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INHIBITION OF CALCIUM OXALATE CRYSTALLIZATION BY THE LEAVES EXTRACT OF KALANCHOE PINNATA

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ABSTRACT

In the present study the leaves of *Kalonchoe pinnata* have been selected for their antiurolithiatic activity. The extract was subjected to phytochemical screening and found to contain flavanoid, phenolic compounds and saponins. The given research work was studied under *in vitro* conditions using two critical assays such as crystal nucleation and aggregation assay. The inhibitory potential alcoholic extract of the *Kalonchoe pinnata* were tested for the Calcium oxalate crystal formation, which are predominantly present in most of the kidney stones, under in vitro conditions. The nucleation and aggregation of calcium oxalate crystals were measured separately using Spectro -photometric methods. A result obtained showed that alcoholic extract *Kalonchoe pinnata* has the higher capacity to inhibit the crystal formation and aggregation.

KEYWORDS: Kidney stones, *Kalonchoe pinnata*, nucleation assay, aggregation assay.

INTRODUCTION

Renal or urinary calculi also called urolithiasis, is a condition which involves the process of formation and retention of stone(s) in kidney, bladder and/or urethra that results in renal colic, urine retention and pain in the abdomen and flank.[1] It is estimated to occur in approximately 12% of world population and 50% of recurrence rate in 5-10 years of treatment.[2] The pathogenesis of urolithiasis involves the imbalance between promoters and inhibitors of crystallization in the kidneys.[3] The mechanism of calculi formation is a complex process concerned with supersaturation, nucleation, aggregation, growth and retention of crystals within the renal tubules.[4] Among urinary stones, majority of stones are

calcium oxalate (CaOx).[5] The etiopathogenesis of renal calculi is multifactorial involving anatomic, environmental, infections, metabolic and dietary lifestyle habits.[6,7]

Medical or surgical stone removal and extracorporeal shock wave lithotripsy are the latest options to manage and treat such stone disorders. But they are expensive, pose various side effects and also do not prevent the recurrence of stone formation.[8,9] Hence, there is a growing interest towards the use of medicinal plants for correction of stone disorders and ailments. Ayurvedic system of medicine also advocates the use of various medicinal plants and their formulations for the treatment of urinary stones and kidney diseases.[10,11]

Bryophyllum pinnatum (Lam.) Oken (Crassulaceae) is a perennial herb. The other synonyms are *Bryophyllum calycinum* and *Kalanchoe pinnata*. It is widely distributed in Madagascar, tropical Africa, tropical America, India, China and Australia.[12] In Ayurveda, the plant is also known as *Pāṣāṇabheda* which means "dissolver of stones".[13] The leaves of *Bryophyllum pinnatum* are widely used in traditional and ethnomedicinal practices for treatment of urinary insufficiency and stone disorders. The fresh leaf juice or along with the powder of 2-3 black peppers (*Piper nigrum* Linn.) is used as folklore medicine by various tribes of Muzaffarnagar (Uttar Pradesh),[14] The alcoholic extract of the leaves of *Kalanchoe pinnata* showed antioxidant activity.[15]

Previously, Yasir *et al*. reported *in-vitro* inhibitory activity of the leaves of *Kalanchoe pinnata* on calcium oxalate crystallisation.[16] In view of the traditional and ethnomedicinal use of leaves of *Kalanchoe pinnata* for the treatment of kidney and bladder stones, and urinary insufficiency, it was thought worthwhile to investigate its effect on experimentally induced *in-vivo* lithiatic model. Hence, the present study evaluates the effects of alcoholic extract of *Kalanchoe pinnata* leaves on formation of urinary calculi (urolithiasis).

MATERIALS AND METHODS

Plant material: The leaves of *Kalanchoe pinnata* were collected from Salem, Tamil Nadu. About 1.0 kg of shade-dried coarse powders of the plant material were extracted with 80%v/v aqueous ethanol by maceration at room temperature for 72 h. After the completion of each extraction, the extracts were filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50°C). The residues were stored in a vacuum desiccator for further use.

Preliminary phytochemical screening: For preliminary phytochemical screening, the extracts was tested for the presence of alkaloids, flavonoids, phenols saponins, steroids, terpenoids, anthraquinones, proteins and aminoacids following the standard procedures.[17]

Experimental protocol: The effect of extracts on CaOx crystallization was determined by the time course measurement of turbidity changes due to the crystal nucleation and aggregation. The precipitation of calcium oxalate at 37 °C and pH 6.8 has been studied by the measurement of turbidity at 620 nm. A spectrophotometer UV/Vis was employed to measure the turbidity of the formation of calcium oxalate.

