

## Estimation of In-Vitro Antiurolithiatic Activity of Herbal Plants

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### ABSTRACT:

Despite advances in modern medicine, the development and growth of calculi continues to be a source of concern for mankind, as there is no effective treatment for kidney stones. In the present study we investigated antiurolithiatic activity of different herbal plants against calcium oxalate crystals in vitro.

Kidney stone is a complex that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the kidneys. Calcium oxalate is the most frequent urinary stone component; it exist in three different crystalline forms. So in the present review aims to give data highlighting the present trends in research of medicinal plants accredited with antiurolithiatic activity. In this experiment we majorly focused on the herbal plants like Amla, Lemon, Pashanabhed to check antiurolithiatic activity on calcium oxalate crystals by evaluating with standard drug cystone. The evaluation is done by two methods. i.e by titrimetric method and UV Spectroscopic method. When compared with standard it was found that lemon showed good antiurolithiatic activity on calcium oxalate crystals then Amla and Pashanabhed.

**KEYWORDS:** Antiurolithiatic, Herbal, Kidney stones, Calcium oxalate, in vitro.

### I. INTRODUCTION:

Kidney stones are a multi-factorial disease resulting from the combined influence of epidemiological, biochemical, and genetic risk factors. Stone formation is thought to be caused by an abnormal increase in urinary calcium, oxalate, and uric acid levels, which reduces urinary citrate levels. Citrate and magnesium are major inhibitors of stone formation in the urinary tract

and reduced levels or absence of these inhibitors in the urine lead to stone formation. The formation of stones or lithiasis is characterized by calculiformation. Calcium oxalate is more common, these stones are hard and occasionally dark.(1)

Stones often form and accumulate in the renal pelvis and calyces. The composition of kidney stones may vary due to various factors such as metabolic abnormalities, climatic conditions, and presence of infection. They are more common in men than women. They vary in size from <1mm to large stones capable of dilating entire renal pelvis.(2) The most common kidney stones are calcium oxalate (CaOx) crystal stones. CaOx crystals, the main component of human kidney stones, exist as CaOx monohydrate (COM) and CaOx dihydrate (COD). Stone formation requires supersaturated urine, which depends on urine pH, ionic strength, concentration of solutes and complexes. In India, in the Ayurvedic system of medicine, the plants of the 'Pashanabheda' group claim to be useful in the treatment of urinary stones(3)

Pashana = stone, bheda = to break. Therefore, this research has attempted to list the studies conducted on these plants. The most common types of commercial formulations used as Pashanbhed are B.ligulata (rhizomes), C.aromaticus (leaves), and K.pinnata (leaves)(4)

Various allopathic drugs are currently used to treat urolithiasis. A combination of physical methods such as ultrasound and laser are often used in combination with conventional medicine. In the current scenario, the demand for herbal products is growing exponentially around the world, and major pharmaceutical companies are currently conducting extensive research into plant materials for their potential medicinal value(5)

The other herbal drugs apart from Pashanabheda selected for the study in this research are Citrus limon(lemon) and Embilica officinalis (Amla). Citrate consumption prevents stone formation not only because it increases urine volume, but also because it is high in potassium and citric acid. Citrate prevents stone formation through two mechanisms. First, it binds to calcium in the urine, thereby reducing urine supersaturation. It also binds to calcium oxalate crystals and prevents crystal growth. Patients with low urinary citrate should be endorsed to boom their intake of mealsexcessive in citric acid, inclusive of lemon(6,7)

**PHARMACOLOGY OF KIDNEY STONES**

These are small & hard deposit that forms in the Kidney &produces pain when passing through urological morphology.

**Types of Kidneys Stone:**

**1. Calcium oxalate stone:**

Solid masses form in the kidney when there is a high level of calcium oxalate, cystine, phosphate & too little liquid.

**2. Uric Acid Stone:**

These Stones After formation mainly stays in the kidney or travel down the urinary tract into the ureter.

**3. Struvite Stone:**

These are mainly caused by UTI from Bacteria.

