



Research Article

EFFECT OF SEED EXTRACTS OF *Elettaria cardamomum* ON CALCIUM OXALATE CRYSTALLIZATION

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ABSTRACT

Kidney stone has tormented humans since the earliest records of civilization. Urinary stones are one of the oldest and the most common afflictions in humans. Calcium containing stones are the most common comprising about 75% of all urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) or a mixture of both (45%). 10% of men and 3 % of women have a stone during their adult lives. A number of plants have been mentioned in the Indian ayurvedic system, which can prevent formation or dissolve kidney stones. In the present study, the inhibitory potency of different extracts of seeds of *Elettaria cardamomum* were evaluated on various stages of formation and on the growth of calcium oxalate crystals *in vitro*. A result obtained showed that alcoholic and aqueous extract has the higher capacity to inhibit the crystal formation and aggregation as compared to ethyl acetate and petroleum ether extracts.

KEY WORDS: Calcium oxalate, *Elettaria cardamomum*, antiurolithiasis.

INTRODUCTION

Urolithiasis is a recurrent renal disease affects 4-8% in UK 15% in US and 11% in India. There are several types, most commonly consisting of calcium phosphates and calcium oxalates; others are composed of agnesium ammonium phosphate, uric acid or cystine. Epidemiological data suggest that 60-80% of stone is composed mainly of calcium oxalate. Stones formation occurs when urinary concentrations of stone forming salts, exceed the limit of metastability for that salt in solution. This most often reflects excessive excretion of one or more stone constituents, deficient inhibitory activity in urine, or simply a low urine volume resulting in excessively concentrated urine.(1)

In the present investigation, CaOX crystals were grown in the artificial urine technique. The effect of different extract of seeds of *Elettaria cardamomum* was studied on the growth and inhibition of CaOX crystals. In this work, we performed an *in vitro* crystallization study enabling the specification of kinetic and thermodynamic conditions of formation and growth of crystalline species. The slow and controlled diffusion of species to the growing crystals is very useful to study the growth and inhibition of Whewellite crystals *in vitro*. Different experimental procedures have been proposed using synthetic, diluted or natural supersaturated aqueous solutions of urine (2). Crystallization can be triggered by adding, to reaction medium calcium, oxalates or phosphates, or by crystalline germination of the species under investigation. Crystallization can also take place, by changing the pH of substances having pH – dependent solubility (3). Therefore, it is worthwhile to look for an alternative to these means by using medicinal plants (4). In this regard, many plants have been used to treat kidney stones and showed to be effective among them medicinal plants. The plants studied are Mediterranean traditional medicinal plants widely used in India to treat lithiasis. Here we studied invitro antiurolithiatic activity of seeds of *Elettaria cardamomum*.

MATERIALS AND METHODS

Plant material and preparation of extract

Elettaria cardamomum fruits were collected. seeds were separated out and authenticated. The seeds were ground to coarse powder. The aqueous extracts were prepared by decoction while alcoholic (methanol), ethyl acetate and petroleum ether extract by Soxhalation. The extracts were evaporated under vacuum and stored in the airtight container.

Nucleation assay

Solution of calcium chloride and sodium oxalate were prepared at the final concentrations of 5 mmol/L and 7.5 mmol/L respectively in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950 μ L of calcium chloride solution mixed with 100 μ L of herb extracts at the different concentrations (100 μ g/ml to 1000 μ g/ml). Crystallization was started by adding 950 μ L of sodium oxalate solution. The temperature was maintained at 37 °C. The Optical Density of the solution was monitored at 620 nm. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control (5,6).

The growth of crystals was expected due to the following reaction:

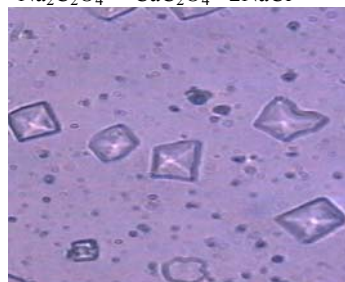
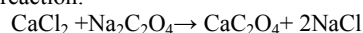


Figure 1: Micrograph of CaOX crystals induced in solution by adding NaOx solution in absence of herb extract.

