



## EVALUATION OF ANTI-UROLITHIATIC POTENTIAL OF PONGAMIA PINNATA (L.) LEAF EXTRACTION: AN IN VITRO STUDY

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### Article Info

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### ABSTRACT

Urolithiasis, commonly known as kidney stone disease, is a prevalent urinary tract disorder characterized by the formation and accumulation of mineral crystals within the urinary system. The present study was undertaken to evaluate the **anti-urolithiatic potential of Pongamia pinnata (L.) leaves using an in vitro model**. The leaves were subjected to maceration extraction using ethanol, and the obtained extract was evaluated for its phytochemical constituents. Preliminary phytochemical screening revealed the presence of alkaloids, glycosides, phytosterols and triterpenoids, saponins, phenolic compounds, flavonoids, carbohydrates, proteins and amino acids, and fixed oils and fats. For the evaluation of anti-urolithiatic activity, experimental calcium oxalate stones were prepared by homogeneous precipitation. The dissolution potential of the plant extract was assessed using a semi-permeable egg membrane model, followed by titrimetric estimation of the remaining calcium oxalate using potassium permanganate. The phytochemical constituents present in the extract may contribute to its potential Urolithiatic activity. The findings of this study suggest that **Pongamia pinnata leaf extract possesses promising anti-urolithiatic potential** and may serve as a basis for further investigation into its role in the prevention and management of urinary stone formation.

**KEYWORDS:** Urolithiasis, Pongamia pinnata, Anti-urolithiatic activity, Calcium oxalate, Phytochemical screening, Ethanolic leaf extract, In vitro study, Egg membrane model.

### INTRODUCTION

Urolithiasis, commonly referred to as kidney stone disease, is a common urinary tract disorder that can affect people of all age groups. It is a significant health problem worldwide, and in India<sup>[1]</sup>, nearly 12% of the population is estimated to be affected by kidney stones. The condition also has a high tendency to recur, with recurrence rates reported to be around 70–80% in males and 47–60% in females<sup>[2]</sup> globally, urinary calculi<sup>[3]</sup> are estimated to affect approximately 4–15% of the population.

Kidney stones develop when certain minerals and salts present in urine become highly concentrated and start to form crystals. These crystals can gradually come

together and grow into larger clusters. When these clusters remain within the kidneys or urinary tract<sup>[2,4]</sup>, they may eventually develop into kidney stones. Calcium and phosphate are among the major minerals commonly involved in stone formation.

The development of urolithiasis is influenced by several factors, including changes in lifestyle and dietary habits, metabolic abnormalities, bacterial infections, genetic factors, and certain medications. The symptoms may vary depending on the size and location of the stone. Common symptoms include blood in the urine, abdominal or flank pain, changes in urine colour, and a burning sensation during urination.<sup>[3]</sup> Because of its high prevalence and frequent recurrence, urolithiasis remains

an important health concern and requires effective approaches for prevention, treatment, and management.

One such plant is *Pongamia pinnata* (L.) Pierre (Family: Leguminosae) is an important non-edible minor oilseed tree that grows mainly in the semiarid regions. It is probably originated from India and grows naturally in India, Pakistan, Bangladesh, Malaysia, Vietnam, Thailand, Florida, Australia, and Sri Lanka and also in northeastern Australia, Japan, Fiji, and the Philippines. In India, billions of *Pongamia* trees exist where *Pongamia* trees are cultivated commercially and seed is available from December to April.<sup>[2]</sup>



Fig 1: Tree.



Fig 2: Leaves.



Fig 3: Shells.

## MATERIALS AND METHODOLOGY

1. Extraction of *pongamia pinnata* by maceration method.
2. Evaluations for anti-urolithiatic Activity of *Pongamia pinnata* leaf.
1. Extraction of Leaves of *Pongamia pinnata* by Maceration Method<sup>[7]</sup>

50gm of dried powder is added to 200 ml of 70% Ethanol in a beaker and the mixture is macerated at 3 days, after the maceration the mixture was filtered by muslin cloth or filter paper. Then the filtrate is collected in a China dish and subjected for evaporation. After the evaporation the residue was collected and stored. All the prepared extracts were subjected to qualitative chemical tests to detect the presence of different classes of phytoconstituents.

### 2. Evaluations for anti – urolithiatic Activity<sup>[8]</sup>

#### Step-1: Preparation of experimental kidney stones (calcium oxalate stones) by homogenous precipitation

Equimolar solution of Calcium chloride dehydrate (AR) in distilled water and Sodium oxalate (AR) in 10ml of 2N H<sub>2</sub>SO<sub>4</sub> were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate. The resulting precipitate was calcium oxalate. Equimolar solution of Calcium chloride dehydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10ml of 2N H<sub>2</sub>SO<sub>4</sub>, was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium phosphate. Both precipitate freed from trace of sulphuric acid by Ammonia solution. Washed with distilled water and dried at 60°C for 4 hours.

