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PHYTOCHEMICAL EXAMINATION AND *IN VITRO* INVESTIGATION ON ANTI UROLITHIATIC ACTIVITY OF COCONUT COTYLEDON

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ABSTRACT

Aim: Coconut cotyledon, a plant part belongs to Arecaceae family rich in nutrients and bioactive compounds such as alkaloids, flavonoids, tannins, phenols, glycosides, and saponins, which are known to contribute to antioxidant and effects but rarely explored for medicinal applications. Urolithiasis, or kidney stone formation, is a widespread disorder caused by the accumulation of insoluble crystalline substances such as calcium oxalate in the renal system. Our aim is to evaluate the *in vitro* anti-urolithiatic activity of the Ethanolic extract of coconut cotyledon. **Material and methods:** The cotyledons were collected, authenticated, and extracted using the hot percolation method with ethanol as solvent. Preliminary phytochemical screening was performed. The *in vitro* anti-urolithiatic activity was investigated through crystal inhibition, nucleation, aggregation, and calcium oxalate dissolution assays. **Result:** In the inhibition assay, the extract achieved a maximum inhibition of 66.46% at 1000 µg/ml, comparable to the standard drug Cystone (71.28%). The nucleation and aggregation assays also showedsignificant inhibition, with the highest concentration (1000 µg/ml) yielding 68.13% and 62.16% inhibition, respectively, closely matching Cystone's activity. Furthermore, the EECC (Ethanolic Extract of Coconut Cotyledon) promoted the dissolution of preformed calcium oxalate crystals, achieving a maximum dissolution of 48.53% at 1000 µg/ml, suggesting a potential effect. **Conclusion:** In conclusion, the ethanolic extract of coconut cotyledon exhibits significant *in vitro* anti- urolithiatic activity by inhibiting the formation and aggregation of calcium oxalate crystals and promoting their dissolution. These encouraging results provide preliminary scientific evidence supporting its traditional use and warrant further investigation in animal models to confirm its efficacy and safety in a biological system.

KEYWORDS: *Cocos nucifera*, Urolithiasis, Calcium oxalate crystallization, *In vitro* anti-urolithiatic activity, Ethanolic extract.

INTRODUCTION

Urolithiasis, commonly known as kidney stone disease, is a chronic and recurrent disorder of the urinary tract characterized by the formation of crystalline concretions within the kidneys or other parts of the urinary system. It represents a significant global health concern, with a steadily rising incidence across different age groups and geographical regions.[1,2] Epidemiological studies indicate that calcium-containing stones, particularly calcium oxalate calculi, account for nearly 70–80% of all urinary stones.[3] The high recurrence rate of urolithiasis, even after successful stone removal, poses a major therapeutic challenge and emphasizes the need for effective preventive strategies.[4]

The pathogenesis of calcium oxalate stone formation is a complex, multistep process involving urinary supersaturation, crystal nucleation, growth, aggregation, and retention within renal tubules.[5] These events are further influenced by oxidative stress, urinary

macromolecules, dietary factors, metabolic abnormalities, and renal epithelial injury.[6] Although modern treatment modalities such as extracorporeal shock wave lithotripsy and surgical interventions are effective in stone removal, they do not adequately prevent recurrence and may be associated with adverse effects and high treatment costs.[7] Pharmacological agents used for prevention often exhibit limited efficacy or undesirable side effects, reinforcing the demand for safer and multi-targeted therapeutic alternatives.[8]

Medicinal plants have long been utilized in traditional systems of medicine for the management of urolithiasis, owing to their diuretic, antioxidant, anti-inflammatory, and crystallization-inhibitory properties.[9] Several plant-derived compounds are known to interfere with calcium oxalate crystallization by inhibiting nucleation and aggregation or by promoting the dissolution of preformed crystals.[10] These properties make phytotherapeutic agents attractive candidates for the development of complementary or alternative approaches to urolithiasis management.

Cocos nucifera L. (Arecaceae), commonly known as coconut, is a widely cultivated tropical plant with well-documented nutritional and medicinal value. Various parts of the coconut plant, including the water, kernel, oil, husk, and inflorescence, have been reported to possess antioxidant, nephroprotective, and anti-inflammatory activities.[11,12] However, the coconut cotyledon—an embryonic tissue formed during seed germination—has received minimal scientific attention despite its rich content of bioactive phytochemicals such as flavonoids, phenolic compounds, tannins, alkaloids, and saponins.[13] These constituents are known to modulate oxidative stress and crystal–cell interactions, which play a critical role in the initiation and progression of kidney stone formation.[14]

Given the limited data available on the pharmacological potential of coconut cotyledon, systematic evaluation using established *in vitro* urolithiasis models is warranted. *In vitro* assays such as calcium oxalate crystal inhibition, nucleation, aggregation, and dissolution provide valuable preliminary evidence for assessing anti-urolithiatic activity and screening novel therapeutic candidates.[15] Therefore, the present study was undertaken to investigate the phytochemical profile and *in vitro* anti-urolithiatic potential of the ethanolic extract of coconut cotyledon, with the aim of providing scientific validation for its possible role in the prevention and management of calcium oxalate urolithiasis.

