turn brick red, or they dissolve and produce a reddish-colored solution.

H) Test for steroid:

a) Salkowski reaction:

Add 2 ml of chloroform and 2 ml of concentrated H₂SO₄ to 2 ml of extract. Shake firmly. Acid layer exhibits fluorescence that is greenish yellow whereas the chloroform layer appears red.

I) Test for Tannin:

a) 5% FeCl₃ solution:

Add a few drops of 3% ammonia solution to 2-3 ml of the extract to produce a strong blue-black hue that shows the presence of tannin. (13)

2.5 Thin Layer Chromatography Analysis:

Analyses using thin layer chromatography were performed in accordance with the procedure outlined by Harborne (1998). Glass plates were covered in silica gel G and let to dry before being activated in a hot air oven at 110°C for 10 min. The plates were then utilized as the stationary phase. On the TLC plate, Spot was put. Plates were air dried after TLC development using the mobile phase Chloroform: Methanol: Ethyl Acetate (3:7:6). The retention factor (RF) values of the active chemical, which were calculated as (Fig.2):

RF = Distance travelled by solute/Distance travelled by solvent front

2.6 Column Chromatography:

Solvents were utilized as the mobile phase in column chromatography, with silica gel F254 (60–120 mesh) serving as the stationary phase. Silica gel was used to treat the hydro alcoholic extract of *Bryophyllum pinnatum* (Lam.). The fractions were obtained using an elution system consisting of chloroform, methanol, and ethyl acetate (3:7:6). By using TLC and GCHRMS, isolated column fractions were discovered.[13] (Fig 3, d). TLC of collected fractions were done (figure) and RF value shown in Table 3.

2.7 TLC of Column chromatography fractions:

1)	Sample	Column chromatography fractions
2)	Mobile phase	Chloroform:Methanol:Ethyl acetate (3:7:6)
3)	Adsorbent	Silica gel-G
4)	UV light	366nm

2.8 GCHRMS Analysis:

By using GCHRMS analysis, the hydroalcoholic stem extract of *Bryophyllum pinnatum* (Lam.) was chemically profiled. The Sophisticated Analytical Instrument Facility (SAIF) Office, Indian Institute of Technology Bombay, Powai, Mumbai, conducted the GCHRMS analysis.

2.9 Ethylene glycol-induced urolithiasis model in rats:

The Development of Renal Calculi

The previously reported procedures were used to provide ethylene glycol (EG) in drinking water ad libitum for 28 consecutive days in order to generate renal calculi.[14]

Experimental strategy

- Six groups of rats each were given an injection of EG (0.75% in drinking water) daily for 28 days.
- Group I served as the standard control and received nothing but drinking water.
- Group II functioned as the lithiatic control and received the extracts in that group.
- Cystone (750 mg/kg), a conventional medication, was also administered to Group III. [15]
- Co-treatment of Group IV with Aqueous at doses of 200 mg/kg body weight
- Groups V received concurrent Ethyl acetate treatments at dosages of 200 mg/kg body weight.
- Group VI received Hydroalcoholic extract treatment at dosages of 200 mg/kg body weight.
- Every dose was made in distilled water with 1% tween 80 used as a suspending agent, and it was given daily for 28 days. Each rat's weekly body weight during the experiment was noted. On the 29th day, the kidney tissues' histology, oxidative stress parameters, and urine and serum biochemical values were assessed.

Group	Group Type	Experimental Design
I	Normal Control	Received normal water
II	Urolithiatic Control	Urolithiasis was induced by administrating 0.75% of ethylene glycol in drinking water
III	Standard	Urolithiatic rats received cystone (750mg/kg)
IV	Test	Urolithiatic rats received Aqueous extract (200mg/kg)
V	Test	Urolithiatic rats received Ethyl Acetate extract (200mg/kg)
VI	Test	Urolithiatic rats received Hydro alcoholic extract (200mg/kg)

Body Weight:

Each rat's body weight was noted twice during the experimental period—once before the therapy and once each week while it was being administered. Each animal's weekly percentage change in body weight was determined as follows:

$$\% \text{Change in BW} = \frac{\Delta \text{BW(g)}}{\text{IB(g)}} \times 100$$

Where IB is the rat's initial body weight at the start of therapy, and BW is the difference in body weight at one time interval. (Table 4)

