Assessment of Antiurolithiatic activity of *Bryophyllum Pinnatum* Leaves- *In-vitro*

Rahul Jodh[1]*, Mukund Tawar[1], Sudarshan Behere[1], Tanvi Raut[1], Swaraj Wankhade[1], Chaitali Thotange[1]

[1]Department of Pharmacology, P.R. Pote Patil College of Pharmacy, Amravati-444602, India.

ABSTRACT

The present study aimed to evaluate effects of *Bryophyllum Pinnatum* extract on calcium oxalate and calcium phosphate by in vitro methods. The leaves of *Bryophyllum Pinnatum* were sequentially extracted by using the hot maceration method, with a solvent such as ethanol, water and hydroalcoholic solution in various concentrations. The obtained extract was subjected to phytochemical screening and the test for alkaloid, flavonoid, saponin, phenol and triterpenoid saponin. For the in vitro study, experimentally calcium phosphate and calcium oxalate stones were prepared and compared with standard drug. Cystone is used as standard drugs. *Bryophyllum Pinnatum* is rich in phytochemicals such as alkaloids, saponin, glycoside, kamferol, and flavonoids and has a substantial capacity to dissolve calcium phosphate and calcium oxalate. These flavonoids inhibit calcium Phosphate and calcium oxalate deposits from forming in the renal tubules. The leaf extract contains anti-urolithiasis therapy and preventative capabilities and lowers the size of stones. In addition to diuretic and antiurolithic, antidiabetic, anticancer, anti-ulcer, anti-microbial, and wound healing activities, Kalanchoe pinnata leaf extract includes phenolic chemicals, tannin, and titerpenes. The main goal of the study is to find out how the Bryophyllum Pinnatum herb, especially its leaves, can prevent and treat health problems like renal stones, which are becoming more common in younger people because they don't exercise and eat poorly. The ability of the extract to get small particles out of the kidney and out of the urinary tract reduces the chance that they will get stuck in the urinary tract and form stones.

Key Words: *Herbal medicine, Kidney stone, Urinary calculi, Antiurolithiasis, Renal calculi*

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INTRODUCTION

One of the oldest and most common disorders is kidney stone production. People in various states of India use different herbs to treat urolithiasis. The urinary tract is thought to be the third most frequent ailment. The Urinary stone disease affects the biggest number of individuals worldwide, accounting for about 4–15 percent of the global population. Urinary stones are predicted to impact 12% of the population in India, with 50% of those affected suffering from kidney failure or injury. Urolithiasis is a multifactorial illness that is very unexpected and has a complicated etiology. A stone is an accumulation of urine solute components such as calcium, phosphate, uric acid, and oxalate. Calcium oxalate is determined to be the most common element of urolithiasis in India [1]. India's population suffers from urinary tract and kidney stones caused by calcium, phosphate, and oxalates deposits. These stones can last indefinitely, generating subsequent issues with catastrophic ramifications for the patient's life [2]. It is excruciatingly uncomfortable, and a correct remedy is required to resolve the issue. Depending on where they are detected, kidney stones are referred to as urinary calculi, urinary tract stone disease, renal calculi, ureterolithiasis nephrolithiasis, and urolithiasis. Appropriate and timely treatment of kidney stones can prevent major complications such as partial or complete renal function loss [3]. Urine is a chemical solution containing a range of chemical components, urinary tract stones are a common issue. These chemicals crystallize readily and expand in size to create stones. Because there is no adequate medical treatment for such stone diseases, Except for few composite herbal medications and plants, the rationale behind their usage in ancient systems of

Corresponding author: Rahul Jodh
Address: Department of Pharmacology, P.R. Pote Patil College of Pharmacy, Amravati-444602.
E-mail: ✉ jodhrahul@gmail.com
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medicine, including Ayurveda, has not been adequately established via rigours pharmacological and clinical investigations [4]. These plant components are said to be useful in lowering the reappearance rate of urinary stones. In indigenous medicine, the leaves of *Bryophyllum pinnatum* are used to cure a range of diseases, including urinary stones. There has been no scientific research published on the in vitro anti-urolithiatic efficacy of different extracts of *Bryophyllum pinnatum* leaves against calcium stones [5].

MATERIALS AND METHODS

Materials

Chemical

Drangendroff reagent, 1% Ferric chloride, 2% Lead acetate, Ethanol Rankem PVT. LTD. Distilled water purchased from Shree Ganesh scientific syndicate, Amravati, Maharashtra, India.