Aggregation assay

The method was described by Atmani and Khan (6). With some minor modifications. 'Seed' CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both solutions were equilibrated to 60°C in a water bath for 1 hr and then cooled to 37°C overnight. The crystals were harvested by centrifugation and then evaporated at 37°C. CaOX crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments

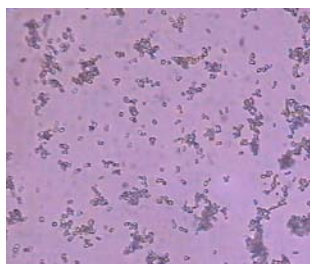
were conducted at 37°C in the absence or presence of the plant extract after stopping the stirring. The percentage aggregation inhibition rate was then calculated by comparing the turbidity in the presence of the extract with that obtained in the control using following formula (7):

$$I_r = \left(1 - \frac{\text{Turbidity}_{\text{sample}}}{\text{Turbidity}_{\text{control}}} \right) \times 100$$

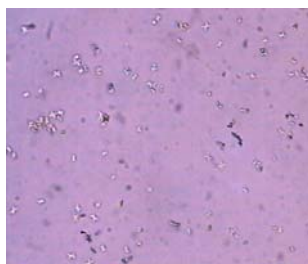
RESULT**Table 1: Effect of *Elettaria cardamomum* seed extracts on different stages of crystallization.**

| Concentration (µg/ml) | <i>Elettaria cardamomum</i> | | | | | | | | | | | |
|--------------------------|-----------------------------|----|----|---------------|----|----|-------------------------|----|----|-----------------------|----|----|
| | Alcoholic extract | | | Water extract | | | Petroleum ether extract | | | Ethyl acetate extract | | |
| | N | G | A | N | G | A | N | G | A | N | G | A |
| Control | + | + | + | + | + | + | + | + | + | + | + | + |
| 100 | + | + | + | + | + | + | +- | +- | + | + | + | + |
| 200 | + | + | + | + | + | + | + | + | + | +- | +- | + |
| 400 | +- | +- | +- | + | + | + | + | + | + | +- | +- | + |
| 600 | +- | - | +- | +- | +- | +- | + | + | + | + | + | + |
| 800 | - | - | - | - | - | - | + | +- | +- | + | +- | +- |
| 1000 | - | - | - | - | - | - | + | - | - | + | - | - |

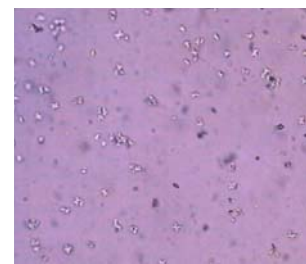
(+) = Action on the stage, (-) = No action on the stage, (+-) = More or less action
N= Nucleation, G= Growth, A= Aggregation.