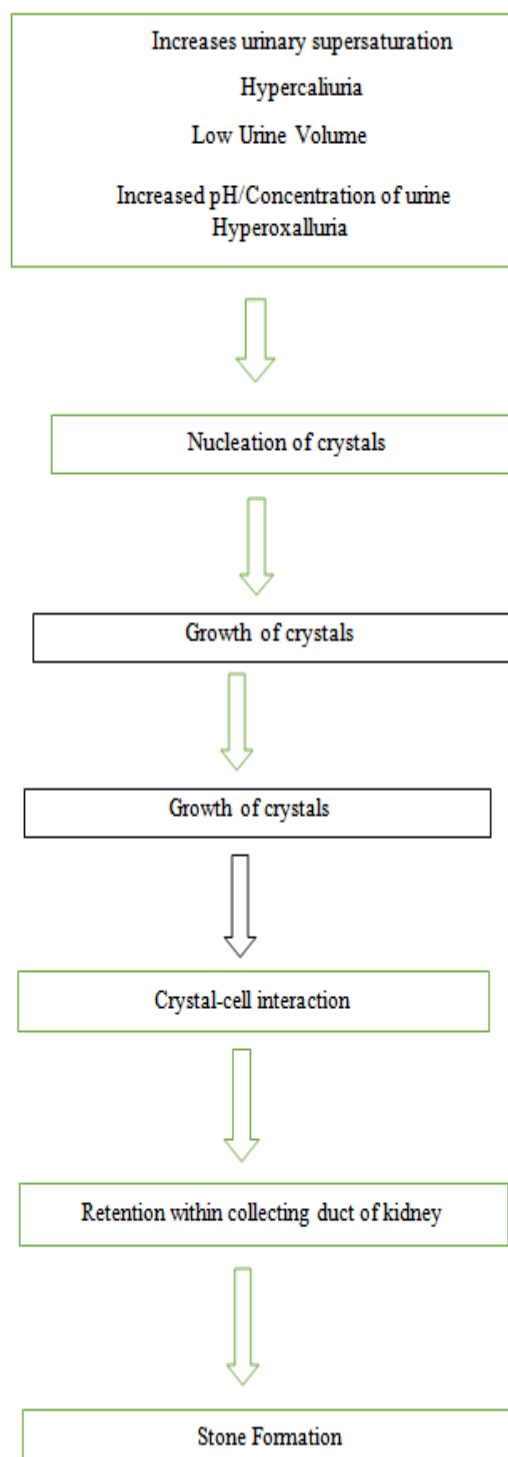
**4. Cystine Stone:**

These are rare conditions, mainly are inherited Disorder called Cystinuria.(8)

**• Factors responsible for Stone formation:**

- 1) Infection: (causative organism: proteus, pseudomonas, klebsiella)
- 2) Hot climate & Dehydration
- 3) Alteration in Dietary factor
- 4) Medical conditions: Hyperparathyroidism & Gout
- 5) Immobilization
- 6) Low level of urinary citrate
- 7) Urinary Stasis
- 8) Randall’s Plaque(9)

**Pathophysiology:**(5,10)



**Fig 1. Flow-chart showing mechanism of stone formation.**

- **Clinical features of kidney stones(11):**
- Guarding and Rigidity
- Dull acting pain

- Hematuria
- Nausea
- Vomiting
- Fever
- Chills
- Recurrent UTIs
- Ureteric colic
  
- **Complications Includes:**
  - 1) Calculus Hydronephrosis
  - 2) Calculus Pyelonephrosis
  - 3) Renal Failure
  - 4) Squamous cell carcinoma
  
- **Laboratory & Imaging evaluation/ Diagnosis:**
  1. S. Urea
  2. S. Creatinine
  3. Urine Culture & sensitivity test
  4. Metabolic workup
  5. X-ray (KUB)
  6. USG (Abdomen + Pelvis)
  7. IV Urogram
  8. CECT- Scan
  
- **Treatment:**
  - 1) Non-operative [If Stone <= 5 mm]:

Class	Drugs
Alpha Blocker	Tamsulosin
Uric Acid Reducers	Potassium Citrate
Xanthine Oxidase Inhibitor	Allopurinol
Calcium Channel Blocker	Nifedipine
NSAIDs	Diclofenac,
Diuretics	Furosemide, Acetazolamide

**Table 1. Drugs used in treatment of kidney stones(12)**

- 2) Operative [If Stone => 5 mm]:
  - a. Endoscopic Approach (PCNL-Percutaneous nephrolithotomy)
  - b. Open Surgical Approach
    1. Pyelolithotomy
    2. Nephrolithotomy
    3. Extended Pyelolithotomy
    4. Pyelonephrolithotomy
    5. Partial Nephrectomy

6. Nephrectomy

**METHODS AND MATERIALS:**

We have adopted two methods for in vitro anti-urolithiatic activity studies of selected herbal drugs.

1. Titrimetry Method
2. UV Spectroscopic Method

**TITRIMETRY METHOD FOR ESTIMATION OF ANTI-UROLITHIATIC ACTIVITY OF HERBAL DRUGS(13)**

**Chemicals used :**

Sodium oxalate, calcium chloride, Tris buffer, Potassium permanganate (KMnO<sub>4</sub>), Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>).

