

# Novel *In Vitro* Approaches for Screening and Identification of Anti- Urolithiatic Activity: A Comprehensive Review

**ABSTRACT:** Urolithiasis is characterised by the formation of renal calculi remains a significant global health concern, with high recurrence rates and limitations in current treatment options. The major types of renal stones include calcium oxalate, calcium phosphate, uric acid, struvite and cystine stone, each associate with distinct metabolic or infectious aetiologies. the pathophysiology of stone formation involves super saturations of urine with lithogenic substances, crystal nucleation, growth, aggregation and retention within renal tubules. Current treatment strategies such as extracorporeal shock wave lithotripsy, ureteroscopy, and percutaneous nephrolithotomy effectively fragment or removes stones but often fail to prevent recurrence and carry procedural risks. Many medicinal plants exhibit anti-urolithiatic effects attributed to their phytoconstituents, including flavonoids, saponin, phenols, tannins, and alkaloids with antioxidant, diuretic, and anti-inflammatory properties. In-vitro studies are conducted on egg membrane to dissolve calcium oxalate crystals by using ethyl acetate extract of *Bryophyllum pinnatum* and *Aerva lanata*. These extracts show anti-urolithiatic activity mainly due to the presence of phytochemicals such as flavonoids and tannins.

**KEYWORDS:** Anti-urolithiatic activity, Types of kidney stone, Pathophysiology of nephrolithiasis, Anti-urolithiatic plants, Phytochemicals, Methodologies, Chemical test, current treatment of kidney stone, *Aerva lanata*, *Bryophyllum pinnatum*.

## 1. INTRODUCTION:

Urolithiasis commonly referred to as kidney stones, is the one of the most prevalent disorders affecting the urinary tract. The condition involves the formation of hard, crystalline masses within the kidney stones or urinary systems often composed of calcium oxalate, phosphate, uric acid or other minerals. kidney stones are not only painful but can also leads to severe complication if not diagnosed and managed properly. The mechanism behind the formation of kidney stones is multi factorial contributing factors include genetic predisposition, metabolic disorders, dietary habits, dehydration, infection and lifestyle factors such as low physical activity. Crystallization begins when the urine becomes supersaturated with stone -forming constituents, leading to nucleation, growth, aggregation and retention of crystals within urinary tract.in light of limitation s and side effect of conventional therapies, there has been increasing interesting use of plant remedies and natural supplements to prevent and manage kidney stones. Traditional system of medicine such as Ayurveda, have long utilised medicinal plants their Anti-urolithiatic properties. It works through multiple mechanisms. such as inhibiting stone formation, reducing crystal aggregation, promoting diuresis and easing the expulsion of stones. phytochemical such as flavonoids, saponins, alkaloids, tannins, glycosides and terpenoids are commonly found in these plants and are responsible for their therapeutic effects. these

compound exhibit antioxidant, ant-inflammatory antispasmodic and antimicrobial properties, all of which contribute to the inhibition of stone formation and improvement of urinary health. Research indicates that many of these herbal remedies work by altering the urinary environment, reducing concentration of stone forming substances and enhancing the solubility of salts. some plants increase urine output, these flushing out small crystals before they have the chance to aggregate and form large stones. others may act by modulating oxalate metabolism, decreased urinary calcium excretion, or increasing magnesium levels, which help prevent calcium oxalate crystallization<sup>[1,2]</sup>.

Herbal plants play a significant role in ancient methods of medicine, so plants are the primary source for producing cost effective medicines. *Aerva lanata* is the plant that have ability to cure a broad range of disease naturally. It includes in Amaranthaceae family and it is commonly seen in tropical planes of India as a normal weed. It is commonly used as antimicrobial, antidiabetic, diuretics, expectorant, anti-inflammatory and urolithiasis. It consists of wide range of phytochemicals like alkaloids, flavonoids, steroids, amino acid, terpenoids, tannins and proteins<sup>[11]</sup>.