Alcoholic extract of Elettaria cardamomum

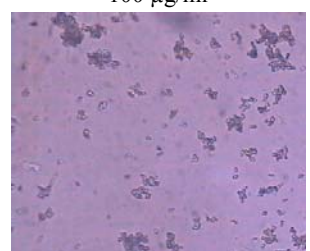
100 µg/ml



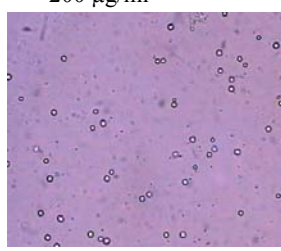
200 µg/ml



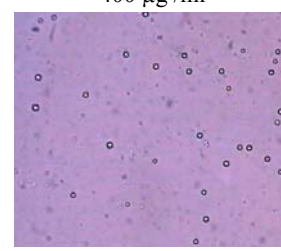
400 µg/ml



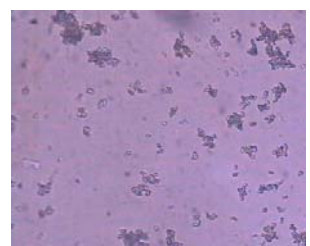
600 µg/ml



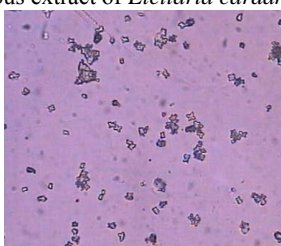
800 µg/ml



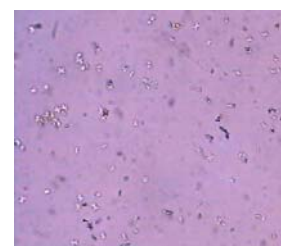
1000 µg/ml

Aqueous extract of Elettaria cardamomum

100 µg/ml



200 µg/ml



400 µg/ml

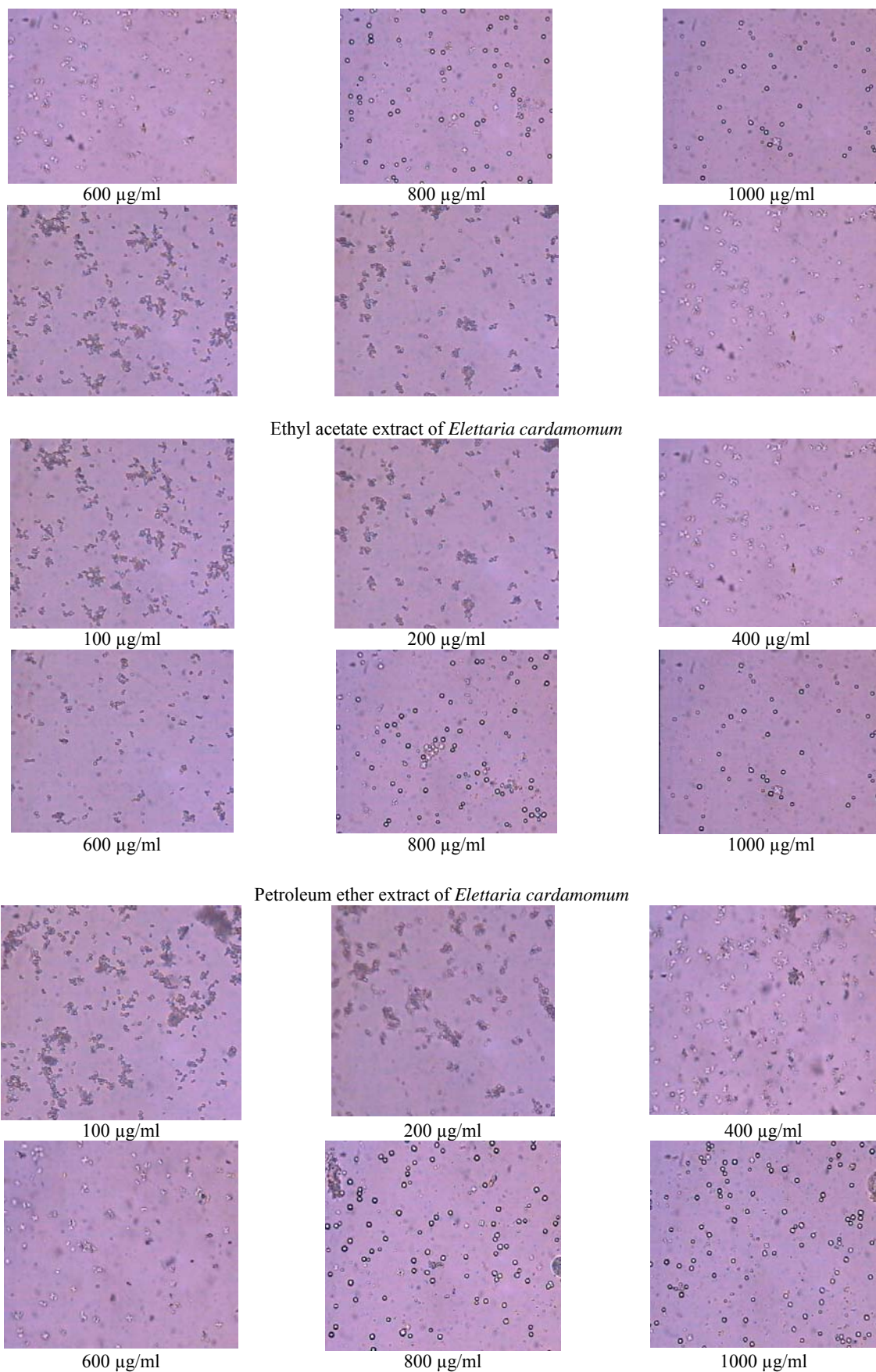


Figure 2: Micrograph of CaOX crystals induced in solution by adding NaOX solution in presence of various extract of *Elettaria cardamomum* at different concentrations.

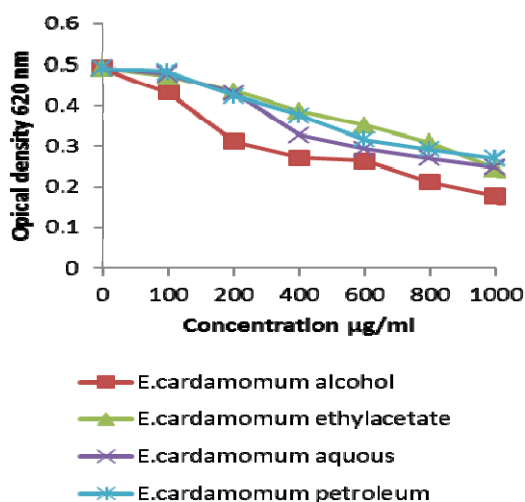


Figure 3: The effect of different concentration of *Elettaria cardamomum* extracts on CaOX crystal nucleation.