Urine Biochemical Parameter:

On the 28th day of the experiment, each animal was housed separately in a metabolic cage with free access to water. 24 hour urine samples were gathered. The amount of urine produced and its pH were measured using Urodip pH strips from ERBA diagnostics Mannheim, GmbH, Germany. The urine was mixed with a drop of concentrated HCl before being chilled to -20°C. Using commercially available biochemical assays, the elements uric acid, calcium, phosphorus, magnesium, and creatinine were determined in urine. Oxalate was calculated using spectrophotometry.[16] The following formula was used to compute creatinine clearance (ml/min):[17] (Table 5,6,7,8,9,10)

$$\text{Creatinine clearance (ml / min)} \\ = \frac{(\text{mg creatinine / dl urine}) \times (\text{ml urine / 24h})}{(\text{mg creatinine / dl serum}) \times 1440}$$

Histopathology of harvested kidney section and staining method:

The kidneys of the rats were removed after sacrifice. 0.1 M phosphate buffered saline (pH 7.4) was used to thoroughly cleanse the left kidney. To make a 10% w/v suspension, it was blotted dry and homogenised in 1.15% KCl. At 0°C, a cooling centrifuge was used to centrifuge this suspension at 16000 g. The resulting supernatant was used to estimate levels of superoxide dismutase (SOD) (31), catalase (CAT)(30), glutathione (GSH) (29), malondialdehyde (MDA) (28), and glutathione (MDA) in addition to other compounds. Using the Biuret method, the supernatant's protein concentration was calculated.MDA and GSH were expressed in terms of nmol/mg protein, whilst SOD and CAT were expressed in terms of U/mg protein (49). The right kidney of each animal was stored in 10% neutralised formalin for additional histological research in order to validate the prevalence of lithiasis. (Fig 4)

2.10 Statistical Analysis:

The statistical analysis was carried out by one way ANOVA followed by Tukey's post hoc test, and a value of P <0.05 was regarded as significant in all cases. The data were expressed as mean ± SEM (n = 6).

3. RESULTS

3.1 Authentication and Collection of plant material

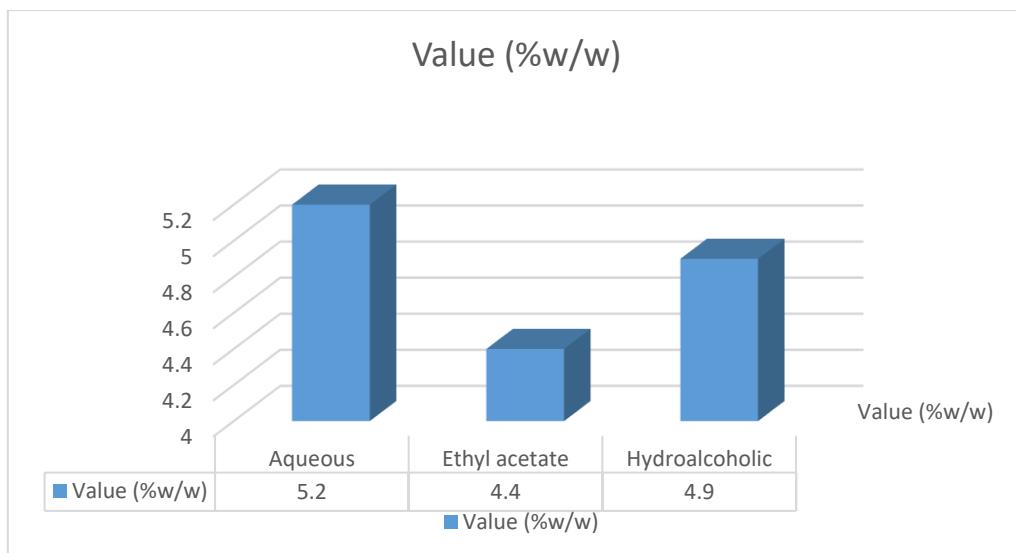


Fig: 1 Authentication Certificate of *Bryophyllum pinnatum*

3.2 Extraction of Plant material:

Sr. No.	Extraction yield	Value (%w/w)
1	Aqueous	5.2
2	Ethyl acetate	4.4
3.	Hydroalcoholic	4.9