**Preparation of plant extract :**

The extract was prepared by maceration process. The powders were kept in 5 times the volume of water for 2 days. The extract was filtered through muslin cloth. The filtrate was kept on electric water bath for evaporation of the content to get the concentrated extract. Later the extract were stored in refrigerator for further use



**Fig 2. Herbal extracts before filtration**



**Fig 3. Herbal extracts after evaporation and evaporation**

**Preparation of Calcium oxalate crystals**

The experimental kidney stones of calcium oxalate (CaOx) were prepared in the

laboratory by taking an equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2N H<sub>2</sub>SO<sub>4</sub>. Both were allowed to react in sufficient quantity of distilled water in a beaker, the resulting precipitate was calcium oxalate. The precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water and dried at 60 °C.(13)



**Fig4. Dried calcium oxalate powder**

**Fig5. Calcium oxalate prepared by mixing CaCl<sub>2</sub> and sodium oxalate**

#### Removal of semi-permeable membrane from egg

The eggs were decalcified by keeping in 10% Acetic acid solution for 24 hours. The outer calcium layer dissolves, leaving behind egg content enclosed in thin membrane. Membrane is ruptured by pin and the content squeezes out. The semi-permeable was washed and used.(13)



**Fig 6. Decalcified egg**

**Fig 7. Eggs kept in 10% acetic acid solution for 24 hours for decalcification**

#### Investigation of in vitro antiurolithiatic activity test by titrimetry

The dissolution percentage of calcium oxalate was evaluated by taking exactly 5 mg of calcium oxalate and 50 mg of the extract, packed it together in the semi-permeable membrane of the

egg as shown in the model designed given below. This was allowed to suspend in a beaker containing 100 ml of 0.1M Tris buffer. The first group served as blank containing only 5 mg of calcium oxalate. The second group served as a positive control containing 1 mg of calcium oxalate and along with the 10 mg standard drugs, i.e. Cystone syrup by Himalaya. The 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> groups along with 1 mg of calcium oxalate contain aqueous extracts of herbal drugs under study. The conical flasks of all groups were kept in an incubator preheated to 37 °C for 2 h. Remove the contents of semi-permeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuric acid to each test tube and titrated with 0.2 N KMnO<sub>4</sub> till a light pink colour end point obtained. The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment, in the beginning, to know the total quantity of dissolved calcium oxalate by various solvent extracts.(13)



**Fig8. Calcium oxalate and inhibiting extracts enclosed in semipermeable and suspended in Tris buffer**

#### UV SPECTROPHOTOMETRIC FOR IN VITRO CALCIUM OXALATE CRYSTALLIZATION INHIBITION:

##### Sample preparation

The various extraction of plant material was ready to use.

**Experiment:** The precipitation of calcium oxalate at 37°C and pH 6.5 has been studied by the measurement of turbidity at 620nm. A UV visible spectrophotometer was employed to measure the developed turbidity due to the formation of calcium oxalate.(7)

##### Chemicals used:

Chemicals used were of pure and analytical grade.

1. Calcium chloride dehydrate (CaCl<sub>2</sub>.2H<sub>2</sub>O)
2. Sodium oxalate (Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub>)

### 3. Sodium chloride (NaCl)

### NaCl solution

#### Procedure:

#### Study without inhibitor:

The solutions of CaCl<sub>2</sub>.2H<sub>2</sub>O (10mM) and Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> (4mM) were prepared using sodium chloride solution (0.15M). A volume of 1.5ml of calcium chloride dehydrate was transferred in to the cell and blank reading was taken. 1.5ml of sodium oxalate solution was added to the previous volume, and the turbidity measurement was immediately started for a period of 10min. For each experiment, six replicates were taken.(7)

#### Study with inhibitor:

The inhibitor (100%) was prepared by taking 0.5g of the extract with 60ml of sodium chloride (0.15mM). From this inhibitor, prepare diluted inhibitory solutions (10%, 50%) added to the previous volume, and the turbidity measurement was immediately started for a period of 5 min.

Study with inhibitor: The inhibitor (100%) was prepared by taking 0.5g of the extract with 60ml of sodium chloride (0.15mM). From this inhibitor, prepare diluted inhibitory solutions (10%, 50%) using solvent such as sodium chloride solution (0.15mM). A mixture of 1ml of calciumchloride dehydrate (10mm) and 1ml of inhibiting solution was versed in the cell. Take a blank reading, and then a volume of 1ml of sodium oxalate (4mM) was added and the measurement was immediately started for a period of 10minutes.