MATERIALS AND METHODS

Collection of plant material: Germinated coconut (Cocos nucifera) was procured from local markets of Nellore district, Andhra Pradesh. The plant material was authentified by Dr. K. Madhava chetty, Asst. Prof, Department of Botany, S.V. University, Tirupathi, Andhrapradesh, India. It was preserved for future reference in our Pharmacognosy department. Just after collection, the coconut embryo was washed thoroughly with running tap water. It was kept away from direct sunlight to avoid destruction of active compounds, shade dried at room temperature and was grounded mechanically into a coarse powder.

Preparation of plant extract

The cotyledons were separated from the shell sliced into pieces (0.5 cm^3) and was subjected to extraction. The experiment was started by building a rig using stands and clamps to support the extraction apparatus. Following this the solvent (ethanol- 500ml) was added to the round bottom flask, which was attached to a soxhlet extractor and condenser on an isomantle. The plant material was loaded onto the thimble, and was placed inside the soxhlet extractor. The side arm was lagged with glass wool. The solvent was heated using the isomantle and it started to evaporate, moving through the condensor. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon tube it was poured back into the flask and the cycle begins again. The process was runned for 16 hours. The alcohol was evaporated using a rotary evaporator, leaving a small yield of extract (3.78%) in glass bottom flask**.** and the extract will be stored at 4-5^0 C. Double-distilled water was used to redissolve the extract prior to experimentation to evaluate anti-cataract activity.[16]

The Percentage yield of extract was calculated

$$\text{Yield}[\%] = \frac{wt\ of\ solvent\ free\ extract\ (g)}{Dried\ extract\ wt} X100$$

Preliminary Phytochemical Screening

Standard screening test of the Ethanolic extract of coconut cotyledon (EECC) was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites using standard procedures.[17]

***In vitro* Models for Anti-urolithiatic Activity**

1. Inhibition assay

In a beaker, 50 mL of the Ethanolic extract of coconut cotyledon was placed, and the two salt-forming solutions was added in drops with the aid of burettes forming the reservoir. After that the mixture was boiled for 10 min on a heating mantle, of the Ethanolic extract of coconut cotyledon cooled to room temperature. The precipitate was collected by centrifuging small volumes at a time and the supernatant was discarded in a preweighed centrifuge tube. After drying in a hot air oven, the precipitate-filled tube was cooled to room temperature and weighed with a weighing balance until it reached a constant weight. The precipitate's weight was calculated. Water was used as the blank in this experiment. All of the tests was carried out at room temperature. The following formula was used to calculate the percentage efficiency of both individual and of the Ethanolic extract of coconut cotyledon extracts. Whereas, WB stands for

the weight of blank tubes, and WS stands for the weight of test sample tubes.[18]

$$Percenta\,ge\ Inhi\,biti\,on = \frac{(WB - WS)}{WB} \times 100\%$$

Whereas, WB stands for the weight of blank tubes, and WS stands for the weight of test sample tubes.

2. Nucleation assay

A buffer containing 0.05 M Tris–HCl and 0.15 M NaCl at pH 6.5, a solution of calcium chloride (5 mM) and sodium oxalate (7.5 M) will be prepared. A 9 mL calcium chloride solution was combined with 1 mL extracts at various concentrations (250,500,750,1000 mg/mL). The crystallization process began with the addition of 950 mL of sodium oxalate solution. The temperature was maintained at 37◦C. After 30 min, the optical density of the solution was measured at 620 nm. The rate of nucleation was calculated by comparing the induction time in the presence of test samples to the induction time in the absence of test samples. The following reaction was predicted to cause crystal growth.[19]

3. Aggregation assay

The technique use for aggregation was modified and evaluated using a multiple electrode aggregometer. CaOx crystals will be used at a final concentration of 0.8 mg/mL in a buffered solution containing 0.05 M Tris–HCl and0.15 M NaCl at pH 6.5. Experiments was carried out at 37◦C in the presence or absence of test and control samples. The percentage aggregation inhibition was calculated by comparing the turbidity in the presence of test samples at various concentrations (250,500,700,1000mg/mL) to the turbidity in the control using the formula.[20]