## MORPHOLOGY

[Fig.1] *Pongamia pinnata* is a glabrous evergreen deciduous tree which grows up to 15–25 meter; it's a legume tree with a huge spreading canopy with linear or curved stem.

[Fig. 2] of 50–80 cm diameter and smooth or vertical grey-brown bark. The branches are hairless and have pale stipulate scars. The alternate imparipinnate leaves.

[Fig. 3] of *Pongamia pinnata* are elliptical in shape and covered with a hard shell.

### Step-2: Preparation of semipermeable membrane from farm egg

The semi-permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the egg in 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole is made on the top and the content squeezed out completely from the decalcified egg. Then egg membrane washed thoroughly with distil water, and placed it in ammonia solution, in the moisten condition and rinsed with distilled water. Stored in refrigerator at pH of 7-7.4.

### Step-3: - Estimation of Calcium oxalate by Titrimetric

Weighed exactly 1mg of calcium oxalate and 10mg of extract and packed it together in semi evaluation. Permeable by suturing as shown in model design fig. This was allowed to suspend in a beaker containing 100ml of buffer. One group served as negative control (containing only 1mg of calcium oxalate). Placed the conical flask containing all groups in incubator at 37°C for 2 hrs. Remove the content of semi permeable membrane from each group into a test tube. Added 2 ml of 1 N Sulphuric acid and titrated with 0.9494 N KMnO<sub>4</sub> to obtain pale pink as an end point. 1ml of 0.9494 N KMnO<sub>4</sub> equivalent to 0.1898mg of Calcium. The amount of undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning; to know how much quantity of calcium oxalate actually test substance could dissolved.

## RESULTS AND DISCUSSION

### 6.1. Identification, Collection and Authentication of plant material

The fresh plant leaves were collected in the month of June-July from the near home garden, Mandya district, Karnataka, India. It was duly identified and authenticated by Botanist, Dr. Thejesh Kumar M.P, Head of the

Department of Botany, Bharathi College. After collection the leaves was washed thoroughly with running tap water. Cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The voucher specimen was preserved in laboratory for further reference.

**Table No. 1: percentage yield of leaf extract obtained by solvent extraction.**

| Plant name              | Part used | Method of extraction | Solvents    | Colour     | Average extractive value (%w/w) |
|-------------------------|-----------|----------------------|-------------|------------|---------------------------------|
| <i>Pongamia Pinnata</i> | Leaves    | Maceration method    | Ethanol 70% | Dark brown | 22.8%                           |

### 6.6. Evaluation of Anti urolithiatic activity of *Pongamia pinnata*.

| Sl. no. | Group                                                              | Final weight of stone | Weight difference | % Dissolution |
|---------|--------------------------------------------------------------------|-----------------------|-------------------|---------------|
| 1       | Blank (1mg calcium oxalate)                                        | 1mg                   | 0mg               | -             |
| 2       | 1mg calcium oxalate +10 mg extract                                 | 0.59mg                | 0.41mg            | 41%           |
| 3       | 1mg calcium oxalate + 20 mg extract                                | 0.38mg                | 0.62mg            | 62%           |
| 4       | 1mg calcium oxalate + 1mg calcium phosphate+10mg extract           | 1.36mg                | 0.62mg            | 32%           |
| 5       | 1mg calcium oxalate +1mg calcium phosphate+20mg extract            | 0.9mg                 | 1.1mg             | 55%           |
| 6       | 1mg calcium phosphate +10 mg extract                               | 0.61mg                | 0.39mg            | 39%           |
| 7       | 1mg calcium phosphate +20 mg extract                               | 0.44mg                | 0.56mg            | 56%           |
| 8       | Standard 1mg calcium oxalate+10mg Cystone)                         | 0.13mg                | 0.87mg            | 87%           |
| 9       | Standard (1mg calcium phosphate+10mg Cystone)                      | 0.16mg                | 0.84mg            | 84%           |
| 10      | Standard (1mg calcium oxalate+1mg calcium phosphate+10 mg Cystone) | 0.6mg                 | 1.4mg             | 70%           |



**Fig. No 4: Semipermeable membrane.**



**Fig. No 5: Before Incubation.**

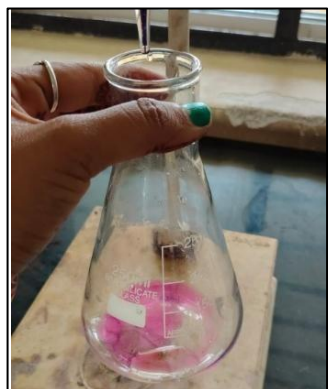


**Fig. No 7: Titrimetric Analysis.**



**Fig. No 6: After Incubation.**

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