Fig9. Diluted solutions of extracts in 0.15M

The % of inhibition was calculated using the following formula:

$$I (\%) = [1 - Ti / Tc] \times 100$$

Where,

Ti is turbidimetric slope with inhibitor,

Tc is turbidimetric slope without inhibitor.(14)

## II. RESULT

Drug therapy has developed in response to population health care needs. There are many crucial areas in medicine such as liver diseases, arthritis, old age related problems, certain viral infections and cancer where the conventional medicine is devoid of satisfactory treatment. These are among the promising areas of research and development of medicines from the vast highly potential plant resources.(15) Plants are also attractive sources for the development of novel and very effective and safe therapeutic agents against kidney procumbens. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects(16). Unlike allopathic medicines which target is only one aspect of urolithiatic pathophysiology, most of the plant-based therapy has been shown to be effective at different stages of stone pathophysiology(17). About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials. Plant-based drug discovery programmes continue to provide an important source of new drug leads(18) Lithiasis (stone formation) is an important cause for acute and chronic renal failure, includes both nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or both). Among the various kinds of stones identified, calcium stones occur mainly in Men, while phosphate stones formation is more in women. (19)

### % Dissolution of Calcium Oxalate by Titrimetry Method

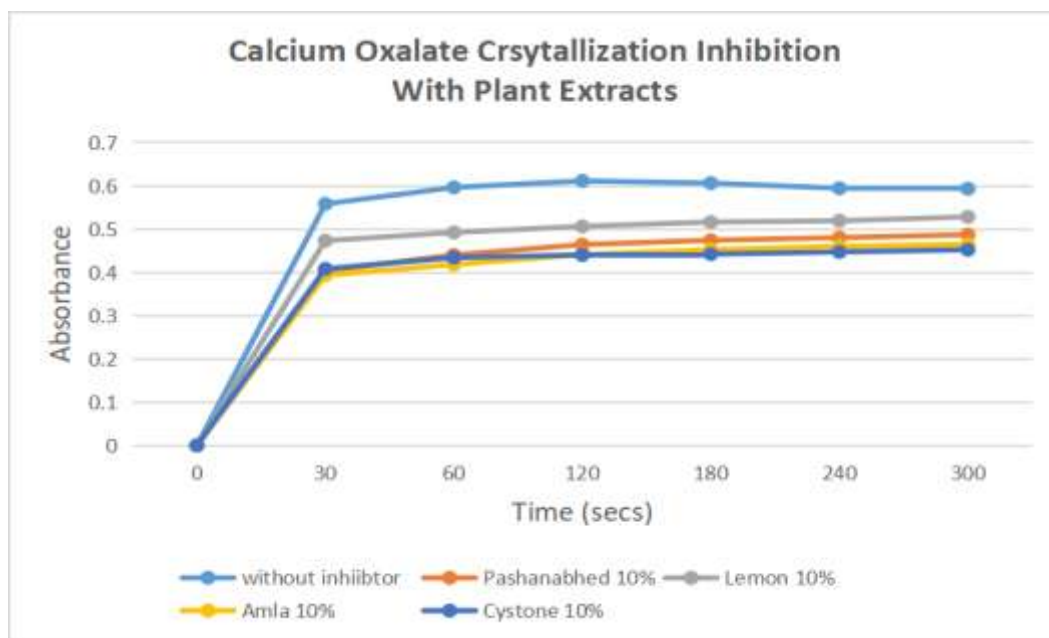
Extract	Volume of Titrant (0.1M KMnO <sub>4</sub> in mL)	% Dissolution
Standard (Cystone Syrup)	0.4	48.9
Lemon	0.6	23.2
Amla	0.7	10.4
Pashanabhed	0.7	10.4

Table 2 . % Dissolution activity of calcium oxalate by Titrimetry method

**UVSpectrophotometric Analysis of Calcium Oxalate Crystallization Inhibition with Plant Extracts**

Time (secs)	Absorbance				
	Without inhibitor	Pashanabhed 10%	Lemon 10%	Amla 10%	Cystone 10%
30	0.558	0.403	0.473	0.398	0.408
60	0.596	0.44	0.492	0.418	0.434
120	0.611	0.464	0.506	0.441	0.44
180	0.606	0.474	0.516	0.452	0.442
240	0.594	0.48	0.519	0.46	0.447
300	0.593	0.487	0.528	0.464	0.452