Collection of plant material and authentication

In February 2022, the leaves of *Bryophyllum pinnatum* were taken from the nursery in the Amravati in the state of Maharashtra and the plant is authenticated at P.R.Pote Patil College of Agriculture, Amravati (281/2021) dried in the shade and A botanist made sure that the plant was real. The dried leaves were ground up into a rough powder (passed through sieve no. 40). After complete drying, the dried leaves were extracted using hot maceration with solvent ethanol, distilled water, and hydroalcoholic solution in various concentrations.

Plant Profile

- Botanical Name: Kalanchoe Pinnata
- Scientific name: Kalanchoe sect. *Bryophyllum*
- Family: Crassulaceae
- Kingdom: Plantae [6].
- Subkingdom: Tracheobionta—Vascular plants
- Subclass: Rosidae
- Genus: Kalanchoe, *Bryophyllum*
- Species: Bryophyllum pinnatum [7].

Bryophyllum pinnatum gets its name from the Greek terms bryo, which means sprout, and phyllon, which means leaf, implying the ability to spread by leaf cutting. Pinnatum is derived from the Latin word pinna, which means winged or feathered [8]. The reddish-brown leaves of this species are thick, meaty, elliptical, curled, and have a crenate or serrated edge. The leaves are simple at the bottom of the stem and imparipinnate at the top. They have 3 to 5 pairs of fleshy lobes and are 10–30 cm (4–12 in) long [9]. The ability of the leaves to produce bulbs is incredible. Adventitious buds grow on the tooth's borders and produce roots, stalks, and leaves. Plantlets that fall to the ground grow roots and grow into larger plants [10].

Method

Extraction

The plant leaves were shade dried and powdered in a mixer grinder and stored in an airtight jar for study. The extraction was performed using the hot maceration technique according to the standard procedure [11]. The solvent used was ethanol and distilled water. First, the samples were divided into five groups for extraction, each containing a sample of 30g. The first sample was extracted with 200ml of pure ethanol, then in the second group, samples were extracted with pure distilled water and hydroalcoholic preparations were prepared for the remaining samples in different concentration ratios. The first was 70:30, which contained 140ml of ethanol and 60ml of water; the second was 50:50, which contained 100ml of ethanol and 100ml of distilled water; and the last one was 70:30, which contained 140ml of distilled water and 60ml of ethanol. Each extract was subsequently filtered, and the filtrate was evaporated. All five samples were assembled for hot maceration for 24 hours and after that, the filtrate was collected and kept for evaporation. On evaporation, the solid form was obtained as a product.

Evaluation of antiurolithiatic activity for bryophyllum pinnatum

Preparation of experimental kidney stone (calcium Phosphate, calcium oxalate)

In 100 ml distilled water, 1.47 gm calcium chloride dihydrate was dissolved, while 1.42 gm disodium hydrogen phosphate was dissolved in 100 ml of 2N H2SO4. To precipitate calcium phosphate, all were mixed evenly in a beaker with agitation. The calcium phosphate that resulted was washed with distilled water and dried for 2 hours. The calcium phosphate stone was then punched into tablets and served as stones in the study [2].

In 100 mL of distilled water, 2.94g of calcium chloride dihydrate was dissolved, while 2.84g of disodium hydrogen phosphate was dissolved in 100 mL of 2N H2SO4. To precipitate calcium phosphate, all were mixed evenly in a beaker with agitation. The calcium phosphate that resulted was washed with distilled water and dried for 2 hours. The synthesized calcium oxalate stone was then punched into tablets, which were used as stones in the study [12].

Tablet punching: by direct compression method

Calcium oxalate and calcium phosphate are shaped into spherical kidney stones by direct compression, per the usual method [13]. 160 mg of calcium oxalate and calcium phosphate are combined with the binder HPMC (Hydroxy

Propyl Methyl Cellulose) and then compressed in a tablet compression machine.