Nucleation assay: We chose the classical model for the study of oxalate crystallization because of its simplicity and satisfactory repro-ducibility. This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of any chemical species used. Solution of calcium chloride and sodium oxalate were prepared at the final con-centrations 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. Calcium chloride solution (950 ml) mixed with 100 ml of leaves extract at the different concentrations (100 mg/ml to 500 mg/ml). Crystallization was started by adding 950mL of sodium oxalate solution. The temperature was maintained at 37 °C. The OD 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.

$$\% \text{ inhibition} = (Abs_{Control} - Abs_{Test}) / Abs_{Control} \times 100$$

Where; Abs_{Test}: Absorbance in the presence of inhibitor (Extract), $Abs_{Control}$: Absorbance without inhibitor.

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

$$CaCl_2 + Na_2C_2O_4 \rightarrow CaC_2O_4 + 2NaCl$$

Aggregation assay: The method used was similar to that described by Atmani and Khan.[18] 'Seed' CaOx monohydrate (COM) crystals were prepared by mixing cal-cium chloride and sodium oxalate at 50 mmol/L. Both solutions were equilibrated to 60°C in a water bath for 1h 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. [19]

% aggregation inhibition rate = (1-Turbidity$_{sample}$ / Turbidity$_{control}$) X 100

Scanning Electron Microscope: The CaO crystals were analyzed using a VEGA3 TESCAN (Czech Republic) scanning electron microscope (SEM).

RESULTS AND DISCUSSION

Crystalline calcium oxalate monohydrate (COM) is the most common material that create kidney stones.[20] The main findings of the present study was that extract from *K. pinnata* leaves inhibited the crystallization of CaOx in solution. Fig. 1 showed CaOx crystallization without the addition of extract (control) while Fig. 2 showed CaOx crystallization in the presence of extract in the concentration of 100, 300 and 500 mg/ml respectively. 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.

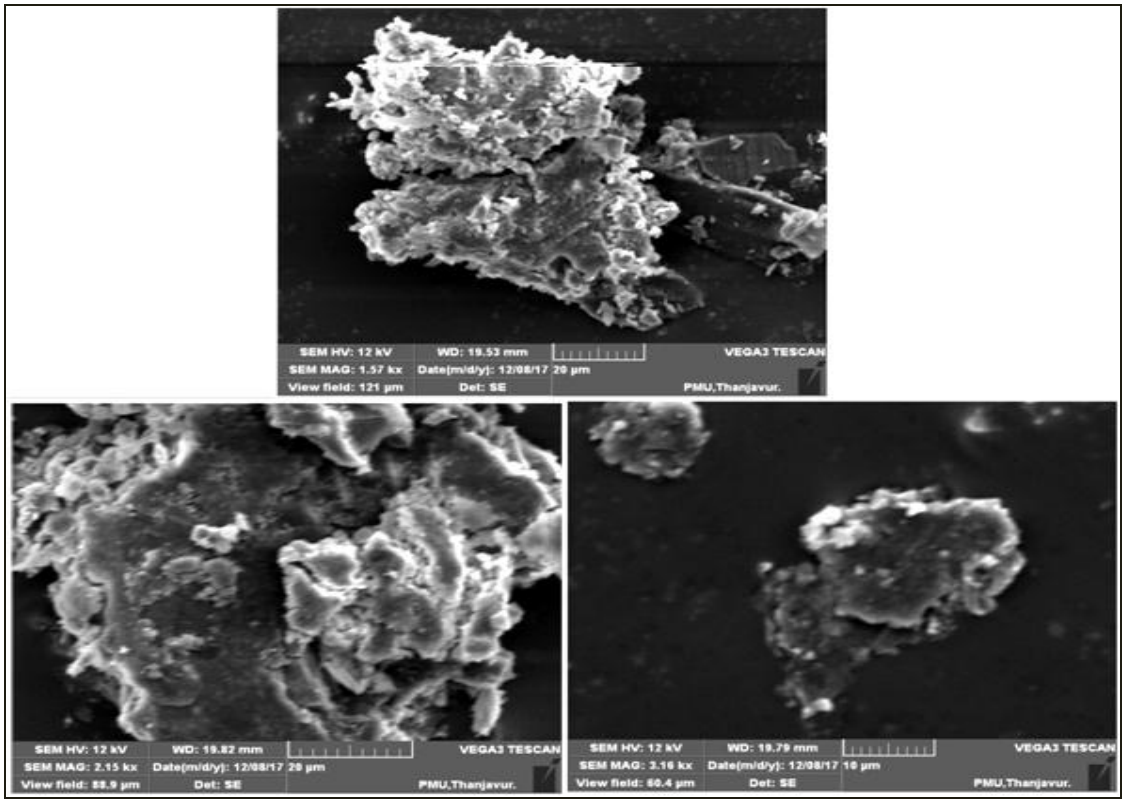

Fig. 1: Scanning electron micrograph of CaOx crystals without plant extract.

