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Research Article

Evaluation of Antiuro lithiatic activity of Celosia argentea seeds by UV Spectroscopic method

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ABSTRACT

Kidney stone formation also as urolithiasis, is a common health problem caused by the crystallization of calcium, oxalate, phosphate, sodium urate that needs effective and safe treatment options. This study examines the ability of Celosia argentea seed extract to prevent kidney stone formation using UV spectroscopic analysis (Shimadzu A1900 i) with the help of two assays, nucleation and aggregation assay, these two assays are in-vitro methods for evaluation of calcium oxalate stones. The extract was tested to see if it could stop calcium oxalate crystals from crystal initiation and crystal growth phase. The study done with control sample of calcium oxalate monohydrate and various concentrations of herb extract as (100, 300, 500, 700, 1000 µg/ml). The results showed that the extract significantly reduced both crystal initiation and crystal growth, suggesting its potential as a natural remedy for kidney stone prevention. These findings support the traditional use of Celosia argentea and encourage further research into its benefits.

INTRODUCTION

Urolithiasis, commonly known as kidney stone disease. It is a significant global health problem characterized by the formation of stones in the urinary tract due to the supersaturation of urine with stone-forming constituents such as calcium, oxalate, phosphate and uric acid. The pathophysiology of kidney stone formation

involves nucleation, crystal growth, aggregation and retention within the renal tubules. Factors such as urinary pH imbalance, reduced citrate levels, oxidative stress, increase calcium levels and renal tubular injury contribute to stone formation. Additionally, metabolic disorders like hypercalciuria, hyperoxaluria and hypocitraturia play a crucial role in urolithiasis.

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Several treatment are available for managing kidney stones, ranging from traditional approaches to minimally invasive and surgical interventions. Extracorporeal Shock Wave Lithotripsy (ESWL) is a non-invasive technique that uses shock waves to break into fragment stones, facilitating their natural removal through urine. However, ESWL may not be effective for harder or larger stones and can sometimes lead to renal injury. Ureteroscopy (URS) involves the insertion of a thin scope through the urethra to directly visualize and remove or break stones using laser lithotripsy. For larger renal stones, Percutaneous Nephrolithotomy (PNL) is often chosen, where a small cut is made in the back to access and remove stones using specialized instruments. In severe cases or when other methods fail, open surgery may be required, although it is less commonly performed due to advancements in minimally invasive procedures. Despite these interventions, reappearance rates remain high and complications such as infections, bleeding and renal damage require the examination of alternative and safer treatment options. (1) (2) Medicinal plants have gained increasing attention for their potential in preventing and managing urolithiasis due to their diuretic, anti-inflammatory and stone-dissolving properties. *Celosia argentea*, a plant belonging to the *Amaranthaceae* family, has been traditionally used for renal disorders. The seeds of *Celosia argentea* contain bioactive constituents such as flavonoids, saponins and alkaloids, which are reported to have antiurolithiatic properties. (3) (4) This study aims to evaluate the antiurolithiatic activity of *Celosia argentea* seeds using a UV spectroscopic method. UV spectroscopy is a reliable analytical tool for quantifying the dissolution of kidney stone-forming constituents under in vitro conditions. By assessing the extract's ability to inhibit or dissolve calcium oxalate crystals, this study seeks to provide scientific evidence supporting the traditional

claims of *Celosia argentea* as a natural remedy for kidney stone.

MATERIALS AND METHODS:

Plant material collection and extraction:

The fresh plant seeds were collected from Maharastra state by gently uprooting the plant. Authenticated by Vilas M. Suroshe Msc., Phd (Ethnobotanist). Seeds collected are allow for shade drying process later grinded into fine powder. Extracted by reflux extraction technique by using 70% ethanol as solvent.

Apparatus used:

Reflux condenser, Mortar-pestle, UV Spectrophotometer A1900i Shimadzu

METHOD:

Preparation of Extracts:

The seeds of *Celosia argentea* were collected from Thane district of Maharastra during the month of October 2024. Seeds of *celosia argentea* were shade dried and grinded to obtain fine powder which then stored in closed container in dark place. The 20 gm of powdered were subjected to reflux extraction in 200 mL of 70% ethanol for 2-4 hours. The obtained extract were filtered and filtrate were evaporated to viscous mass, % yield was calculated and used for further study.