Figure 1. Calcium oxalate and Calcium Phosphate tablets

In vitro antiurolithiatic study

In vitro activity was assessed by changing the conventional procedure [2, 14, 15]. Weight fluctuation and dissolving of calcium phosphate and calcium oxalate tablets were carried out in this study. A pH 7.4 phosphate buffer was made, and accurately weighed calcium oxalate and calcium phosphate tablets were inserted in each extract solution (concentrations of 100 mg/ml and 200 mg/ml, respectively) and packed individually in the semipermeable membrane and sutured. A standard solution was also produced and utilized. The semipermeable membrane was allowed to float in the beaker independently. A negative control (perfectly weighted calcium phosphate and calcium oxalate tablet) was inserted in the semi-permeable membrane with 100 ml of normal saline solution in the same way. For 12 days, all of the beakers were exposed to a percent dissolution investigation. The weight loss of calcium phosphate and calcium oxalate tablets was measured in each bag at a two-week interval. The dissolving rate was calculated by multiplying the beginning and end weights of the tablets using the following formula:

$$\% \text{ Dissolution} = (W \text{ initial} - W \text{ final}) \, 100/W \text{ initial} \quad (1)$$

W – Before and after weights of tablets [16].

Phytochemical constituent

Standard techniques were used to test the extracts for the presence of active phytochemical components.

1. Phenols test: 1 ml of extract was mixed with 1% ferric chloride solution. The green colour showed that phenols were present.
2. Flavonoids test: 3-5 drops of a lead acetate solution with a concentration of 2 Percent were added to 1ml of alcoholic extract. The appearance of orange or yellow colour shows that flavonoid is there.
3. Alkaloid test: Alkaloids were detected by adding one drop of Dragandroff reagent to one millilitre of extract and looking for a yellow precipitate.
4. Triterpenoid Saponins Test: Test of Froth Formation In a test tube, when 1ml of extract was mixed with 1ml of water, foam formed [17].
5. Bendict's Test: The extract mixed with Bendict's reagent and boiled until a red colour showed. This showed that the extract contained carbohydrate.
6. Biuret Test: A few drops of a 5 percent Sodium Hydroxide solution and a 1 percent Copper Sulphate solution were added to 2-3 ml of the extraction. When there is protein, the colour will be violet or pink [18].

RESULTS AND DISCUSSION

Extraction yield: The extraction yield of all extracts in the solvent of different concentrations was different, as we carried out extraction in different solvents at different concentration ratios. All the extraction yield of all extracts of different solvents was in grams, and it was converted into percentages as shown in **Table 1**.

Table 1. Extraction yield of different solvent extracts.

Extracts	Percentage Yield
Pure water	90%
Pure ethanol	20%
70:30 HA	88%
30:70 HA	58%
50:50 HA	62%

From the sample of pure ethanol, we got a 20% yield, and from the sample of pure water and 70:30 HA, we got a high amount of yield, i.e., 90% and 88%.

Phytochemical screening

The result of phytochemical screening is given in **Table 2**. We performed phytochemical screening by standard procedure and we found the presence of bioactive compounds like alkaloid, phenol, flavonoids, carbohydrates, protein, triterpenoid saponin, and alkaloid.

Table 2. Result of Phytochemical Analysis of extracts.

Sr. No	Phytochemicals	Test	Result (Leaves)
01	Alkaloids	Dragandroff test	+
02	Flavonoids	Lead. Acetate test	+
03	Phenols	Ferric Chloride test	+
05	Carbohydrate	Benditc's test	+
06	Protien & Amino acid	Ninhydrin test	+
07	Triterpenoid Saponin	Froth formation test	+

[(+) = Present (-) = Absence]

