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EVALUATION OF ANTIUROLITHIATIC ACTIVITY OF SOLANUM TRILOBATUM AGAINST CALCIUM OXALATE CRYSTAL FORMATION: AN IN VITRO STUDY

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ABSTRACT

The present study was carried out to evaluate the antiurolithiatic activity of the ethanolic leaf extract of Solanum trilobatum against calcium oxalate crystal formation using in vitro methods. The extract was evaluated by nucleation assay, aggregation assay, and titrimetric dissolution assay. In the nucleation assay, the extract exhibited concentration-dependent inhibition of calcium oxalate crystal formation with 20.0%, 39.0%, and 50.4% inhibition at 10, 20, and 30 mg/ml respectively. In the aggregation assay, the extract exhibited 7.65%, 44.59%, and 61.74% inhibition at the respective concentrations. In the titrimetric dissolution assay, the extract produced 45.36%, 50.82%, and 55.73% dissolution at 10, 20, and 30 mg/mL, respectively, whereas the standard drug Cystone showed 50.0%, 60.0%, and 78.0% dissolution, respectively., whereas the standard drug Cystone displayed 78.0% dissolution. The results indicate that the ethanolic extract of Solanum trilobatum possesses significant

antiurolithiatic activity by inhibiting calcium oxalate crystal formation and promoting crystal dissolution in a concentration-dependent manner. These findings scientifically support the traditional use of Solanum trilobatum in the management of urolithiasis.

KEYWORDS: Solanum trilobatum; Antiurolithiatic activity; Calcium oxalate crystals; Nucleation assay; Aggregation assay; Titrimetric methods.

1. INTRODUCTION

1.1. UROLITHIASIS: AN OVERVIEW

The term urolithiasis is derived from the Greek words “ouron” meaning urine and “lithos” meaning stone, and refers to the formation of solid concretions composed of crystalline and organic matrix substances within the urinary tract.[1] It is a significant medical condition that affects approximately 12% of the global population. Urolithiasis is more prevalent between the ages of 20 and 40 years and occurs more frequently in males than females, with a male-to-female ratio of 2: 1.[2] Urinary calculi are generally composed of uric acid, calcium phosphate, cystine, struvite (magnesium ammonium phosphate), and calcium oxalate in monohydrate or dihydrate forms. Other less common stones include those induced by xanthine accumulation and certain medications.[3] Among these, calcium oxalate is considered the major constituent of renal calculi, accounting for nearly 80% of cases worldwide.[4]

1.2. PATHOGENESIS OF CALCIUM OXALATE STONE FORMATION

Kidney stone formation is a complex physicochemical and biological process resulting from an imbalance between stone-promoting and stone-inhibiting factors in urine. The process begins when urine becomes supersaturated with calcium, oxalate, phosphate, and uric acid ions. Under these conditions, crystals are formed through the process of nucleation and subsequently increase in size through crystal growth by the deposition of additional minerals. Individual crystals then combine to form larger crystal aggregates through the process of aggregation. These larger aggregates may become retained within the renal tubules and subsequently adhere to damaged renal tubular epithelial cells or mineralized structures such as Randall’s plaques. Continued deposition of minerals on the retained crystals ultimately leads to the development and enlargement of renal calculi.[5]

Oxidative stress and renal epithelial cell injury further enhance crystal adhesion and retention, thereby promoting stone formation. Natural urinary inhibitors such as citrate, magnesium, nephrocalcin, osteopontin, and Tamm–Horsfall protein can inhibit crystal formation and

aggregation, whereas dehydration, hypercalciuria, hyperoxaluria, hypocitraturia, and oxidative stress promote stone development.[6] Therefore, urinary supersaturation, crystal nucleation, crystal growth, crystal aggregation, and crystal retention represent important sequential events in the initiation and progression of calcium oxalate urolithiasis.