Nucleation assay

The nucleation assay was performed to evaluate the inhibitory effect of *Celosia argentea* seed extract on calcium oxalate crystal formation. This assay mimics the early stages of kidney stone formation, where calcium and oxalate ions supersaturate and nucleate into crystals.

Materials and Reagents



Calcium chloride (CaCl_2) – 5 mmol/L

Sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$) – 7.5 mmol/L

Tris-HCl buffer (0.05 mol/L, pH 6.5)

NaCl solution (0.15 mol/L)

Test extract solutions of *Celosia argentea* (100, 300, 500, 700, 1000 $\mu\text{g/ml}$)

Preparation of Solutions:

5 mmol/L CaCl_2 and 7.5 mmol/L $\text{Na}_2\text{C}_2\text{O}_4$ solutions were prepared in 0.05 mol/L Tris-HCl buffer (pH 6.5) containing 0.15 mol/L NaCl to maintain ionic strength.

Reaction Setup:

Equal volumes of CaCl_2 and $\text{Na}_2\text{C}_2\text{O}_4$ (950mL) solutions were mixed in a reaction medium to initiate calcium oxalate crystallization.

The reaction was carried out in the absence (control) and presence of different concentrations of *Celosia argentea* seed extract (100mL).

Spectrophotometric Measurement:

The rate of nucleation was monitored by measuring the absorbance at 621 nm using a UV-visible spectrophotometer.

A decrease in absorbance compared to the control indicated inhibition of crystal nucleation by the extract.

Calculation of turbidity: The turbidity of calcium oxalate in nucleation was calculated using the formula:

$$\text{Turbidity} = 2.3 \times \text{Abs} / L$$

Aggregation Assay

The aggregation assay was conducted to evaluate the ability of *Celosia argentea* seed extract to

inhibit the growth and clustering of preformed calcium oxalate monohydrate (COM) crystals. Crystal aggregation plays a crucial role in kidney stone formation, as larger aggregates increase the risk of urinary obstruction and stone retention in the renal system.

Materials and Reagents

Calcium chloride (CaCl_2) – 50 mmol/L

Sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$) – 50 mmol/L

Tris-HCl buffer (0.05 mol/L, pH 6.5)

NaCl solution (0.15 mol/L)

Preformed calcium oxalate monohydrate (COM) crystals

Test extract solutions of *Celosia argentea* (100, 300, 500, 700, 1000 $\mu\text{g/ml}$)

Preparation of Calcium Oxalate Monohydrate (COM) Crystals:

Equimolar solutions of 50 mmol/L CaCl_2 and 50 mmol/L $\text{Na}_2\text{C}_2\text{O}_4$ were mixed in Tris-HCl buffer (pH 6.5) containing 0.15 mol/L NaCl to induce calcium oxalate crystal formation.

The formed crystals were filtered and collected to prepare 0.8 mg/ml solution.

Reaction Setup:

The crystal solution was incubated with different concentrations of *Celosia argentea* seed extract.

A control sample was prepared without the extract for comparison.

Spectrophotometric Measurement:

The degree of crystal aggregation was monitored by measuring absorbance at 621 nm using a UV-visible spectrophotometer against extract concentrations.



A decrease in absorbance indicated inhibition of crystal aggregation by the extract.

Calculation of Aggregation Inhibition Percentage:

The percentage inhibition of crystal aggregation was calculated using the formula:

$$I\% = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where, A_{control} is the absorbance of the control (without extract), and A_{test} is the absorbance in the presence of the extract. (5)

RESULT AND DISCUSSION:

Determination of λ max:

Take the plant seed extract and dilute to 100 $\mu\text{g/mL}$, 300 $\mu\text{g/mL}$, 500 $\mu\text{g/mL}$. Scanned 3 samples by UV and λ max was found at 621 nm.

Turbidity calculation by Nucleation assay:

The formula for turbidity as per Lambert and Beers law:

$$\text{Turbidity} = (2.3 \times \text{absorbance}) / L$$

Table 1: Table for Nucleation assay

Conc. of Herb extract	Absorbance at 621nm	Turbidity
100 $\mu\text{g/ml}$	0.937	2.1551
300 $\mu\text{g/ml}$	0.863	1.9849
500 $\mu\text{g/ml}$	0.743	1.7089
700 $\mu\text{g/ml}$	0.652	1.4996
1000 $\mu\text{g/ml}$	0.425	0.9775