*Bryophyllum pinnatum*, known as air plant, life plant etc., belongs to the family Crassulaceae. The leaves and bark of the plant posses a bitter taste which can be used to treat vomiting, diarrhoea, burns, gastric ulcer, urolithiasis. Plant extract is used to cure asthma, cough, headache, convulsion and edema of legs. The phytochemicals present in *B.pinnatum* such as flavonoids, steroids, alkaloids, glycosides, phenols, tannins, saponins, carotenoids and organic acids<sup>[12,13]</sup>.

#### **TYPES OF KIDNEYS STONES:**

Kidney stones are solid masses formed from crystals in urine, and they are classified based on their chemical composition.

Calcium oxalate stones are the most prevalent form, accounting for up to 75% of all kidney stone. These stone form when calcium binds with oxalate in urine. Monohydrate type includes extremely hard and dark brown with a smooth surface and Dihydrate type include more fragile, yellow to light brown in colour, and often have jagged edges.

Calcium phosphate stone comprising about 10% of stones, these are associated with high urinary pH and metabolic condition such as hyperparathyroidism or renal tubular acidosis. They may be off-white grey, or yellowish.

Uric acid stones develop in acid urine and are linked to conditions like diabetes, obesity, and metabolic syndrome. They are often reddish, brown, or yellow colour.

Struvite stones are usually associate with urinary tract infection caused by urease-producing bacteria. These stones often form large staghorn calculi and are found in alkaline urine.

Cystine stones are rare, accounting for only 1-2 % of cases, and result from a genetic condition called cystinuria. These stones are waxy, yellowish, and resistant to standard treatments like shock wave lithotripsy<sup>[9]</sup>.

#### **PATHOPHYSIOLOGY OF NEPHROLITHIASIS:**

The formation of kidney stones is a multifactorial biological process influenced by both intrinsic and extrinsic factors. A critical initiating event in stone formation is urinary supersaturation, where the concentration of certain solute exceeds their solubility threshold. This condition sets the stage for crystal precipitation, particularly when urine is stagnant. Supersaturation is measured as a ratio between the actual concentration of solutes and their saturation point-ratios above 1 indicate a tendency for crystal formation.

The body naturally produces several inhibitors of crystallisation, such as citrate, pyrophosphate, magnesium, and various proteins including uromodulin and osteopontin. These substances help prevent stone formation by binding to stone-forming ions, thereby reducing their availability to form crystals. Low urine volume increases the concentration of stone-forming solutes, amplifying the risk of stone formation. Urinary pH also plays a significant role. Alkaline urine favours calcium phosphate and struvite stones, while acidic urine promote the formation of uric acid and cystine stones.

The presence of supersaturation alone does not enhance stone formation. Crystals typically require a nucleation site, such as damaged epithelial cells or structures known as Randall's plaques-sub epithelial calcium phosphate deposits in the kidney's papillae. These plaques can rupture into urinary tract, providing a surface for calcium oxalate or phosphate crystals to adhere and grow into larger stones through aggregation.

Genetics also influence stone formation. Variants in genes regulating calcium, oxalate, citrate, and uric acid excretion can predispose individual to nephrolithiasis. Also, monogenic disorders like cystinuria, Dent's disease, or primary hyperoxaluria directly cause stone disease through metabolic abnormalities. Such conditions may warrant genetic testing, particularly in individual with early-onset or recurrent stone disease and a positive family history. Anatomical abnormalities and chronic inflammation are recognised contributors, either by promoting urinary stasis or altering the balance of crystal inhibitors and promoters<sup>[8]</sup>.

#### **PHYTOCONSTITUENTS:**

Plants and their phytoconstituents have been widely used against urolithiasis. many natural compounds show potential by inhibiting crystal formation, reducing oxidative stress and enhancing anti-oxidant defence mechanism.

Berberine: it is an isoquinoline alkaloid found predominantly in *Berberis vulgaris* bark, has demonstrated significant anti-urolithiatic effect. It reduces crystal formation by modulating crystallization process and exhibits strong anti-oxidant properties, helping to alleviate oxidative stress associated with kidney stone formation. Berberine is a key component in in several plant species, including *Berberis aquifolium*, *Berberis aristata*, *Mahonia aquifolium*, *cordifolia* and *coptis chinensis*.