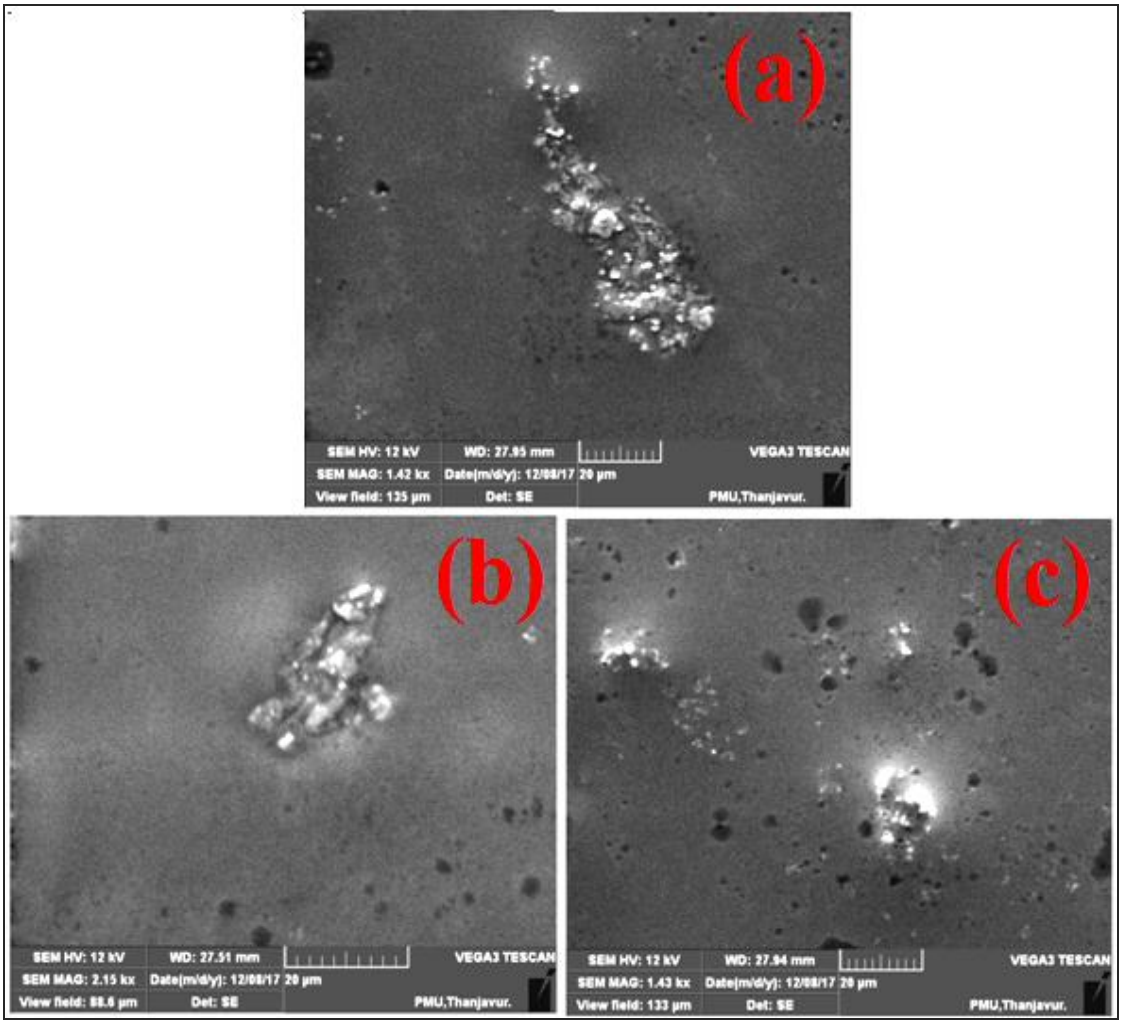

Fig. 2: Scanning electron micrograph of CaOx crystals in presence of plant extract on different concentrations. (a) 100mg/ml, (b) 300mg/ml, (c) 500mg/ml.

There were less and smaller particles with increasing concentrations of extract as shown in various SEM micrographs (Fig.2a-2c). The main findings of the present study that plant extract inhibited the nucleation and aggregation process of kidney stone formation. The leaves extract may contain phytochemicals like saponins, phenolic, flavanoids compounds etc., that inhibit the growth of CaOX crystals, such phytochemicals that inhibit CaOX crystal aggregation. The increasing concentration of plant extracts (100, 200, 300, 400 and 500mg/ml) had inhibited the CaOx crystal growth (Fig. 3 and Fig.4).