4. Egg membrane assay

Preparation of semi-permeable membrane

The semipermeable membrane was prepared from egg. A glass rod was used to puncture the apex of the eggs and the entire contents was squeezed out. Empty egg shells was washed thoroughly in distilled water, after that the shells was placed in a beaker containing 4.0 mL concentrated HCl in 200 mL distilled water. The semi-permeable membrane was completely decalcified after being kept for an overnight period. The next day the semi permeable membranes was carefully removed from the egg shell and washed thoroughly with distilled water. The trace of acid present in the membrane is neutralised by placing the shell mem-branes in an ammonia solution, and rinsed with distilled water. It was kept in a moistened state in the refrigerator at a pH of 7–7.4. Ten milligram of calcium oxalate was suspended in 10.0 mL of distilled water as a negative control. Ethanolic extracts of samples in the amount of 5.0 mL was taken at different concentration 250,500,700,1000 mg/ml. The color coating on a 500 mg Cystone tablet was removed with absolute ethanol, yielding 400 mg. Cystone tablets was crushed into powder and mixed with 100 mL distilled water before being filtered. Cystone filtrate was used as a positive control for anti-urolithiatic activity in vivo.

$$\%\ inhi\,biti\,on = \frac{1 - (Turb\,idity\ \ Samp\,le)}{Turb\,idity\ \ Cont\,rol} \times 100\%$$

Estimation of calcium oxalate by using dissolution model

Each egg semi permeable membrane was packed separately and was suspended in a conical flask containing 100 mL of 0.1 M Tris buffer, by tying the membrane with the thread. In order to make the shell hanging a stick is placed in the mouth of a conical flask and covered with aluminum foil at the other end of the thread. All conical flasks was kept in an incubator for 2 h at 37◦C. Remove the contents of each group's semipermeable membranes into separate test tubes, add 2 mL of 0.5 M sulphuric acid to each tube, and titrate with 0.2 M KMnO4 until a light pink color is achieved. To determine the total amount of dissolved calcium oxalate by various ex-tracts, subtract the amount of remaining undissolved calcium oxalate from the total quantity used in the experiment at the start. Each mL of 0.2 M KMnO4 equals 0.1898 mg of calcium oxalate.[21]

RESULTS

Preliminary Phytochemical Screening

The ethanolic extract *coconut nucifera* cotyledon gave positive results for alkaloids, steroids, flavonoids, flavonones, glycosides, saponins, proteins and tannins etc.

INHIBITION ASSAY

The inhibitory potential of different ethanolic extracts of coconut cotyledon on the growth of calcium oxalate crystals was evaluated, and the results are presented in Table 1. The findings revealed that the extracts effectively suppressed crystal growth in a concentration-dependent manner. The extract at the highest concentration exhibited the maximum percentage of inhibition. Notably, the extent of inhibition observed with the coconut cotyledon extracts was comparable to that produced by the standard commercial formulation, Cystone.

Table-1: Effect of Coconut cotyledon ethanolic extract on CaOx crystals inhibition.

S. No	Concentration (µg/ml)	Percentage of Inhibition by EECE	Percentage of Inhibition by Cystone
1	250	24.28	36.21
2	500	39.11	44.13
3	750	58.14	56
4	1000	66.46	71.28

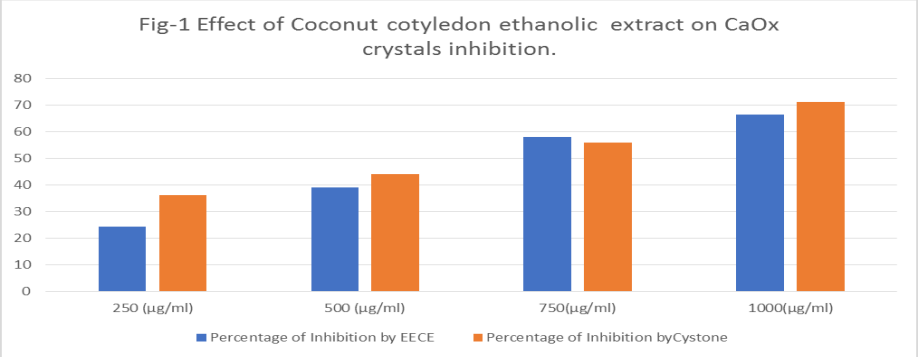
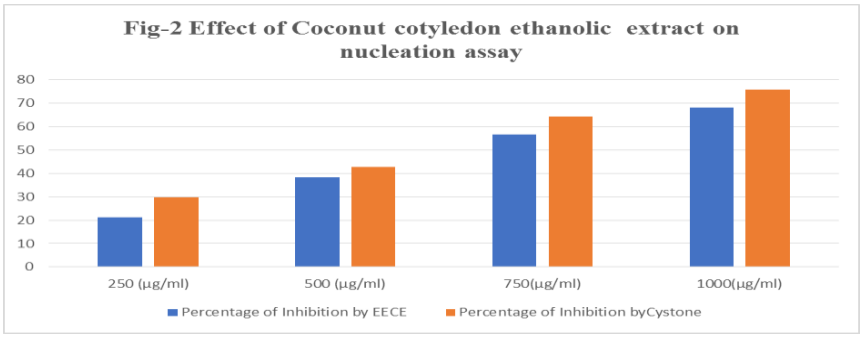