Table 1: Extraction yield



3.3 Preliminary phytochemical Screening:

Sr. No.	Test	Stem extract in		
		Aqueous	Ethyl acetate	Hydroalcoholic
1.	Alkaloids	+	+	+
2.	Carbohydrates	+	+	+
3.	Glycosides	+	-	+
4.	Saponins	+	+	+
5.	Phenol	-	+	+
6.	Flavonoids	+	+	+
7.	Proteins	-	-	-
8.	Steroids	+	+	+
9.	Tannin	-	+	+

Table 2: Preliminary Phytochemical screening test



Fig. (a) Preliminary test of Aqueous



Fig. (b) Preliminary test of Ethyl acetate



Fig © Preliminary test of Hydroalcoholic extract

3.4 Thin Layer chromatography analysis:



Fig. (2) Thin layer chromatography

3.5 Column Chromatography:



Fig. (3) Column Chromatography



Fig. (d) Fractions collected

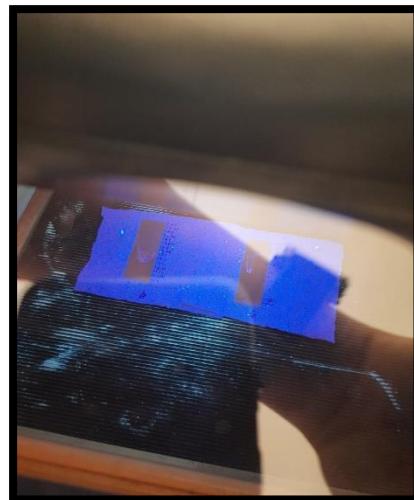


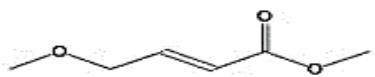
Fig. (e): TLC of Collected

Sample	Rf value
Sample A	0.5
Sample B	0.57

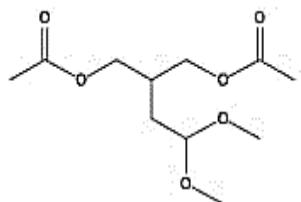
Table 3: RF Value of collected fractions

3.6 GCHRMS Analysis:

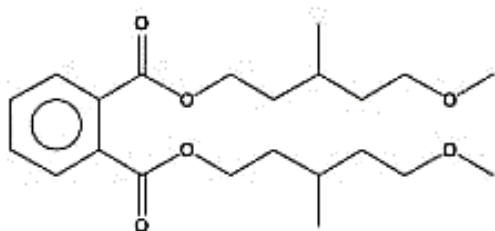
1. 2-butanoic acid, 4-methoxy, methyl ester
Common Name- Terpenoid



2. Acetate 2-[acetyl(oxy)methyl]-4,4-dimethoxybutyl ester



3. Pthalic acid:



3.7 Experimental effect :

Effect on Body Weight:

In the lithiatic control group, EG administration resulted in a considerable ($P < 0.01$) body weight loss when compared to the normal control group. When compared to the lithiatic control group, treatment with cystone or Aqueous E and Ethyl acetate Extract and Hydro alcoholic E (200mg/kg) considerably reduced the reduction in body weight of the animals. [Table 4]

Effect of *Bryophyllum pinnatum* leaf extracts on the body weight of rats against ethylene glycol-induced renal calculi:

Group	Treatment	Dose mg/kg	Body weight	Change in Body weight		
				Day 0	Day 7	Day 14
I	Normal Control	-	121.34±0.11	124.12±0.11	130.17±0.34	119.82±0.29*
II	Urolithiatic Control	-	132.12±0.40	134.13±0.98	140.17±0.12	125.62±0.11
III	Standard	750 mg/kg	119.18±0.90	121.11±0.99	130.17±0.18	120.73±0.22**
IV	Aqueous	200 mg/kg	117.10±0.45	124.16±0.23	130.17±0.10	125.26±0.31
V	Ethyl acetate	200 mg/kg	141.25±0.50	144.14±0.21	148.17±0.25	132.23±0.50***
VI	Hydroalcoholic	200 mg/kg	127.11±0.24	134.12±0.32	130.17±0.11	123.22±0.38

