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## Evaluation of *In Vitro* Antiurolithiatic Activity of *Syzygium cumini* Seeds

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### ABSTRACT

The present study was undertaken to evaluate the *in vitro* antiurolithiatic activity of the medicinal plant *Syzygium cumini* seeds. Ethanolic extract showed their maximum efficiencies in the dissolution of calcium oxalate crystals. Our results have clearly indicated that the Ethanolic seeds extract of *S. cumini* were quite promising for further studies in this regard. In this study Neeri was used as standard drug.

**KEYWORDS:** *In vitro* antiurolithiatic activity, Ethanolic extract, Urolithiasis, *Syzygium cumini*, Neeri.

### INTRODUCTION

Urinary stone occupy an important place in everyday urological practice. The average life time risk of stone formation has been reported in the range of 5-10 % in which there is a predominance of men over women that can be observed with an incidence peak between the fourth and fifth decade of life. Reoccurrence of stone formation is a common part of the medical care of patients with stone disease [1]. These stones may be classified on the basis of their constituent i.e. Calcium-containing stones, specially calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate are the most commonly occurring ones to an extent of 75-90%, magnesium ammonium phosphate (Struvite) to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1%. Out of all the types most common type is calcium oxalate or magnesium ammonium phosphate type which generally occurs commonly [2,3]. Many medications and remedies have been used during the past many years to treat urinary stones. Generally in the traditional systems of medicine, the majority of the remedies are based on plants and they were proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs and plants. Pharmacotherapy can reduce the recurrence rate. The use of plant products with claimed uses in the traditional systems of medicine assumes importance.

In the Ayurveda system of medicine in India, plants which belongs to 'Pashanabheda' group are claimed to be useful in the treatment of urinary stones. 'Pashanabheda' is the Sanskrit term used for a group of plants with diuretic and antiurolithiatic activities [4,5].

Drugs with multiple mechanisms of protective action may be one way forward in minimizing tissue injury in human disease [6]. Herbal medicines contain several phytoconstituents and exert their beneficial effects by multiple mechanisms like:

- By increasing the urine volume, pH and anti-calcifying activity (Diuretic activity) helps in spontaneous passage.
- By balancing the process of Inhibition and promotion of the crystallization in urine it affects the crystal nucleation, aggregation and growth (Crystallization inhibition activity)
- Relieves the binding mucin of calculi (Lithotriptic activity)
- By Improving renal function
- Regulation of oxalate metabolism
- Regulates the crystalloid colloid imbalance and improve renal function, thus prevents recurrence of urinary calculi.
- Improve renal tissue antioxidant status and cell membrane integrity and prevent reoccurrence (Antioxidant activity)

- ACE and Phospholipase A2 Inhibition
- Exerts significant anti-infective action in against the major causative organisms (Antimicrobial activity)
- Reveals marked improvement in symptoms of urinary calculi like pain, burning micturition and haematuria (Analgesic and anti-inflammatory activity) [7].

*Syzygium cumini* Linn (family Myrtaceae), commonly known as "Jamun" is widely distributed in tropical and subtropical regions. *S. cumini* has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties, which is widely used in folk medicine for the treatment of various diseases [8]. The therapeutic value of *S. cumini* has been recognized in different system of traditional medication for the treatment of different diseases and ailments of human beings. It contains several phytoconstituents belonging to the category of alkaloids, glycosides, flavonoids and volatile oil. It has been reported as a digestive, astringent, blood purifier and anthelmintic. It is reported as antibacterial, analgesic, anti-inflammatory, antioxidant, as well as gastro protective agents. It is also reported for the treatment of bronchitis, asthma, thirst, biliousness, dysentery, ulcers, diabetes. Several studies using modern techniques have authenticated its use in diabetes and shown promising results [9].

## MATERIALS AND METHODS

### Plant Material

The seeds of *S. cumini* seeds were collected in the month of march 2019 from Venkatapur, Medak dist. of Telangana, India. The plant was authenticated by M. Malla Reddy [M.sc,M.phil in botany] retired lecturer in botany, Vikarabad, Telangana. The seeds were washed with tap water and dried under shade.

### Preparation of Plant Extract

The seeds were shade dried and powdered. The crude plant extract was prepared by soxhlet extraction method. 50 g of powdered plant material was extracted with 500 ml of ethanol. The process of extraction was carried out up to 6 cycles, till the solvent in siphon tube of an extractor became colorless. The extract was filtered and evaporated to dryness using rotary evaporator. Further the dried extract was maintained in a refrigerator at 4°C for further antiurolithiatic activity.