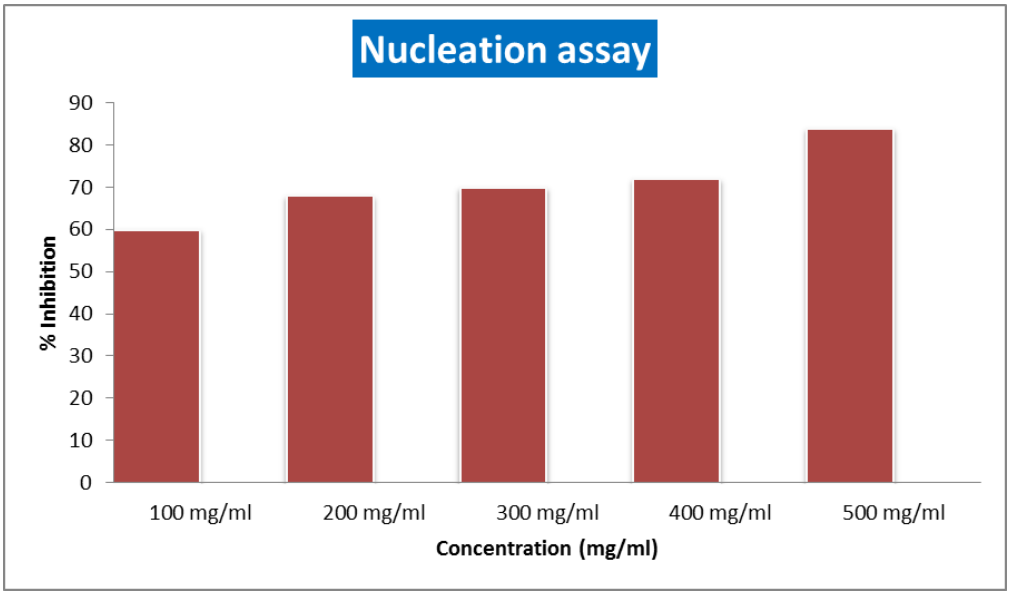

Fig.3: Effect of different concentrations of leaf extract of *K.pinnata* CaOx crystallization.

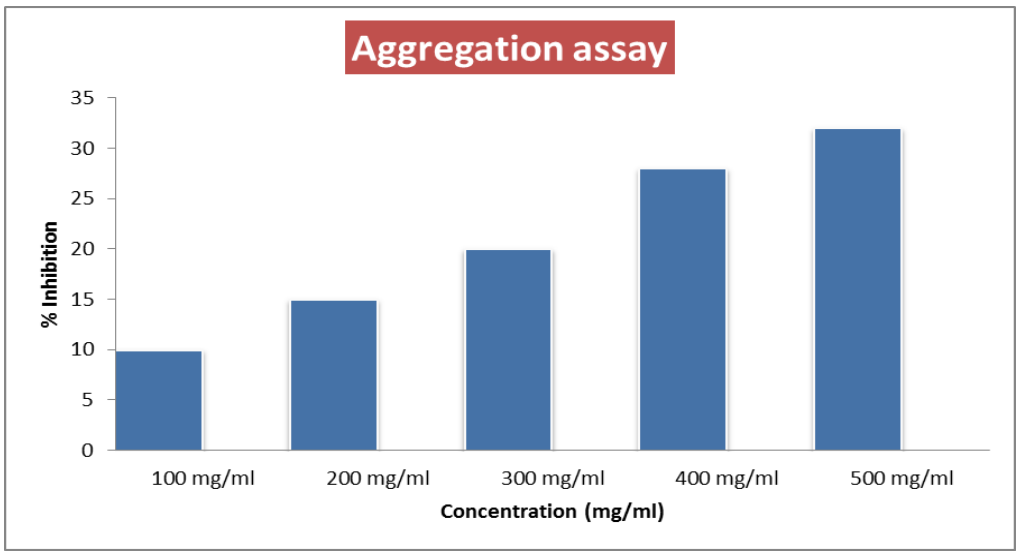

Fig.4: Effect of different concentrations of leaf extract of *K.pinnata* CaOx crystallization.

Maximum inhibition of nucleation 84 % was observed for *K.pinnata* leaves extract at concentration of 500 mg/ml (Fig.3). The plant extract causes fewer numbers of crystals in solution, thereby reduced supersaturation and the size of the particles. This property of the extract is therefore, advantageous in preventing urinary stone formation by inducing the excretion of small particles from the kidney and reducing the chance of retention in urinary tract.

CONCLUSION

The main findings of the present study were that *K.pinnata* leaves extract 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. From the results obtained, it is concluded that *K.pinnata* leaves extract has the higher capacity to inhibit the calcium oxalate crystal formation and aggregation.

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