Table-2: Effect of Coconut cotyledon ethanolic extract on nucleation assay.

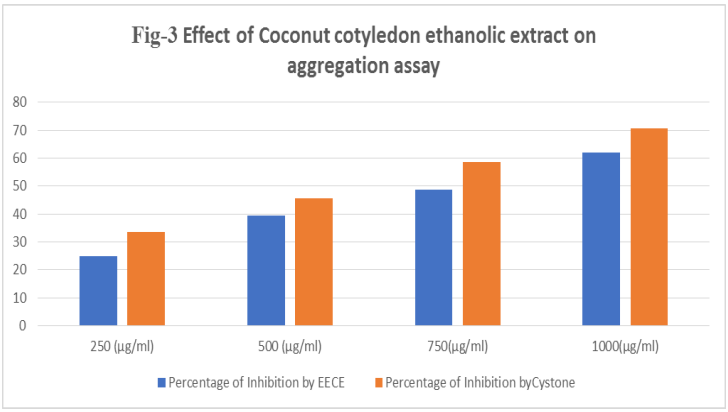
S.No	Concentration (µg/ml)	Percentage of Inhibition by EECE	Percentage of Inhibition by Cystone
1	250	21.30	29.69
2	500	38.25	42.81
3	750	56.49	64.23
4	1000	68.13	75.66



To evaluate the antiurolithiatic potential of the extract, a series of *in vitro* assays were conducted targeting the key stages of stone formation—nucleation, crystal growth, and aggregation. The results of the crystal nucleation assay are illustrated in Figure 2. Various concentrations of the ethanolic extract of coconut cotyledon (250 µg/ml, 500 µg/ml, 750 µg/ml, and 1000 µg/ml) were tested along with the standard drug. All tested concentrations demonstrated a concentration-dependent reduction in calcium oxalate crystal formation during nucleation. Notably, the extract at 1000 µg/ml exhibited the highest percentage of inhibition, achieving 68.13% reduction in crystal nucleation and it is comparable with that of standard drug cystone(75.66%).

Table-3: Effect of Coconut cotyledon ethanolic extract on aggregation assay.

S. No	Concentration (µg/ml)	Percentage of Inhibition by EECE	Percentage of Inhibition by Cystone
1	250	24.77	33.41
2	500	39.52	45.72
3	750	48.71	58.62
4	1000	62.16	70.57



The aggregation assay was performed to assess the inhibitory effect of the ethanolic extract of coconut cotyledon on calcium oxalate crystal aggregation. Four different concentrations of the extract (250 µg/ml, 500 µg/ml, 750 µg/ml, and 1000 µg/ml) were evaluated alongside the standard drug. A concentration-dependent reduction in calcium oxalate aggregation was observed for both the extract and the standard. The standard drug at 1000 µg/ml exhibited the highest inhibition, achieving 70.57% reduction, while the extract at the same concentration demonstrated a slightly lower but comparable inhibition of 62.16%. These results indicate that the anti-aggregation effect of the ethanolic extract of coconut cotyledon is close to that of the standard drug. The findings were presented in Table 3 and Figure 3.

Table-4: effect of Ethanolic Extract of Coconut Cotyledon on Dissolution of Calcium Oxalate.

S. No	Concentration (µg/ml)	%(Dissolution of Calcium oxalate) EECC
1	250	21.19
2	500	28.21
3	750	40.52
4	1000	48.53
5	Positive Control (cystone 1000 µg/ml)	59.83

The egg membrane permeability method was utilized to assess the dissolution capacity of calcium oxalate crystals in the presence of the ethanolic extract of coconut cotyledon (EECC). As illustrated in Table 4, an increase in extract concentration led to a gradual rise in the percentage of calcium oxalate dissolution. The extract exhibited 21.19% dissolution at 250 µg/ml, which progressively increased to 28.21% and 40.52% at 500 µg/ml and 750 µg/ml, respectively. The maximum dissolution was observed at 1000 µg/ml, with 48.53% activity.

