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Evaluation of *In Vitro* Anti-Urolithiatic Activity of *Elettaria cardamomum*

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ABSTRACT

The present study explores that evaluation of *in vitro* antiurolithiatic activity of *Elettaria cardamomum* seeds. It was observed that the highest calcium oxalate crystals dissolution was observed in the ethanolic extract of seeds of *E. cardamomum*. It was found that ethanolic extract of *E. cardamomum* has more efficient to dissolve calcium oxalate. In this study Neeri was used as standard drug.

INTRODUCTION

Kidney stones are a common cause of blood in the urine and pain in the abdomen, flank, or groin. Kidney stones occur in 1 in 20 people at some time in their life. The development of the stones is related to decreased urine volume or increased excretion of stone-forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate. The stones form in the urine collecting area (the pelvis) of the kidney and may range in size from tiny to staghorn stones the size of the renal pelvis itself. The process of stone formation, urolithiasis, is also called nephrolithiasis [1]. When the urea-splitting organisms infect the urinary tract, bacteria disintegrate the urea excreted in urine in the presence of urease enzyme, which subsequently trigger the formation of ammonia rendering the urine alkaline. In alkaline state, urine leads to contain precipitated crystals of calcium oxalate, magnesium phosphate and calcium carbonate in large amount thereby leading to a strong tendency to form calculi. Bacterial infection may induce stone formation by crystal adherence. Most of the urea-splitting organisms belong to species *Proteus* but, organisms such as *Pseudomonas*, *Staphylococcus*, *Escherichia coli* and even *Mycoplasma* were reported to be capable of producing urease [2].

The worldwide incidence of urolithiasis is quite high, and more than 80% of urinary calculi are calcium oxalate stones alone or calcium oxalate mixed with calcium phosphate [1]. However, the presence of certain molecules raise the level of supersaturation of salts needed to initiate crystal nucleation or reduce the rate of crystal growth or aggregation and prevents stone formation [3]. Calcium oxalate stones represent up to 80% of analyzed stones [4]. Calcium phosphate account for 15-25%, while 10-15% is mixed stones. The others are struvite 15-30%, cystine 6-10%, and uric acid stones 2-10% [5]. Calcium oxalate stones are of primary two types, calcium oxalate monohydrate (whewellite) and calcium oxalate dihydrate (weddellite). The occurrence frequency of whewellite is 78% while that of weddellite is 43% [6]. Though technological advancements have made dramatic improvement in the removal of urinary stones still some of the drawbacks of these methods exists which includes their being too costly for a commonman and recurrence of stone formation along with a number of other side effects [7]. Many medications and remedies have been used during the past many years to treat urinary stones. Endoscopic stone removal and extracorporeal shock wave lithotripsy have revolutionized the treatment of nephrolithiasis, but do not avoid the possibility of new stone formation [8]. Various therapies including thiazide diuretics and alkalictrate are being used in an attempt to prevent the recurrence of hypercalciuria and hyperoxaluria induced calculi, but scientific evidence for their efficacy is less convincing [9].

Medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheap source of drugs for majority of World's population. Several pharmacological investigations on the medicinal plants used in traditional antiurolithiatic therapy have revealed their therapeutic potential in the *in-vitro* or *in-vivo* models [10].

Herbal medicines have several phytoconstituent and exert their beneficial effects urolithiasis by multiple mechanisms like:

- Helps in spontaneous passage of calculi by increasing urine volume, pH and anti-calcifying activity (Diuretic activity)
- Balance the Inhibitor and promoter of the crystallization in urine and affects the crystal nucleation, aggregation and growth (Crystallization inhibition activity)•Relieves the binding mucin of calculi (lithotriptic activity)
- Improved 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) [11]

Despite the fact that the currently available stone removal techniques like Extracorporeal Shock Wave Lithotripsy (ESWL), Uteroscopy (URS), and Percutaneous Nephrolithotomy (PNL), are considered to be effective, but they are costly, making them an option of choice for limited number of patients and a compelling data suggest that these techniques have some serious side effects. Moreover, the high rate of recurrence in stone formation, which is around 50% at a 5 years follow-up, makes it a chronic condition which underscores the importance of preventive therapy. In spite of substantial progress in the study of the biological and physical manifestation of urolithiasis, its mechanism is still not clearly understood and there is no satisfactory drug available for the treatment of urolithiasis, especially for the prevention of recurrence of the stones [12].