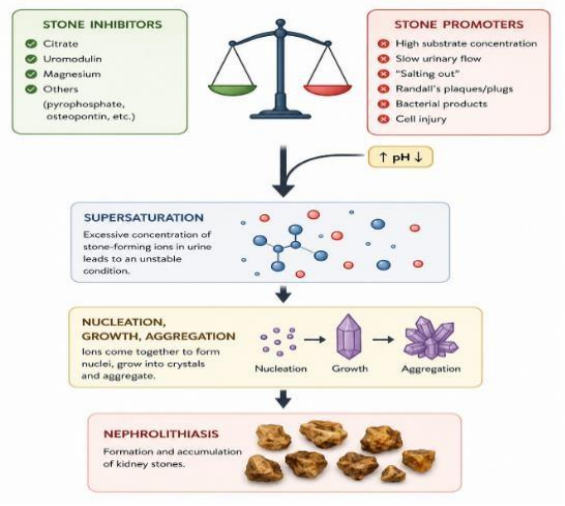

Figure 1: Schematic presentation of the mechanism of calcium oxalate kidney stone formation.

1.3. ROLE OF MEDICINAL PLANTS IN UROLITHIASIS

Various medicinal plants have been traditionally used in different countries and cultures for the treatment and prevention of urolithiasis. These plants are preferred because they are inexpensive, readily available, associated with fewer side effects, and contain a variety of phytochemicals that may exhibit beneficial effects against urinary stone formation.[7] Several medicinal plants have demonstrated potential antiurolithiatic effects through antioxidant, anti-inflammatory, diuretic, nephroprotective, and anti-crystallization activities. In particular, inhibition of calcium oxalate crystal nucleation, growth, and aggregation may prevent the initiation and progression of urinary stone formation. In addition, the ability of plant extracts to promote the dissolution or reduction of preformed calcium oxalate crystals may contribute to their therapeutic potential against established urinary calculi.

Furthermore, the World Health Organization (WHO) has recommended the scientific investigation of medicinal plants for the prevention of diseases and the development of safe and effective therapeutic agents.[8] Therefore, systematic evaluation of traditionally used medicinal plants may provide valuable scientific evidence for the development of novel plant-based therapeutic approaches for the prevention and management of urolithiasis.

1.4. SOLANUM TRILOBATUM LINN. AS A POTENTIAL ANTIUROLITHIATIC AGENT

Solanum trilobatum Linn. (Family: Solanaceae) is a thorny medicinal climber characterized by blue-purple flowers and a scandent armed habit. This valuable medicinal plant is widely distributed in Southern India and has been extensively used in traditional systems of medicine, particularly Siddha and Ayurveda. It is commonly known as “Thuthuvalai” in Tamil, “Alarka” in Sanskrit, “Alarkapatramu” in Telugu, and “Tutuvalam” in Malayalam. Different parts of the plant, including its leaves, berries, flowers, and roots, are traditionally used for various medicinal purposes.[9]

Solanum trilobatum is traditionally employed in the treatment of respiratory disorders such as cough, bronchial asthma, and tuberculosis. Traditional medicinal literature also reports its use in the management of urinary tract disorders and urinary calculi. Phytochemical investigations have revealed the presence of several bioactive constituents, including alkaloids, flavonoids, glycosides, saponins, steroids, phenolic compounds, and other secondary metabolites.[10]

These phytoconstituents have been associated with diverse pharmacological activities, including antioxidant, anti-inflammatory, nephroprotective, and diuretic properties, which may contribute to the prevention of kidney stone formation.

The flavonoids, saponins, phenolic compounds, and steroidal alkaloids present in S. trilobatum may interfere with different stages of calcium oxalate crystallization, including crystal nucleation, growth, and aggregation. Furthermore, their antioxidant properties may help reduce oxidative stress and renal epithelial injury, thereby potentially decreasing crystal adhesion and retention.[11] These properties suggest that S. trilobatum may possess promising antiurolithiatic potential.