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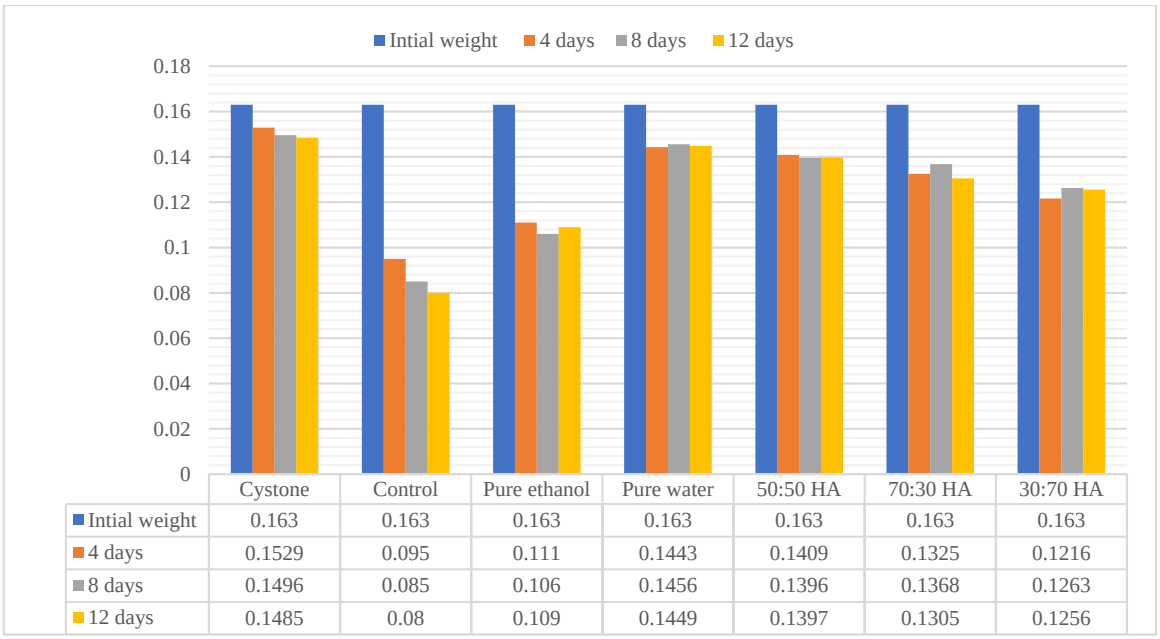
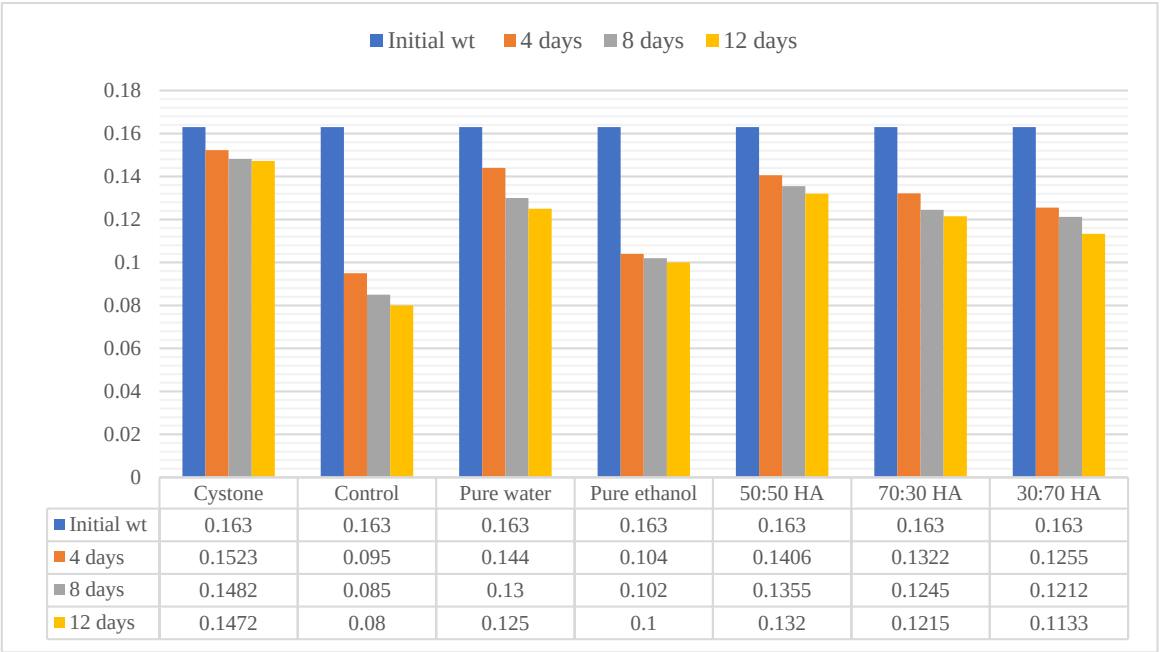
Base on this fraction, we compared the in vitro anti-urolithiatic activity of several Bryophyllum Pinnatum leaf extracts with the standard Cystone against calcium phosphate and calcium oxalate stones. The second table compares the dissolving of calcium phosphate tablets by Bryophyllum pinnatum extract with Cystone.