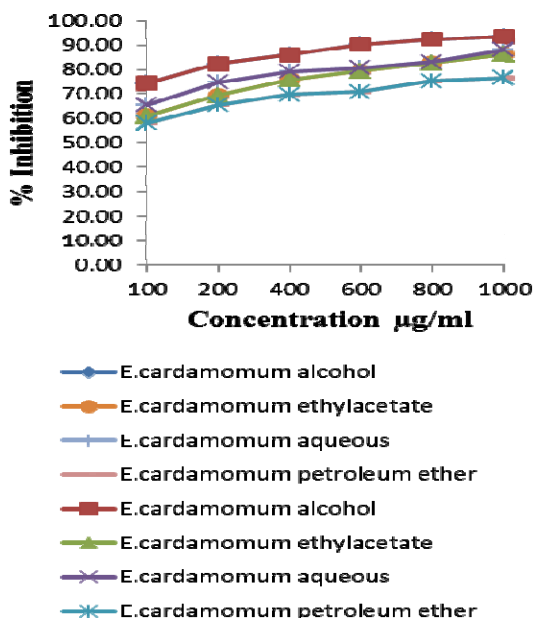


Figure 4: The effect of different concentration of *Elettaria cardamomum* extracts on CaOX crystal aggregation.

Kidney oxalate stone is the result of supersaturation of urine with certain urinary salts such as calcium oxalate. Since crystallisable oxalate species are pH independent, the crystallization of oxalate in the absence of an inhibitor, led to the formation of calcium oxalate monohydrate monitored by light microscope (Magnus MIPS (camera), Magnus MLX (microscope)), the process of calcium oxalate crystallization in control without the addition of inhibitors is shown in (Figure 1).

In the crystal growth experiments sown nucleation, growth and aggregation, the rate of crystallization is usually controlled by the number of crystals of calcium oxalate as a function of time, following the introduction of seed crystals. Entitled constant volume against time in the composition calcium oxalate experiments determined that the

rate of growth of crystals was made in the absence and presence of plants extract.

We followed the same experimental procedure for the study of crystallization in the presence of inhibitors. In order to assess the inhibiting potential of substances for oxalate crystallization and understand the mechanisms of action of these inhibitors on oxalate crystallization steps viz. nucleation, growth, aggregation, we tested the effectiveness of medicinal plants. As shown in the Table 2 nucleation, growth and aggregation were gradually decreased as the concentration increased. Alcoholic and aqueous extract has a greater capacity to reduce all these crystallization processes as compared to petroleum ether and ethyl acetate extract. (Figure 2)

Figure 3 shows the graph of nucleation as optical density at 620 nm with respect to concentration of herb extracts. The higher concentrations of herb extract were associated with fewer crystals, and the size decreased proportionally. Alcoholic and aqueous extracts have capacity to decrease the nucleation process, so the crystallization as compared to other extracts. (Figure 2)

The % inhibition of turbidity (aggregation) in the presence of herb extracts was lower than in the control, showing that crystals were less aggregated. The inhibited aggregation associated with the extract increased with concentration. This inhibition was found with alcoholic and aqueous extract. (Figure 4).

DISCUSSION

The supersaturation of urine with CaOX, the most common component of kidney stones (8), is an important factor in crystallization, with later factors being nucleation, growth and aggregation. Thus if supersaturation or later steps in crystallization can be prevented, then lithiasis should be avoided. Indeed, several measures are usually taken to reduce supersaturation, e.g. increasing fluid intake and medical therapy. Although treatments have improved considerably, it is generally accepted that better strategies for preventing kidney stones need to be developed (6).

The main findings of the present study were that extracts from plants inhibited the crystallization of CaOX in solution; there were less and smaller particles with increasing concentrations of extract. These results were confirmed in the nucleation assay, which showed that the extract contained nucleation preventing agents.

The limiting factors in stone formation could be those processes that affect the size of the particles formed, because particles may become large enough to occlude the urinary tract, leading to stone formation. The extract of the plants causes fewer numbers of crystals in solution, thereby reduced supersaturation and the size of the particles. This property of the extract is therefore, advantageous, preventing urinary stone formation by inducing the excretion of small particles from the kidney and reducing the chance of retention in the urinary tract.

The herb extracts may contain substances that inhibit the growth of CaOX crystals. This property of plants may be important in preventing kidney stone formation; CaOX crystals induced by urinary macromolecules was less tightly bound to epithelial cell surfaces, which are then excreted with urine (9). The extract may also contain substances that inhibit CaOX crystal aggregation; the agglomeration of particles is a critical step in urinary stone formation, as larger crystals are less likely to pass spontaneously in the urinary tract (10). If the extract keeps CaOX

particles dispersed in solution they are more easily eliminated.

Thus, this study puts forth the possibility of using phytochemicals present in the plants as therapeutic agents to treat urolithiasis, but still it warrants further investigation both, in elaborated experimentation and human trials.

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