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EVALUATION PHARMACOLOGICAL STUDY OF *BRYOPHYLLUM PINNATUM* PLANT FOR ANTILITHIATIC ACTIVITY

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ABSTRACT: The present investigation was directed to assess the counter lithiasis action of ethanolic concentrates of entire plant of *Bryophyllum pinnatum*. Urolithiasis is an infection brought about by the nearness and impacts of uroliths (stones) or calculi or exorbitant measures of precious stones in the urinary tract. The illness is alluded to by numerous names, including cystitis, urethritis, urinary calculi, bladder stones, or kidney stone. Ethanolic concentrate of the plant of *Bryophyllum pinnatum* was exposed to antilithiatic action in rodents where Ethylene glycol (0.75 (v/v)) utilized as the causing lithiasis specialist. A stamped ascend in calcium, oxalates, phosphorus, and creatinine in blood serum level saw in lithiasis (100 and 200 mg/kg) displayed a portion subordinate critical enemy of lithiasis action on treatment. The concentrate portion of 300 mg/kg likewise caused fractional instruction of calcium, oxalates, phosphorus, and creatinine in blood serum level the outcomes were discovered factual irrelevant. The antilithiatic impact of ethanol separate was discovered compelling with that reference standard.

INTRODUCTION: Medicinal plants have been part and parcel of human society to combat diseases since the dawn of human civilization. The earliest description of curative properties of medicinal plants was described in the Rigveda (2500-1800 BC), Charak Samhita and Sushruta Samhita¹. India is a mother hub for the development of Ayurveda, Unani, Siddha; Homoeopathy and other natural herbs based health science (Ayush)². All over the world especially in developing countries, approximately 80% of the population continues to use traditional medicine in primary medical problems.

In the past decade, therefore, research has been focused on scientific evaluation of traditional drugs of plant origin. There is an urgent need to systematically evaluate the plants used in traditional medicine¹⁰. Urolithiasis is the process of forming a stone in the kidney or the urinary tract. The development of the stones is related to decreased urine volume or increased excretion of stone-forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate^{11, 12, 13}.

Plant Profile: *Bryophyllum pinnatum*, (Family: Crassulaceae) also known as Miracle Leaf, Katakataka, Life Plant and Pashan Bheda / Patharchur, is a succulent perennial herb, which grows up to 1m - 2m in height. The plant grows in the tropical, sub-tropical and warm temperate 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.

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This is a branched aromatic herb. The nodes are often hairy in some species. It is grown in the garden for ornamental purposes.

Taxonomical Classification: ³

Kingdom: Plantae
 Subkingdom: Tracheobionta
 Division: Spermatophyta
 Subdivision: Magnoliophyta
 Class: Mangnoliopsida
 Subclass: Rosidae
 Order: Saxifragales
 Family: Crassulaceae
 Genus: *Bryophyllum*
 Species: *pinnatum*

Synonyms: *Bryophyllum calycinum* Salisb.
Cotyledon pinnata Lam. *Crassula pinnata*,
Kalanchoe pinnata, *Sedum madagascariicum*.



FIG. 1: PLANT OF *BRYOPHYLLUM PINNATUM*

The leaves of panfuti have great medicine; value and are used for medicinal purpose both, internally as well externally. The leaves possess various properties like hemostatic, refrigerant, emollient, mucilaginous, vulnerary, depurative, anti-inflammatory, disinfectant and tonic. They are useful in vitiated condition of vata and pitta, cuts and wounds, hemorrhoids, menorrhagia, discoloration of the skin, boils, sloughing ulcers, ophthalmic, burns, scalds, corn, diarrhea, dysentery, vomiting and acute inflammations ⁴.

Externally, the pulp of the leaves or the juice is applied to traumatic injuries to arrest the bleeding and promote the healing of wounds. The juices of its leaves contract the minute arterioles and arrest

bleeding which may be externally or internally. On traumatic wounds, the heated leaves of panfuti are crushed applied. It reduces the edema and promotes the wound healing without leaving a scar. This miraculous haemostatic property of panfuti needs further scientific evaluation. Internally, the leaves juice and cumin seeds are given along with the double amount of ghee in dysentery. It arrests the intestine ⁵. Panfuti is highly recommended in raktapitta bleeding disorders, piles and menorrhagia. The rhizomes of this plant can be used as a preventing of the kidney stone or for the treatment of this. *Bryophyllum pinnatum* is given for the treatment of a cough, asthma, cold with candy sugar ⁶.