In comparison, the standard drug Cystone (1000 µg/ml) showed a higher dissolution rate of 59.83%. Although slightly less effective than the standard, the extract demonstrated a notable concentration-dependent enhancement in calcium oxalate dissolution, indicating its potential role in reducing or eliminating crystal deposits during urolithiasis.

DISCUSSION

Numerous phytochemical investigations on medicinal plants have revealed the presence of bioactive metabolites such as alkaloids, saponins, flavonoids, tannins, steroids, and amino acids, which can be confirmed through qualitative phytochemical analyses. Among these, flavonoids are recognized for their strong antioxidant activity, which contributes to the dissolution of kidney stones by reducing oxidative stress. Saponins demonstrate anti- calcifying properties by disaggregating mucoproteins, which serve as promoters of crystallization.[22,23] Calcium oxalate monohydrate, being an insoluble and thermodynamically stable form of calcium oxalate, is primarily responsible for the formation of kidney stones

Urolithiasis refers to the pathological condition in which calculi develop in the kidneys or urinary tract. The formation of calcium oxalate stones follows a multi-step process involving nucleation, crystal growth, aggregation, and retention.[24] Crystals form when atoms or molecules in a supersaturated solution bond together, minimizing potential energy and leading to nucleation. Nucleation represents the initial and critical stage of crystal formation in solution, which can occur through homogeneous or heterogeneous mechanisms.[25] Most renal calculi are composed of mixed crystalline materials, with calcium oxalate being a predominant constituent.[26]

Although significant advancements have been achieved in the medical removal of kidney stones, frequent recurrences remain a major clinical challenge.[27] Traditional herbal remedies have long been employed for stone prevention and treatment, preceding modern pharmacological approaches. Several Indian medicinal plants have demonstrated significant antilithiatic potential, accompanied by low toxicity and affordability.[28] Various phytochemicals such as phenols and flavonoids, including quercetin, hyperoside, rutin, diosmin, and apigenin, have been identified for their ability to inhibit calcium oxalate stone formation.[29]

Dietary studies on animals and humans have emphasized that a diet abundant in fruits and vegetables helps prevent urolithiasis.[30] Regular consumption of foods rich in phytochemicals elevates urine pH and volume while increasing concentrations of stone inhibitors such as citrate, magnesium, potassium, and phytate, thereby reducing calcium oxalate and uric acid supersaturation.[31]

In the present study, the ethanolic extract of coconut cotyledon (EECC) demonstrated significant anti-urolithiatic activity against chemically synthesized calcium oxalate crystals. Phytochemical evaluation confirmed the presence of alkaloids, flavonoids, flavanones, glycosides, saponins, proteins, steroids, and tannins in the ethanolic extract. The extract showed comparable crystal inhibition to that of the standard drug Cystone. Since crystal aggregation plays a pivotal role in stone retention due to electrostatic and chemical binding of crystals. [32] the ability of EECC to reduce aggregation effectively highlights its potential therapeutic

significance.

The results of the *in vitro* nucleation and aggregation assays strongly suggest that EECC can inhibit calcium oxalate crystal formation at multiple stages, including nucleation, growth, and aggregation. The inhibitory effect increased with extract concentration, indicating a dose-dependent response. Furthermore, EECC facilitated the dissolution of preformed calcium oxalate crystals, demonstrating demineralization comparable to Cystone. These findings indicate that components within EECC interfere with calcium oxalate crystallization mechanisms, potentially through chelation or modulation of crystal surface interactions.

Calcium oxalate stones exist predominantly in two crystalline forms: calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD), both of which are implicated in renal calculi formation.[33] The ethanolic extract of coconut cotyledon significantly inhibited both forms and promoted partial dissolution of preformed crystals, validating its efficacy as an anti-urolithiatic agent. These findings provide preliminary evidence supporting the potential of coconut cotyledon extract in managing urolithiasis and warrant further in vivo investigation to identify the active principles responsible for its therapeutic effect.

CONCLUSION

In conclusion, we have demonstrated, that the ethanolic extract of coconut cotyledon (EECC) effectively inhibited the *in vitro* formation and aggregation of calcium oxalate crystals and promoted dissolution of existing deposits. Although these findings are encouraging, the current study was limited to *in vitro* evaluation. Further research involving animal models is required to confirm its efficacy and safety in biological systems. Moreover, understanding the molecular mechanisms and identifying the specific phytoconstituents responsible for the observed antiurolithiatic activity will be crucial for the future development of standardized formulations.

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