Quercetin: A well-known flavonoid quercetin has shown promising result in experimental model of urolithiasis.it destroy the stone formation because of diuretic

and antioxidant activities. Plants rich in quercetin include *Morus alba*, *Camellia sinensis*, *Centella asiatica*, *Hypericum perforatum*, *Brassica oleracea*, *Coriandrum sativum*, *Lactuca sativa*, *prunus domestica*, *Malus domestica* and *Vaccinium oxycoccos*. these plants effective for ant-urolithiatic therapy.

**Flavonoids:** A type of plant-derived polyphenol are known to possess anti-urolithiatic properties. Which play a role in inhibiting stone formation.it can modulate the inflammatory response, reducing production of pro-inflammatory mediators involved in kidney stone formation. Some flavonoids exhibit diuretic properties, increasing urine production and helping to flush out small stones or precursors of stone formation.

**Triterpenoids:** triterpenoids such as oleanolic acid, lupeol, ursolic acid and betulinic acid lipid-lowering activities relevant to kidney stone prevention. they act by inhibiting calcium oxalate crystallization and reducing stone-inducing factors. These compounds are found in plants like *Crataeva nurvala*, *Aegle marmelos*, *Betula alba*, *Calendula officianalis*, *Cucumins sativus*, *prunusa Amygdalus* and *Nerium oleander*.

**Saponins:** found in plants like *Tribulus terrestris* and *Asparagus racemosus*.it reduce crystallization by increasing urine volume dispersing mucoproteins that promote stone aggregation and exerting anti-inflammatory activity.

**Coumarins:** it interferes with the initial stages of stone formation, preventing crystals from developing urinary tract. Coumarin also prevent the clumping together of crystals, reducing likelihood of stones forming in kidney or urinary tract.it contain plant extract such as those from *Aegle marmelos* and *Kalanchoe laciniata*, can inhibit calcium oxalate crystal formation and growth<sup>[4]</sup>.

## **CURRENT TREATMENT:**

Treating kidney stone can be both painful and costly. The intense pain typically results from the sharp presence of, irregularly shaped crystals, which can damage internal tissues.

**Extracorporeal shock wave Lithotripsy (ESWL):** Extracorporeal shock wave Lithotripsy is method involves directing acoustic shock waves at the kidney stone from outside the body. These waves are delivered at both high and low frequencies to break the stone in to small fragment.

**Ureteroscopy:** Ureteroscopy serves as an alternative to blind basket techniques and open ureterolithotomy. It is used to diagnose and treat stone in upper urinary tract. small stones can be completely removed using this method, while larger ones are fragmented into smaller pieces before removal.

**Percutaneous nephrolithotomy (PCNL):** Percutaneous nephrolithotomy is minimally invasive procedure used to extract kidney stone directly. However, it carries certain risks such as potential kidney injury. Precision and expertise are necessary due to the use of imaging guidance. Nowadays, its use is limited because of growing preference for shock wave lithotripsy.

**Flexible ureterorenoscopy:** Flexible ureterorenoscopy is the technique is primarily used to treat small kidney stones. A flexible fiber-opticscope is interested through the

urinary tract into the kidney, where energy from an external source is used to disintegrate the stones<sup>[2]</sup>.

## **2. MATERIAL AND METHODS:**

### **2.1. General experimental procedure:**

All the chemical and reagents used for this study were collected and prepared before use. Healthy and fresh leaves of *Bryophyllum pinnatum* and flowers of *Aerva lanata* were collected from the regions in Madurai, Tamil Nadu, India and it was stored in an air tight bag.

### **2.2. processing of sample:**

The leaves were washed with tap water to remove the contaminants. The leaves were dried for a week to remove the moisture content. Dried samples were grinded to get fine particles and store in a container<sup>[10]</sup>.