**Table 3. % Dissolution activity of herbal extracts**



**Table 4. % Inhibition of Sample and reference**

Sample	Turbidimetric Slope	% Inhibition
Without Inhibitor	0.0665	-
Cystone 10%	0.0515	22.5
Lemon 10%	0.0557	16.24
Pashanabhed 10%	0.0589	11.42
Amla 10%	0.0607	8.7

This study evaluates the antiurolithiatic activity of Aqueous extract of Citrus limon (lemon), Embilica officinalis (Amla), Pashanabhed and Cystone syrup. The highest percentage i.e. 48.9% of calcium oxalate {CaOx} dissolution was observed in Cystone syrup followed by an Aqueous extract

of lemon which had a percentage dissolution of calcium oxalate was 23.2% followed by Amla (10.4%) and Pashanabhed (10.4%). Both extracts of Cystone and lemon were found to be more effective in the dissolution of calcium oxalate. From this study, it was observed that Aqueous

extracts of Cystone and lemon showed their highest dissolution of calcium oxalate. Cystone syrup was found to be even more effective than aqueous extract in the dissolution of calcium oxalate. This study has given primary evidence for lemon as the plant which possess lithotriptic property. This in vitro study has given lead data and shown that Aqueous and cystone syrup are quite promising for further studies in this regard.

The standard Cystone syrup showed 22.4% crystallization inhibition. While, from the test herbal drugs Lemon showed highest crystallization inhibition activity (16.24%). pashanabhed and amla showed 11.42% and 8.7% respectively.

### III. DISCUSSION :

This study evaluated the antiurolithiatic activities of three herbal extracts—lemon (*Citrus limon*), Amla (*Emblica officinalis*), and Pashanabhed (*Bergenia ligulata*)—using Cystone syrup as a standard comparator(20). The results indicated that lemon extract exhibited the most significant antiurolithiatic activity(21), with a calcium oxalate dissolution rate of 23.2% and a crystallization inhibition rate of 16.24%. This effectiveness is likely due to the high citrate content in lemon, which binds calcium in the urine and prevents the growth of calcium oxalate crystals, making it a potent natural remedy for kidney stones(22).

Amla showed moderate antiurolithiatic activity with a dissolution rate of 10.4% and a crystallization inhibition rate of 8.7%. The presence of antioxidants and polyphenolic compounds in Amla may contribute to its ability to inhibit stone formation, although its efficacy was lower than that of lemon(23). Similarly, Pashanabhed also demonstrated moderate activity, with a dissolution rate of 10.4% and a crystallization inhibition rate of 11.42%. The herb is traditionally used in Ayurvedic medicine for treating kidney stones, and its moderate efficacy in this study aligns with its known properties as a lithotriptic agent.(24)

The standard drug Cystone syrup showed the highest dissolution and crystallization inhibition rates, underscoring its effectiveness as a polyherbal formulation for urolithiasis management.(25) While the herbal extracts demonstrated some potential as natural antiurolithiatic agents, their effects were less potent than Cystone, suggesting that these herbs might be better suited as complementary therapies rather than stand-alone treatments.(26)

Overall, the study highlights the potential of lemon as a promising herbal remedy for kidney stones, with Amla and Pashanabhed offering moderate benefits. Further in vivo studies and clinical trials are needed to confirm these findings and explore the potential of using these herbal extracts in combination or alongside conventional treatments for a more holistic approach to managing urolithiasis.

### IV. CONCLUSION:

The present research illustrates several medicinal plants that are mainly evaluated against calcium oxide type kidney stones. The result of the research shows the effectiveness of the extracts in kidney stones. In vitro urolithiasis has been performed on the selected plant pashanabhed, and the standard drug used is Cystone. The work was performed by using in vitro antiurolithiatic model to calculate the percentage dissolution of kidney stone. The combination was tested in the presence and absence of standard (Cystone) to see if there was any improvement in efficacy that could reduce the dose of the polyherbal combination.(27)

Most recurrent stone formers have no identifiable underlying cause for their condition. A careful medical history can reveal several dietary and lifestyle risk factors that contribute to the risk of stone disease. A more comprehensive understanding of the influence of diet and lifestyle factors. However, intensive preclinical and clinical studies are needed to assess the efficacy and toxicity of these plant products. The main disadvantage of this method is the recurrence of the stones.(28,29)

Herbal products and those derived from their lead compounds as such may not replace these procedures, but they can certainly help reduce the rate of kidney stone recurrence. The main disadvantages in the development of a standard drug can be the multicausal nature of urolithiasis, various biochemical disorders leading to urolithiasis and various chemical variants of kidney stones.(6,30)

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