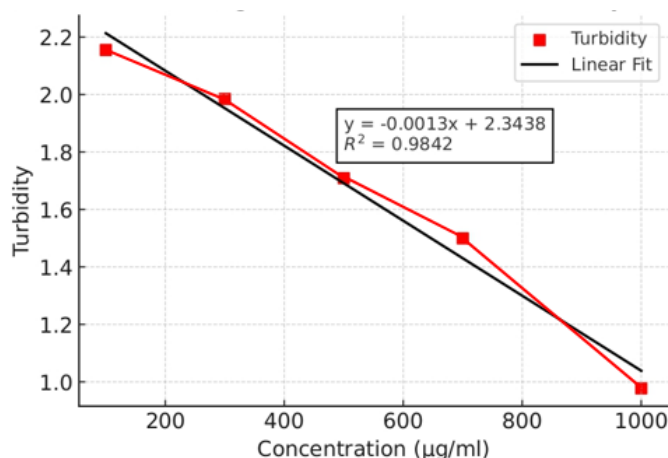


Fig 1: Effect of different concentration of seed extract in nucleation assay.

Aggregation assay:

The calcium oxalate monohydrate crystals prepared are taken in concentration on 0.8mg/ml as control. In control there is no herb extract was present.

Table 2: CaOx crystallization of control

Conc. of Herb extract	Absorbance at 621nm	Turbidity
control	0.938	2.1574

Determination of Rate of Inhibition (IR):

Table 3: Effect of concentration of C. argentea extract on CaOx crystallization inhibition rate:

Conc. of Herb extract	Absorbance	Turbidity	IR
100 $\mu\text{g/ml}$	0.672	1.5456	28.3582
300 $\mu\text{g/ml}$	0.563	1.2949	39.9786
500 $\mu\text{g/ml}$	0.445	1.0235	52.5586
700 $\mu\text{g/ml}$	0.348	0.8004	62.8997
1000 $\mu\text{g/ml}$	0.262	0.6026	72.0682



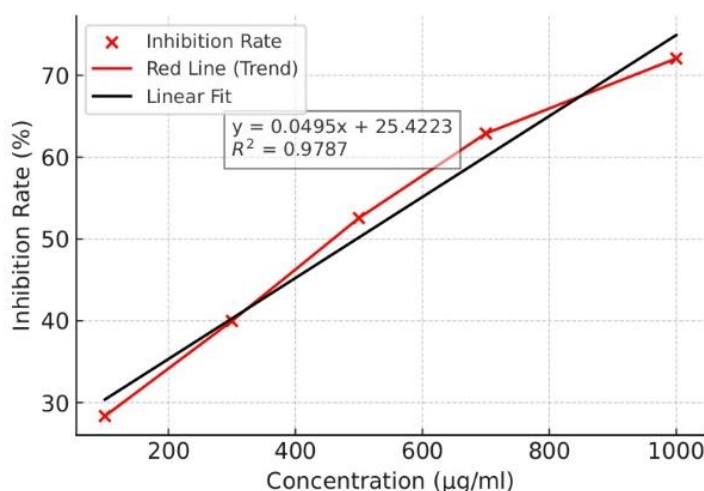


Fig 2: Effect of different concentration of seed extract in nucleation assay

The effect of *Celosia argentea* seed extract was measured by UV Shimadzu A1900 i on calcium oxalate crystallization by two assays Nucleation and Aggregation assays respectively. Both the assays shows positive effect against crystal initiation and crystal growth of calcium oxalate showing that effective anti-urolithiatic drug. Result of nucleation assay shows that increase in herb extract concentration there is decrease in turbidity of calcium oxalate crystals. Turbidity decreases as for 100 µg/ml – 2.1551, 300 µg/ml – 1.9849, 500 µg/ml – 1.7089, 700 µg/ml – 1.4996, 1000 µg/ml – 0.9775. Result of Aggregation assay shows that increase in herb concentration there is increase in Inhibition rate of crystallization of calcium oxalate. The inhibition rate was found 28.3582 for 100 µg/ml, 39.9786 for 300 µg/ml, 52.5586 for 500 µg/ml, 62.8997 for 700 µg/ml, 72.0682 for 1000 µg/ml.

CONCLUSION:

In nucleation assay there is decrease in turbidity of CaOx crystals as increase in concentration of herb extract and in aggregation assay there is increase in inhibition rate of caOx crystallization as increase in concentration of herb extract. So we can conclude that the seed of *celosia argentea* have an inhibitory effect on calcium oxalate

crystallization which is beneficial for treatment of kidney stone.

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