### **2.3. Preparation of leaves extract:**

Leaves extract was prepared by crushing the dried leaves into coarse powder with a domestic mixer. Separation of the component from the extract is made by using percolation method. It is a conical vessel with atop opening and separatory funnel as the percolator. The bottom has an adjustable closure to allow passage of the fluid in a convenient rate with slight pressure. 10 grams of powdered *Bryophyllum pinnatum* and *Aerva lanata* were incubated with 100ml of ethyl acetate at a ratio 1:10. Then it was incubated for a period of 15 days with stirring at regular interval of time. After 15 days, the solvent was filtered by using Whatman no.1 filter paper to remove the debris and the filtered extracts were transferred into petri plate for evaporation of solvents. Then the powdered form of sample extract was transferred into a clean container and stored at -4°C.<sup>[10]</sup>

### **2.4. Phytochemical analysis:**

#### **Test for flavonoids**

The aqueous form of plant extract was blended with 2% NaOH solution, which produce a concentrated yellow colour. On further addition of diluted acid in 2 drops, loss the yellow colour to colourless, this indicates the presence of flavonoids.

#### **Test for phenols**

200mg of powdered plant extract were mixed in distilled water of 20 ml and heated. Then filter it and add 2 drops of 0.1% ferric chloride to it. No red, blue, or green colour of the solution and it indicates the absence of phenols<sup>[14]</sup>.

#### **Test for tannins**

To the 2 ml of ethyl extract, add few drops of FeCl<sub>3</sub> solution and kept undisturbed for few minutes. Appearance of blackish blue colour indicate the existence of tannins<sup>[14]</sup>.

#### **Test for coumarins**

3ml of 10% NaOH solution was mixed with 2ml of plant extract. The extract produced a yellow colour which confirms the presence of coumarin and which does not produce yellow colour which show the absence of coumarin<sup>[14]</sup>.

### **2.5. Evaluation of anti-urolithiatic activity**

**2.5.1. Preparation of synthetic urine:** Due to the simplicity and reproducibility, synthetic urine is commonly used as a model for studying urolithiasis. This urine is formulated to be supersaturated with calcium oxalate and is typically prepared in closed container maintained at 37°C, and the process involves components that mimic the ionic composition of human urine, and distilled water is used as the negative control, and a commercial anti-urolithiatic drug(cystone) served as the positive control<sup>[7]</sup>.

**2.5.2. Preparation of synthetic kidney stone:** Synthetic calcium oxalate stones were prepared using a homogenous precipitation technique. To begin with 1.47 grams of calcium chloride dihydrate were dissolved in 100 ml of distilled water, and 1.34 grams of sodium oxalate were dissolved in 100 ml of 1 M sulfuric acid. These two solutions were mixed in a beaker and stirred to precipitate calcium oxalate crystals. Ammonia solutions were added to remove any residual acid from the crystals. The resulting crystals were thoroughly washed with distilled water and dried at 60°C for 4 hours. These lab-synthesised stones were used for further testing and evaluation of anti-urolithiatic agents<sup>[7]</sup>.

### **2.5.3. egg membrane assay:**

**a. Preparation of semi-permeable membrane:** egg membrane was used as a biological model to simulate the renal membrane for testing Anti-urolithiatic activity. The semi-permeable membrane was extracted by puncturing eggs at the apex and removing their contents. The shells were then washed and soaked in 4 ml of concentrated hydrochloric acid in 200 ml of distilled water to decalcify them. After overnight incubation, the membranes were rinsed with distilled water and neutralised in an ammonia solution. The cleaned membranes were stored in a moistened state at a pH of 7-7.4 in refrigeration<sup>[7]</sup>.

**b. Calcium oxalate dissolution assay:** calcium oxalate crystals are prepared by adding calcium chloride in distilled water and it allowed to react with the mixture of sodium oxalate and 2N sulphuric acid. Both these solutions are allowed to react for sufficient period in presence of distilled water. At the termination of this reaction, calcium oxalate was formed as a precipitate. Then the precipitate was collected and washed with distilled water to remove the impurities and dried at 60°C. Percentage of dissolution was assessed by mixing 10mg of plant extract with 1 mg of calcium oxalate and it was packed in a semi permeable membrane.

100ml of 0.1M tris buffer was taken in a sterile beaker and the egg membrane was transferred into it. The experiment was divided into 4 different classes.