	Cystone	Control	Pure ethanol	Pure water	50:50 HA	70:30 HA	30:70 HA
Intial weight	0.163	0.163	0.163	0.163	0.163	0.163	0.163
4 days	0.1529	0.095	0.111	0.1443	0.1409	0.1325	0.1216
8 days	0.1496	0.085	0.106	0.1456	0.1396	0.1368	0.1263
12 days	0.1485	0.08	0.109	0.1449	0.1397	0.1305	0.1256

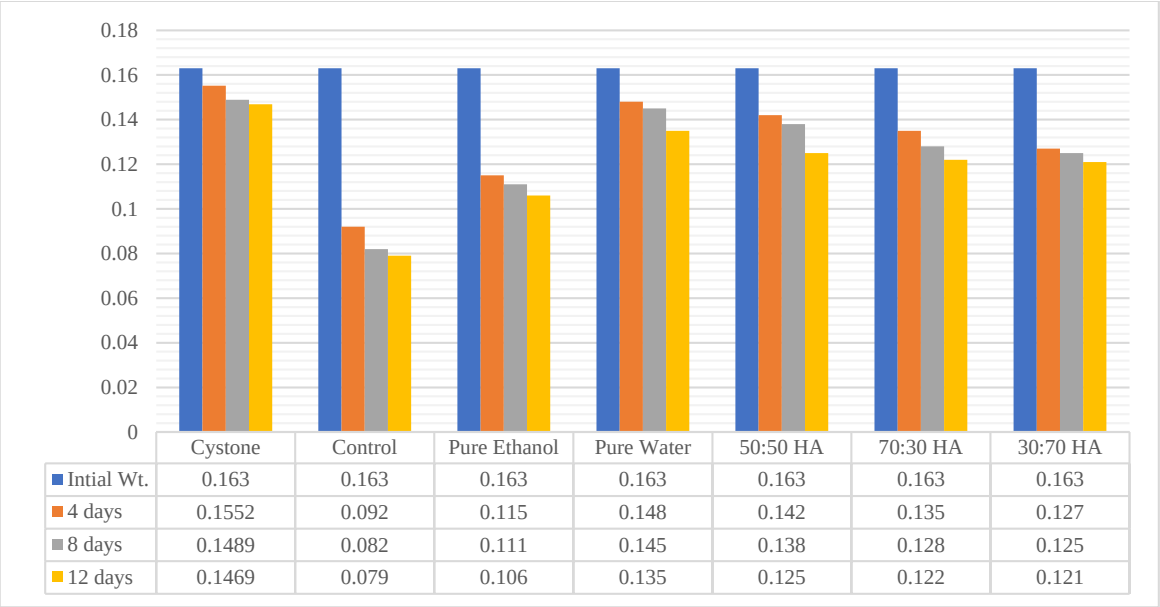
Figure 2. Percent Graphical representation of dissolution of calcium phosphate (100 mg)



	Cystone	Control	Pure water	Pure ethanol	50:50 HA	70:30 HA	30:70 HA
Initial wt	0.163	0.163	0.163	0.163	0.163	0.163	0.163
4 days	0.1523	0.095	0.144	0.104	0.1406	0.1322	0.1255
8 days	0.1482	0.085	0.13	0.102	0.1355	0.1245	0.1212
12 days	0.1472	0.08	0.125	0.1	0.132	0.1215	0.1133