Table 4: effect on Body Weight

Values are expressed as Mean ± SEM (n=6). P values: ***P<0.01 (Highly Significant)

Effect on Urine Parameter:

When compared to the normal control group, the lithiatic control group's urine volume and pH decreased significantly ($p < 0.01$). Comparing the cystone-treated group to the lithiatic control group, a substantial ($p < 0.01$) rise in urine volume and pH was observed. Aqueous E or Ethyl acetate Extract or HalcE therapy at doses of 200 mg/kg resulted in a significant rise in urine volume and pH ($p < 0.01$ to $p < 0.01$, as appropriate). When compared to the lithiatic control group, Aqueous E (200 mg/kg) significantly increased urine volume ($p < 0.01$) without significantly altering pH, but HalcE (200 mg/kg) significantly increased urine volume ($p < 0.01$) and pH ($p < 0.01$).

Urine pH

Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0	7.14±0.23	8.13±0.67	9.19±0.21	3.83±0.42	6.30±0.51	8.21±0.34**

7	8.12±0.67	9.41±0.71	8.71±0.10	3.74±0.11	5.70±0.16	7.51±0.92***
14	9.41±0.34	10.46±0.45	9.70±0.59	1.12±0.37	3.21±0.45	7.48±0.51
21	8.56±0.59	9.56±0.21	7.16±0.32	2.81±0.46	4.90±0.51	5.20±0.12*

Table 5: Effect on Urine pH

Values are expressed as Mean ± SEM (n=6). P values: ***P<0.01 (Highly Significant)

Calcium

Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0	7.21±1.09	7.43±0.34	8.1±0.12	2.12±0.41	4.21±0.71	6.11±0.82*
7	8.1±0.12	8.21±0.17	7.56±0.61	3.41±0.54	5.12±0.40	7.71±0.20***
14	7.41±0.54	11.4±0.62	11.5±0.70	4.32±0.10	5.76±0.16	9.1±0.45**

Table 6: Effect on Calcium level

Values are expressed as Mean ± SEM (n=6). P values: ***P<0.01 (Highly Significant)

Oxalate

Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0	9.11±0.51	8.51±0.31	9.11±0.41	5.31±0.60	7.11±0.28	8.31±0.42**
7	7.12±0.41	7.31±0.27	10.61±0.51	6.81±0.56	9.41±0.41	10.11±0.67***
14	8.31±0.85	9.12±0.32	8.91±0.65	4.81±0.42	6.13±0.70	7.61±0.11*
21	7.61±0.60	8.71±0.31	7.11±0.61	1.41±0.49	3.21±0.78	5.21±0.91

Table 7: Effect on Oxalate level

Values are expressed as Mean ± SEM (n=6). P values: ***P<0.01 (Highly Significant)

Uric acid

Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0	8.18±0.12	7.12±0.12	9.51±0.43	5.81±0.95	6.21±0.11	8.31±0.17
7	9.91±0.17	9.51±0.19	8.21±0.61	4.51±0.21	5.71±0.43	7.21±0.51
14	7.81±0.12	8.61±0.62	7.7±0.51	2.4±0.41	4.61±0.27	6.71±0.12
21	8.41±0.41	9.65±0.31	8.21±0.51	3.80±0.60	5.41±0.71	7.61±0.20

Table 8: Effect on Uric acid level

Values are expressed as Mean ± SEM (n=6). P values: ***P<0.01 (Highly Significant)

Magnesium

Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0	9.11±0.12	8.30±0.54	9.12±0.43	4.13±0.23	6.60±0.67	8.10±0.45***
7	7.71±0.98	7.20±0.43	8.21±0.67	3.63±0.62	5.19±0.19	7.23±0.15**
14	8.7±0.53	9.75±0.72	9.50±0.61	5.15±0.68	6.41±0.12	7.41±0.59*
21	7.12±0.18	8.53±0.14	10.21±0.26	6.12±0.51	7.81±90	8.31±0.37

Table 9: Effect on Magnesium level

Values are expressed as Mean ± SEM (n=6). P values: ***P<0.01 (Highly Significant)