MATERIALS AND METHODS:

Plant Material: The whole plant was collected from the local park (Narayan Bagh) of Jhansi in November 2015. The plant was identified by local people of that park and authenticated by Dr. Rama Shanker National Vrکشayurveda Research Institute Gwalior Road Jhansi Uttar Pradesh. A herbarium specimen of the plant 826 was preserved in the Department of Pharmacognosy of our Institute for further reference.

Extraction of Plant Materials: A total of 250 g coarse powder of air-dried leaves of *Bryophyllum pinnatum* were packed in a muslin cloth and subjected to Soxhlet extractor for continuous hot extraction with petroleum ether and ethanol for 8 h separately. Then each extract was filtered, and the filtrate was evaporated to dryness. The percentage yield of the ethanol extracts was 8.768.

Methods:

Chemical (Drugs & Solutions) and Apparatus: All the chemicals were analytical grade. Cystone was obtained from Himalaya Health Care Ltd. The ethanolic extract of plant *Bryophyllum pinnatum* was given with tween 80 as vehicle administered orally. All chemicals were used of analytical grade available; ethylene glycol was obtained from Merck Ltd., Mumbai, India.

Animals Selection: Male Albino rats of wistar strain weighing between 150-200 gm were selected for the anti-urolithiasis activity. Animals were housed in polypropylene cages with the filter tops and maintained at 25 ± 2 °C; relative humidity $55 \pm$

10% under controlled conditions of 12-h light: 12-h dark cycle. The animals were fed up with commercial rat chow and were given water *ad libitum*. All protocols of the study were approved by the Institutional Animal Ethical Committee with reference number BU/PHARM/IAEC/a/17/02. The IAEC is approved by the committee for control and supervision of experiments on animals (CPCSEA) with registration number 716/02/a/ CPCSEA.

Induction of Experimental Urolithiasis: Ethylene glycol (0.75%) induced hyperoxaluria model was used to induced stone in rats ⁷.

Preparation of Dose: The dose of 100 mg/kg, 200 mg/kg, and 300 mg/kg of ethanolic extract were selected for the test. All the doses were given orally after making a suspension in the vehicle, *i.e.* 1% tween 80 and the standard drug, *i.e.* Cystone was given orally (750 mg/kg) in vehicle ⁸.

Animal Groups: Twenty four rats were divided into 6 groups comprising four animals per groups.

Group 1: Normal, *ad libitum* access to regular food and drinking water administered. Group 2, 3, 4, 5 and 6: *ad libitum* access to regular food and *ad libitum* access to drinking water containing 0.75% (v/v) ethylene glycol (EG) to promote hyperoxaluria and CaoX deposition in the kidneys.

Group 2: Ethylene glycol 0.75% (v/v)

Group 3: Standard drug (Cystone 750 mg/kg).

Group 4: Ethanolic extract of *Bryophyllum pinnatum* (100 mg/kg).

Group 5: Ethanolic extract of *Bryophyllum pinnatum* (200 mg/kg).

Group 6: Ethanolic extract of *Bryophyllum pinnatum* (300 mg/kg).

Design of Work: The rats were housed in cages and divided into 6 groups of four animals each. Group 1 served as control and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75%) in drinking water was fed to group 2 to 6 for induction of renal calculi till 28th day. Group 2 receive only Ethylene glycol 0.75% (v/v), Group 3 receive the standard antilithiatic drug, cystone (750 mg/kg) from the 15th day till 28th day. Group 4 to 6 received as a curative regimen, group 4 received ethanolic extract 100 mg/kg, group 5 received

ethanolic extract 200 mg/kg, and group 6 received ethanolic extract 300 mg/kg from the 15th day till 28th day ⁹.

Assessment of Anti-urolithiasis Activity:

Collection and Analysis of Urine: All animals were kept in individual metabolic cages, and urine samples of 24 h were collected on the 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 40 °C. Urine was centrifuged, and the crystals of urine were analyzed under an optical microscope at 10x to 40x resolution. Urine was analyzed for calcium oxalate and phosphate content.