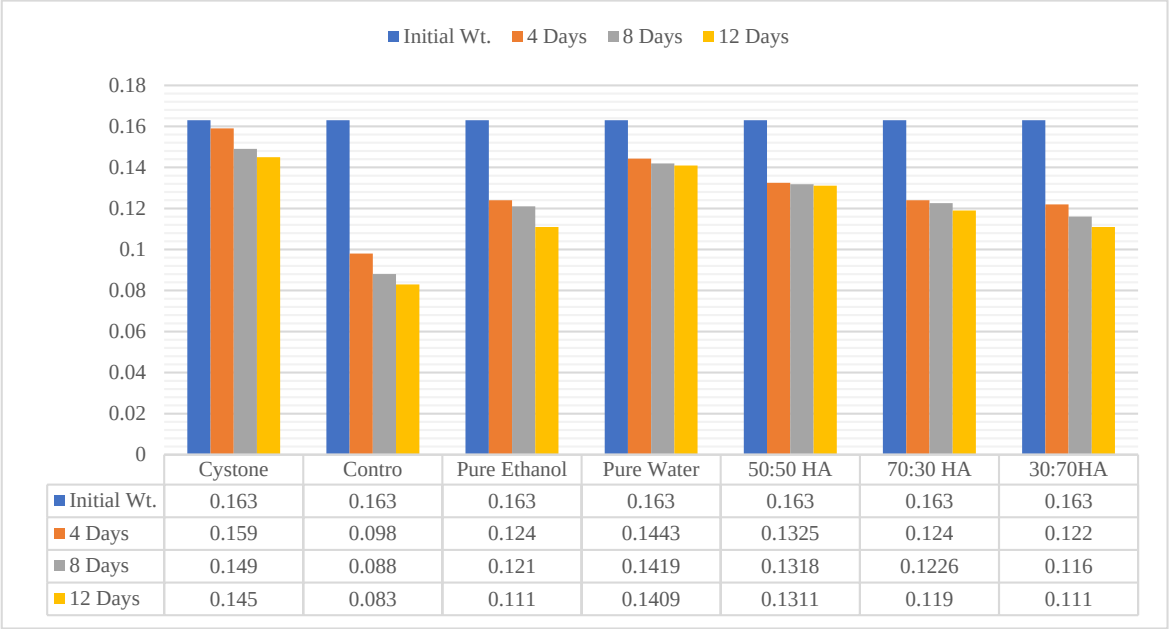
Figure 3. Percent Graphical representation of dissolution of calcium phosphate (200 mg)

Figure 3 that show how much calcium phosphate is dissolved by the in vitro anti-ulolithiatic activity of an extracted fraction of the Bryophyllum pinnatum drug. Compared to other fractions and the standard, pure water and hydroalcoholic solutions had a higher rate of dissolution. standard dissolves more quickly than the others.



	Cystone	Control	Pure Ethanol	Pure Water	50:50 HA	70:30 HA	30:70 HA
Intial Wt.	0.163	0.163	0.163	0.163	0.163	0.163	0.163
4 days	0.1552	0.092	0.115	0.148	0.142	0.135	0.127
8 days	0.1489	0.082	0.111	0.145	0.138	0.128	0.125
12 days	0.1469	0.079	0.106	0.135	0.125	0.122	0.121

Figure 4. Percent Graphical representation of dissolution of calcium oxalate (100 mg)



	Cystone	Contro	Pure Ethanol	Pure Water	50:50 HA	70:30 HA	30:70HA
Initial Wt.	0.163	0.163	0.163	0.163	0.163	0.163	0.163
4 Days	0.159	0.098	0.124	0.1443	0.1325	0.124	0.122
8 Days	0.149	0.088	0.121	0.1419	0.1318	0.1226	0.116
12 Days	0.145	0.083	0.111	0.1409	0.1311	0.119	0.111

Figure 5. Percent Graphical representation of calcium oxalate (200 mg)

Figures 4 and 5 show graphical representations of percent calcium oxalate dissolution by Bryophyllum Pinnatum extract in comparison to cystone pure water and 50:50 hydroalcoholic extract, which produced higher calcium oxalate dissolution than other fractions and the standard. While standards show higher dissolution as compared to others, etanol extract shows the lowest dissolution of calcium oxalate as compared to other.

According to a comprehensive phytochemical investigation, it was shown that the crude extract of Bryophyllum pinnatum leaves contain a significant number of bioactive components. These chemicals include alkaloids, phenols, flavonoids, carbohydrates, proteins, and saponins. An in vitro technique for measuring the dissolving percentage of kidney stones was used to test the anti-urolithiatic activity of *Bryophyllum pinnatum*, and the results were compared to those of a reference medication. It has been shown that many extracts are the most efficient in urolithiatic activity.

CONCLUSION

The study's goal is to learn more about the beneficial effects of the *Bryophyllum Pinnatum* plant, particularly leaves, in preventing and treating medical issues such as kidney stones, which are becoming more common in the younger population as a result of sedentary lifestyles and poor eating habits. The aqueous extract of leaves was

discovered to have strong antilithiatic efficacy in the current investigation. Calcium phosphate and calcium oxalate stones dissolve well in an aqueous extract of *Bryophyllum Pinnatum.* The decrease in calcium phosphate and calcium oxalate stones is reduced in ethanolic extract and other hydroalcoholic solutions (70:30, 50:50, 30:70). As a result of the above research, we can infer that the antilithiatic activity of *Bryophyllum Pinnatum* leaves was satisfactorily assessed.

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