Phosphorus

Days	Group I	Group II	Group III	Group IV	Group V	Group VI
0	7.41±0.52	8.31±0.42	9.51±0.81	5.12±0.50	7.17±0.72	8.11±0.43**
7	8.30±0.12	7.6±0.21	8.75±0.41	4.91±0.80	6.11±0.21	7.90±0.44***
14	7.60±0.45	8.31±0.62	9.40±0.73	3.70±0.21	7.21±0.90	8.10±0.63*
21	9.20±0.42	9.70±0.38	7.55±0.75	3.21±0.38	5.19±0.79	6.30±0.54

Table 10: Effect on Phosphorus level

Values are expressed as Mean ± SEM (n=6). P values: ***P<0.01 (Highly Significant)

3.8 Effect on histopathology of kidney:

Fig 4: Demonstrates example photomicrographs of kidney microscopic findings. In the normal group of rats, there were no histological alterations in the renal tubules, glomeruli, or blood vessels [Figure 4a]. The EG-induced lithiatic group demonstrated CaOx crystals in tubule lumen along with inflammation and cast formation, which results in tubule and blood vessel dilatation. The occurrence of crystals in interstitial spaces along with mild to severe glomerular congestion and tubular degeneration was also seen in the lithiatic group [Figure 4b]. When compared to the lithiatic control group, the cystone [Figure 4c], Aqueous Extract [Figure 4d], Ethyl acetate Extract [Figure 4e], and HAlcE [Figure 4f] treated groups showed very little to no crystal deposition and changes in the architecture of the kidney/s.

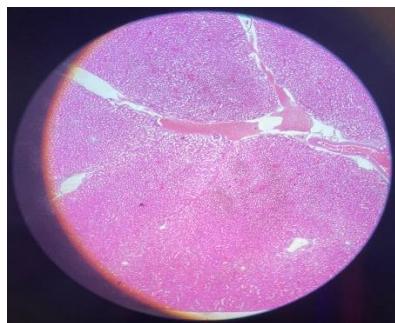


Fig 4 (a)



Fig 4 (b)

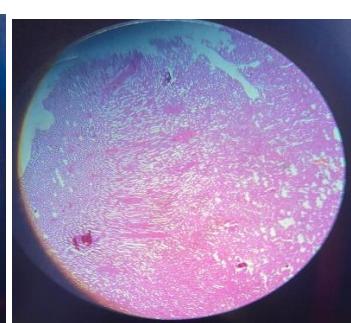


Fig 4 (c)

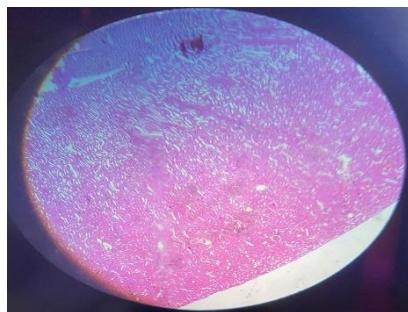


Fig 4 (d)

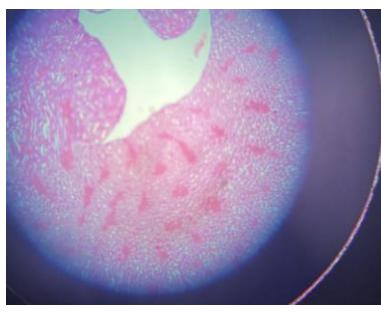


Fig 4 (e)

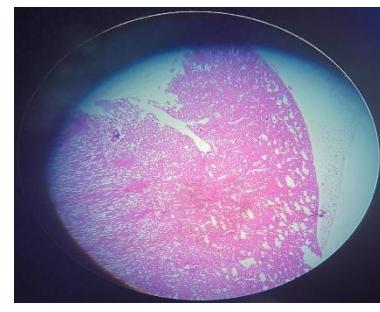


Fig 4 (f)

Effect of *Bryophyllum pinnatum* leaf extract on histology of kidney. 4(a) normal control showing normal cellular structure, 4(b) lithiatic control, showing CaOx crystal indicated by arrow with moderate to marked cellular degeneration, 4(c) cystone treated showed no crystal deposits and nearly normal renal architecture, 4(d) Aqueous Extract 200mg/kg treated show very few crystals, 4(e) Ethyl acetate extract 200 mg/kg treated showed very few to no crystal deposits, 4(f) HAlc Extract 200 mg/kg treated show no crystal deposition and normal structure.