Serum Analysis: The blood was collected from the retro-orbital sinus under the anaesthetic condition, and serum was separated by centrifugation at 10,000 g for 10 min and analyzed for creatinine and uric acid. The creatinine kit (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kit (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid levels respectively.

Kidney Histopathology and Homogenate

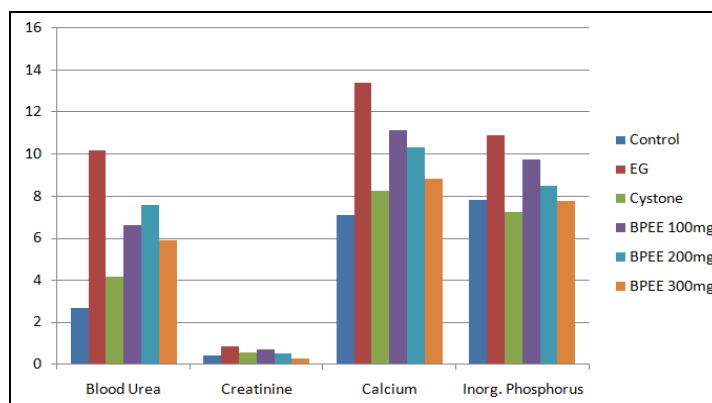
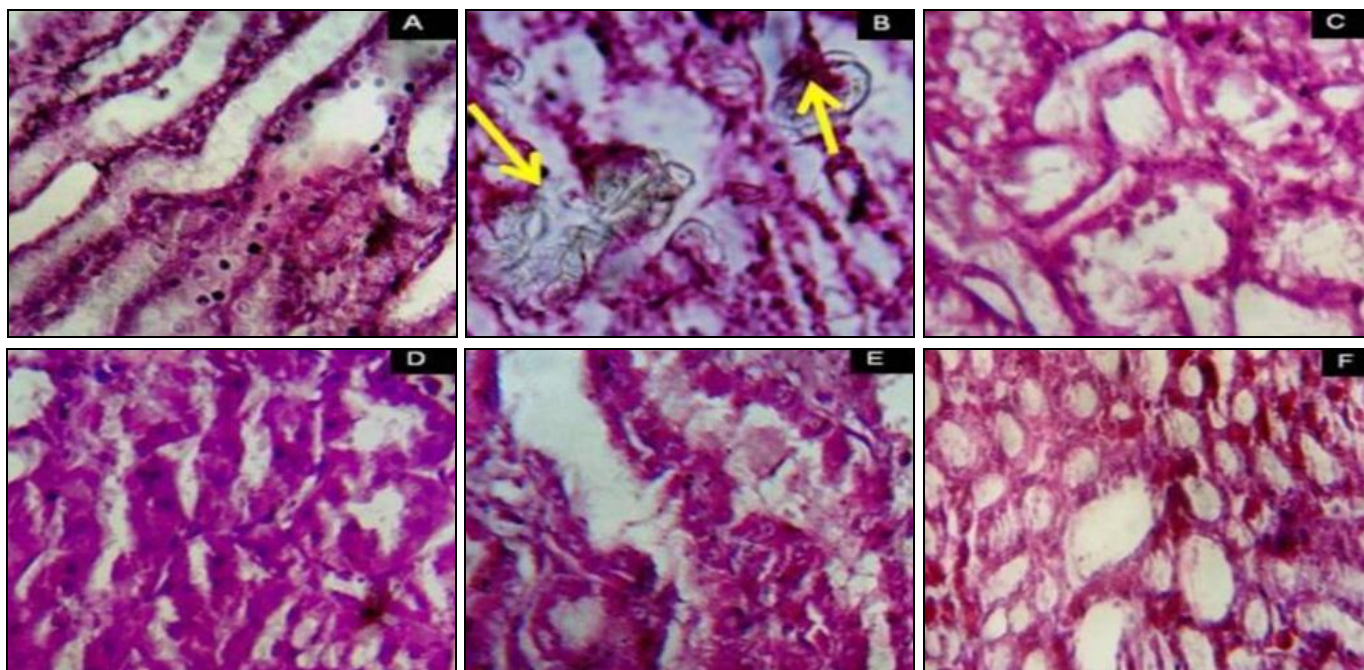
Analysis: The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and rinsed in ice-cold physiological saline. The right kidney was fixed in 10% neutral buffered formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 μ m and stained with H and E (Haematoxylin and Eosin) for histopathological examination.