### Chemicals Used

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

### Investigation of *In Vitro* Antiurolithiatic Activity Test By Titrimetry

The experimental kidney stones of Calcium Oxalate (CaOx) were prepared in the laboratory by taking equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2 N 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 H<sub>2</sub>SO<sub>4</sub> by ammonia solution, washed with distilled water and dried at 60°C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of CaOx and 10 mg of the extract, packed it together in semi permeable membrane of egg as shown in the model designed given below. This was allowed to suspend in a conical flask containing 100 ml of 0.1 M Tris buffer. First group served as blank containing only 1 mg of calcium oxalate. The second group served as positive control containing 1 mg of calcium oxalate and along with the 10 mg standard drugs, i.e. Neeri. The 3<sup>rd</sup> group along with 1 mg of CaOx contain methanolic and aqueous, extracts. 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 1 N H<sub>2</sub>SO<sub>4</sub> to each test tube and titrated with 0.9494 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 [10].

## RESULTS AND DISCUSSION

Drug therapy has developed in response to population health care [11] 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. 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 [12]. Unlike allopathic medicines which target is only one aspect of urolithiatic pathophysiology, most of plant based therapy have been shown to be effective at different stages of stone pathophysiology [13]. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials [14]. Plant based drug discovery programmes continue to provide an important source of new drug leads [15]. 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 [16].

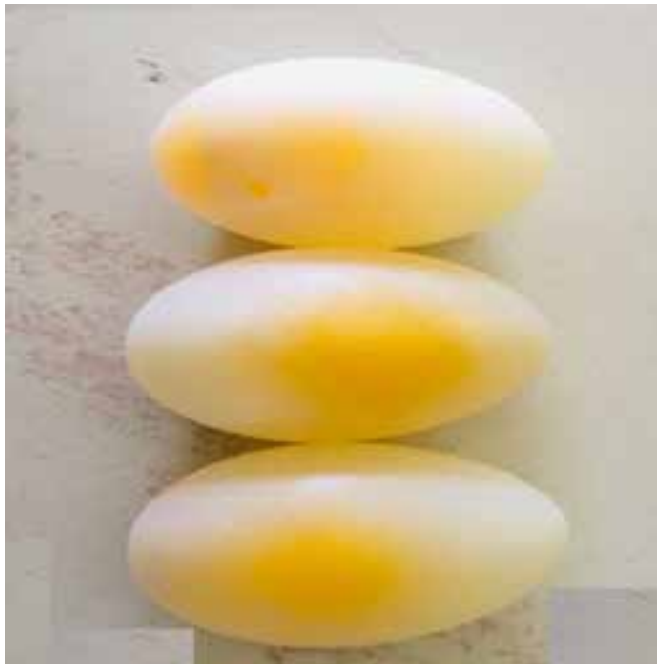
This study evaluates the antiurolithiatic activity of Ethanolic extract of *S. cumini* seeds. The highest percentage i.e. 98% of CaOx dissolution was observed in Ethanolic extract. Ethanolic extract of *S. cumini* seeds was found to be more effective in dissolution of calcium oxalate than standard drug Neeri. From this study, it was observed Ethanolic extracts of *S. cumini* seeds showed dissolution of calcium oxalate. This study has given primary evidence for *S. cumini* seeds as the plant which possess lithotriptic property. This *in vitro* study has given lead data and shown that Ethanolic extracts are quite promising for further studies in this regard.

**Table 1:** Shows % dissolution of Calcium Oxalate (CaOx) by *Syzygium cumini* seeds extracts

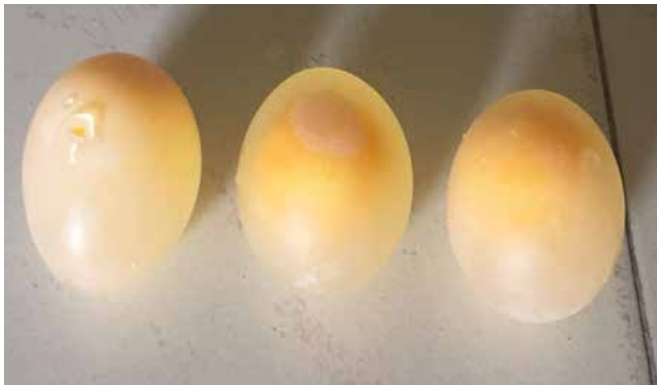
% of dissolution of calcium oxalate		
S. No	GROUPS	<i>Syzygium cumini</i> seeds
1.	Blank	0
2.	Positive Control	81
3.	Methanolic extract	98



**Figure 1:** *In vitro* experimental model setup to evaluate antiurolithiatic activity



**Figure 1(a):** Decalcification of egg shell in 10% Acetic acid overnight



**Figure 1(b):** Decalcified Eggs



**Figure 1(c):** Egg membrane along with the contents suspended into the 0.1 M Tris buffer

## CONCLUSION

In vitro Urolithiasis has been performed on the selected plant *S. cumini* by using the standard drug, Neeri. The work was performed by using in vitro antiurolithiatic model for calculating percentage dissolution of kidney stone. Ethanolic seeds extracts of *S. cumini* shows highest dissolution than standard drug Neeri. This study has

given primary evidence for *S. cumini* as the plant which possess antiurolithiatic property.

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