4. DISCUSSION:

In Indian traditional medical systems, *Bryophyllum pinnatum* is referred to as Pattharcatta, which suggests that it has stone-crushing properties. For the treatment of stones, tribal and other populations frequently use the plant's leaves. Despite its extensive historical use, there have only been a few scientific studies to identify its antilithiatic effect. The present study was attempted to substantiate the ethnomedicinal use of leaves of *Bryophyllum pinnatum* in kidney stones and urine insufficiency in traditional practices. By halting EG-induced changes in body weight, urine and serum biochemical parameters, oxidative stress, and kidney histology, the current study demonstrated the beneficial effects of alcoholic and hydro-alcoholic extracts of *Bryophyllum pinnatum*.

The lithiatic control group's lower body weight is a sign of EG toxicity, which led to oxalate deposition in intracellular spaces, which led to metabolic disruptions and cellular damage.[21,22] Treatment with Aqueous E, Ethyl acetate E and HAlcE stopped the weight loss brought on by EG.

CaOx crystal formation and retention were the causes of the reduced urinary output in lithiatic control rats. Formation of CaOx crystals causes reduction in glomerular filtration rate, which further lowers excretion of Na⁺, Cl⁻ and Ca⁺², and promotes stone formation.[18,23] Treatment with Aqueous E, Ethyl acetate extract and HAlcE led to more frequent urination and avoided the supersaturation of salts that produce kidney stones in the urinary system. In lithiatic rats, higher quantities of uric acid, phosphorus, calcium, and oxalate were found in the urine. Since hyperoxaluria is recognised as a higher risk factor for the development of renal calculi than hypercalciuria, the urine oxalate levels are relatively more significant than those of calcium.[24] The solubility of calcium oxalate is also affected by increasing uric acid levels in urine, and it binds to and weakens the effect of glycosaminoglycans, an endogenous substance that prevents the formation of stones.[18] The levels of these stone-forming compounds in urine were reduced by AlcE and HAlcE. The results of the present study are supported by earlier research using aqueous extract of the plant, which demonstrated an inhibitory effect on urinary oxalate.[25] Creatinine accumulation in the blood, which further raises the risk of urolithiasis, is indicated by the decline in creatinine clearance and urine magnesium levels. Treatment with AlcE and HAlcE increased urinary magnesium concentration and creatinine clearance while lowering crystallisation propensity.

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Extensive CaOx crystal deposition and cellular damage are brought on by EG therapy in several renal organs of rats, together with oxidative damage. [27] In lithiatic control rats, microscopic examination of kidney sections revealed CaOx crystals in the tubular and interstitial regions along with glomerular congestion and tubular necrosis. Treatment with cystone, Aqueous E, Ethyl acetate extract and HAICe inhibited the deposition of CaOx crystals in several renal tubule locations, maybe by accelerating the breakdown of existing stones or by halting the growth of new crystals. The results are supported by an aqueous extract of the plant that demonstrated CaOx crystal deposition prevention. [25] Large CaOx crystals could prevent renal tubular flow, resulting in glomerular congestion and tubular degeneration. By preventing the buildup and retention of CaOx crystals in the renal tubules, Aqueous E, Ethyl acetate extract and HAICe reduced the risk of renal injury. These effects of extracts point to their capacity to dissolve stones. Bryophyllum pinnatum extract has previously demonstrated an in-vitro anti-crystallization action, supporting the present findings.[20] According to reports, plants' saponin concentration enhances their capacity as diuretics and stone dissolvers.[26] Research indicated that plants' antioxidant activities also had a significant impact in their ability to operate as an antilithiatic.[19] Quantitative analyses of Bryophyllum pinnatum extracts in the current study reveal the presence of saponins and antioxidant phytochemicals including flavonoids and polyphenols that may have contributed to antilithiatic activity by stone dissolving research is required.

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