The slides were examined under a light microscope to study the light microscopic architecture of the kidney and calcium oxalate deposits. The left kidney was finely minced, and 20% homogenate was prepared in Tris-HCl buffer (0.02 mol/l, pH 7.4). Total kidney homogenate was used for assaying tissue calcium, oxalate and lipid peroxidation activity.

Statistical Analysis: The results were expressed as mean \pm standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test and P<0.05 was considered significant.

RESULTS:**Serum Analysis:****TABLE 1: SERUM BIOCHEMICAL DATA**

Parameter Unit (mg/dl)	Group 1 Control	Group 2 (EG)	Group 3 Cystone	Curative regimen (BPEE)		
				Group 4 100 mg	Group 5 200 mg	Group 6 300 mg
Blood Urea	2.67±0.45	10.21±0.20	4.18±0.32	6.64±0.37	7.60±0.37	5.90±0.26
Creatinine	0.42±0.02	0.89±0.07	0.59±0.07	0.72±0.02	0.54±0.02	0.33±0.05
Calcium	7.13±0.5	13.39±0.28	8.28±0.34	11.15±0.10	10.35±0.33	8.86±0.17
Inorg. Phosphorus	7.84±0.06	10.91±0.20	7.27±0.46	9.79±0.29	8.54±0.20	7.80±0.27

**FIG. 2: GRAPHICAL REPRESENTATION OF ANTILITHIATIC ACTIVITY OF *BRYOPHYLLUM PINNATUM*.** n = 6, values are expressed as Mean ± SEM P<0.05 when compared with the control group.**Kidney Histopathology and Homogenate Analysis:****FIG. 3: MICROSCOPIC ARCHITECTURE AND CALCIUM OXALATE DEPOSITS IN THE KIDNEY SECTION.** Kidney sections of (A) Vehicle control (B) Urolithic (C) Cystone treated (D) Treatment with BPEE at the dose of 100 mg/kg (E) Treatment with BPEE at the dose of 200 mg/kg (F) Treatment with BPEE at the dose of 300 mg/kg.

DISCUSSION: In the present *in-vivo* study, renal stone inducing treatment to male Albino wistar rats resulted in hyperoxaluria. There were an increased oxalate, calcium, phosphate, creatinine and uric

acid excretion. However, supplementation with BPEE significantly prevented these changes in urinary oxalate, calcium, phosphate, creatinine, and uric acid excretion dose-dependently.

Ethanollic extract of *Bryophyllum pinnatum* was subjected to antilithiatic activity in rats where ethylene glycol (0.75% (v/v)) used as the causing lithiasis agent. A marked rise in calcium, oxalates, phosphorus, and creatinine in blood serum level observed in lithiasis compare standard to different curative doses of rats.

Ethanollic extract of *Bryophyllum pinnatum* (at 100 and 200 mg/kg) exhibited a dose-dependent significant antilithiatic activity on treatment. The extract dose of 100 mg/kg also caused reduction of calcium, oxalates, phosphorus, and creatinine in blood serum level the results were found statistically significant. The antilithiatic effect of ethanol extract was found significant than the reference standard.

CONCLUSION: The outcome demonstrated the nephroprotective impact of concentrate of *Bryophyllum pinnatum* in ethylene glycol actuated urolithiasis show. In this manner, *Bryophyllum pinnatum* may anticipate measurably noteworthy calcium oxalate precious stone testimony in the kidney by forestalling hyperoxaluria-initiated peroxidative harm to the renal cylindrical film surface (lipid peroxidation), which thus can avoid factually critical calcium oxalate gem connection and the resulting improvement of kidney stones.

Urinary science is one of the essential factors in deciding the sort of precious stone shaped and the idea of macromolecules included on the outside of the gems. Thus, the investigation of the urinary science identified with the calculi framing minerals will give a decent sign of the degree of stone arrangement. In this manner; the concentrate of *Bryophyllum pinnatum* is useful to keep the repeat of the illness as it demonstrated its impact on beginning times of stone improvement.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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