First class(blank): 1mg calcium oxalate.

Second class (positive control): 1mg calcium oxalate with 10mg standard drug(*Neeri*).

Third class: 1mg calcium oxalate with 10 mg of *Aerva lanata* extract.

Fourth class: 1mg calcium oxalate with 10 mg of *Bryophyllum pinnatum* extract.

The beakers of all classes are incubated at 37°C for 2 hours. After the incubation period, contents are removed from semipermeable membrane and transferred it into a clean tube. The

contents are then diluted with 2ml of 1N sulphuric acid and it was titrated against  $\text{KMnO}_4$ . Titration is continued till the colour change into pink. The undissolved calcium oxalate was deducted from the initial concentration taken at the beginning of the procedure. The difference gives an idea about the dissolution of calcium oxalate crystals by the ethyl extract of *Aerva lanata* and *Bryophyllum pinnatum*<sup>[10]</sup>.

### 3. DISCUSSION:

Urinary supersaturation is one of the main reason responsible for the stone forming constituents. Phytoconstituents existing in the sample played a major role in the urolithiatic activity. The plant extract shows significant anti-urolithiatic activity due to existence of flavonoids and tannins. The dissolution of calcium oxalate in ethyl acetate extract of *Bryophyllum pinnatum* at concentration of 10 mg is more than the ethyl acetate extract of *Aerva lanata* at concentration of 10 mg. the unique bioactive compound called Bufadienolides may also be a reason for showing the anti-urolithiatic property by *Bryophyllum pinnatum*. The study shows that *Aerva lanata* and *Bryophyllum pinnatum* are the plant which possesses anti-urolithiatic activity. Compared to previously published studies, the current discovery offers distinct advantages. Earlier report often lacked comprehensive mechanistic studies and relied on singular assays or parameters. In contrast, this research employed a multi-tiered evaluation including *in-vitro* assays (nucleation, aggregation, growth), egg membrane dissolution test, and *in-vivo* rat models, supplemented with biochemical, histopathological, and statistical analysis. The use of decalcified egg membrane and crystal growth inhibition at multiple stages provides novel insight into anti-urolithiatic potential of test extracts. The observed dose-dependent reversal of lithogenic markers and restoration of renal architecture further undergoes the therapeutic promise of the herbal formulation used<sup>[10]</sup>.

### 4. CONCLUSION:

Urolithiasis, the formation of urinary stones, remains a significant global health issue due to its higher recurrence rate, associated morbidity, and limited efficacy of conventional therapies. Despite the availability of allopathic treatment such as diuretics, alkalinizers, acidifiers, and crystallization inhibitors, their long-term use often leads to side effects including electrolyte imbalance, gastrointestinal discomfort, and renal complications. Moreover, many conventional drugs focus on symptom management rather than addressing the underlying metabolic imbalances that leads to stone formation. Surgical interventions, though effective in removing stones, do not prevent recurrence and may entail significant cost and patient discomfort. This highlights a major limitation in current treatment modalities.

In summary, while modern medicine offers short-time relief, its limitations necessitate alternative approaches. The phytochemical-rich, plant-based therapies highlighted in this study not only address the root causes of stone formation, but also offer a holistic, cost-effective, and safer route for long-term management of urolithiasis. This integrated model of scientific validation and traditional knowledge sets a new benchmark in anti-urolithiatic drug discovery.

Anti-urolithiatic activity was performed in-vitro on the plants such as ethyl acetate extracts of *Aerva lanata* and *Bryophyllum pinnatum* and standard drug (*Neeri*) was used as positive control. This study confirms that the ability of *Aerva lanata* and *Bryophyllum pinnatum* to dissolve the calcium oxalate crystals. This plant extract is suitable for treating kidney stone disease serves as an eco-friendly, cost effective therapy. Flavonoid and tannins associated with the plant extract are showing the major phytochemicals present in anti-urolithiatic activity. The study conclude that the plant extract shows a promising result in dissolution of calcium oxalate crystals than the standard drug (*Neeri*).

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