Cardamom, the fruits of *Elettaria cardamomum* Maton. (Zingiberaceae) commonly known as "Heel khurd" is used in Unani system of medicine to treat gastrointestinal disorders. Cardamom is widely used for flavoring purposes in food and as carminative. In medicine it is used to treat gastrointestinal disorders. Despite its wide uses, little information has been reported on their pharmacological properties, which showed antioxidant and anti-inflammatory activity [13].

MATERIALS AND METHODS

Plant Material

The seeds were collected from market, Narsapur (Mdl), Medak (Dist) of Telangana in the month of February 2019. The plant was authenticated by M. Mallareddy (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 of plant were dried under shade and crushed in pulverize and powdered. These powdered plant material was extracted with Ethanol in a soxhlet apparatus for 72 hours. After complete the extraction, the extracts were cooled at room temperature and filtered and evaporated to dryness using rotary evaporator.

Chemicals Used

Neeri, sodium oxalate, Tris buffer, calcium chloride, Potassium Permanganate (KMnO_4), Sulphuric acid (H_2SO_4).

Investigation of *In Vitro* Antiurolithiatic Activity By Titrimetry

The experimental kidney stones of Calcium Oxalate (CaOx) were prepared in the laboratory by taking equimolar solution of calcium

chloride dihydrate in distilled water and sodium oxalate in 10 ml of 2 N H_2SO_4 . 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. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed together in semipermeable 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 CaOx and along with the 10 mg standard drugs, i.e. Neeri. The 3rd group along with 1 mg of CaOx contain Ethanolic extracts. The conical flasks of all groups were kept in an incubator preheated to 37°C for 2 h. Remove the contents of semipermeable membranes from each group into separate test tubes, add 2 ml of 1 N H_2SO_4 to each test tube and titrated with 0.9494 N KMnO_4 till a light pink colour end point obtained. The amount of remaining undissolved CaOx 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 [14].

RESULTS AND DISCUSSION

In the present study, Titrimetry method was used to assess the antiurolithiatic activity of Ethanolic extract of seeds of *E. cardamomum*. this study, it was observed that Ethanolic extract *E. cardamomum* seeds showed antiurolithiatic activity more than that of the standard This study has given primary evidence for the plant which possess lithotriptic property. This *in vitro* study has given lead data and shown that Ethanolic extract of *E. cardamomum* is quite promising for further studies in this regard.

Table 1: Shows % dissolution of Calcium Oxalate (CaOx) by *in vitro* antiurolithiatic activity of *Elettaria cardamomum* fruits extracts

% of dissolution of calcium oxalate		
S. No	GROUPS	<i>Elettaria cardamomum</i>
1.	Blank	0
2.	Positive Control	81
3.	Methanolic extract	99



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

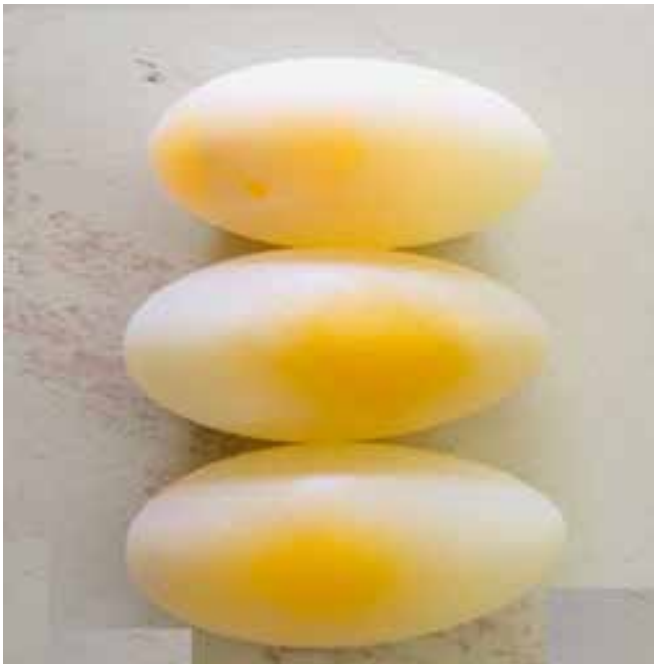


Figure 1(a): Decalcification of egg shell in 10% Acetic acid overnight



Figure 1(b): Decalcified Eggs



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

CONCLUSION

In the present work, the dissolution of calcium oxalate crystals by Ethanolic extract of seeds of *E. cardamomum* was studied by using the standard drug, cystone. The work was performed by using *in vitro* antiurolithiatic model for calculating percentage dissolution of kidney stone. This study has given primary evidence for *E. cardamomum* as the plant which possess antiurolithiatic property.

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