1.5. RATIONALE AND AIM OF THE STUDY

Although Solanum trilobatum has been traditionally used for various medicinal purposes and possesses several pharmacologically active phytoconstituents, its potential against calcium oxalate crystallization requires further scientific validation. In particular, systematic evaluation of its effects on the different stages of calcium oxalate crystal formation and preformed crystal dissolution may provide useful evidence regarding its antiurolithiatic potential. Therefore, the present study was undertaken to evaluate the antiurolithiatic activity of Solanum trilobatum against calcium oxalate crystal formation using an in vitro model, with particular emphasis on

its effects on crystal nucleation, aggregation, and dissolution. The study aims to provide scientific validation for its traditional use in urinary disorders and contribute to the development of novel plant-based therapeutic approaches for the prevention and management of calcium oxalate urolithiasis.[12]

2. AIM AND OBJECTIVE

2.1. AIM

To evaluate the in vitro antiurolithiatic activity of Solanum trilobatum leaf extract against calcium oxalate crystallization.

2.2. OBJECTIVES

- To collect and authenticate the leaves of Solanum trilobatum.
- To prepare the ethanolic extract of Solanum trilobatum leaves by a suitable extraction method.
- To perform preliminary phytochemical screening of the prepared extract for the identification of major phytoconstituents.
- To prepare calcium oxalate crystals for in vitro crystallization studies.
- To evaluate the inhibitory effect of Solanum trilobatum extract on calcium oxalate nucleation.
- To evaluate the inhibitory effect of Solanum trilobatum extract on calcium oxalate crystal aggregation.
- To evaluate the ability of the Solanum trilobatum extract to dissolve preformed calcium oxalate crystals.
- To determine the concentration-dependent antiurolithiatic activity of the extract.
- To compare the activity of the plant extract with a suitable standard antiurolithiatic agent.
- To assess the potential of Solanum trilobatum as a natural source for antiurolithiatic activity.

3. PLANT PROFILE

3.1. SYNONYMS

- Solanum trilobatum Linn.
- Purple Fruited Pea Eggplant
- Climbing Brinjal

3.2. VERNACULAR NAMES

- Tamil : Thuthuvalai
- Sanskrit : Alarka
- Telugu : Alarkapatramu
- Malayalam : Tutuvalam
- English : Purple Fruited Pea Eggplant

3.3. TAXONOMICAL CLASSIFICATION

- Kingdom : Plantae
- Division : Magnoliophyta
- Class : Magnoliopsida
- Order : Solanales
- Family : Solanaceae
- Genus : Solanum
- Species : Solanum trilobatum Linn**.**

3.4. BOTANICAL DESCRIPTION

3.4.1. PLANT

- Solanum trilobatum is a thorny perennial medicinal climber.
- It is commonly Found in tropical and subtropical regions of India, especially in South India.
- The plant possesses a scandent or climbing habit and grows extensively in dry wastelands and hedges.

3.4.2. ROOT

- The root system is well-developed and consists of a taproot with lateral branches.
- Roots are used in traditional medicine for various therapeutic purposes.

3.4.3. STEM

- The stem is green, woody, branched, and climbing in nature.
- It is armed with numerous curved prickles that aid in climbing and protection.

3.4.4. LEAVES

- Leaves are simple, alternate, and deeply lobed.
- They are dark green in colour and possess sharp prickles on the veins.

- Leaves are the most commonly used part of the plant in traditional medicine.

3.4.5. FLOWERS

- Flowers are blue to purple in colour and arranged in small clusters.
- They are bisexual and possess a characteristic star-shaped appearance.

3.4.6. FRUITS

- Fruits are small berries, green when immature and bright red when ripe.
- The berries contain numerous seeds and are used medicinally.

3.4.7. SEEDS

- Seeds are small, yellowish, and flattened.
- They are enclosed within the fleshy berry fruit.

3.5. PHYTOCHEMICAL CONSTITUENTS

The plant contains various bioactive constituents including:

- Alkaloids (Solasodine, Solasonine, Solamargine)
- Flavonoids (Quercetin, Kaempferol, Rutin)
- Glycosides (Steroidal glycosides)
- Saponins (Steroidal saponins)
- Steroids (β-Sitosterol, Stigmasterol)
- Phenolic compounds (Gallic acid, Caffeic acid, Chlorogenic acid)
- Tannins (Hydrolysable and condensed tannins)
- Terpenoids (β-Amyrin, Lupeol)

3.6. PHARMACOLOGICAL ACTIVITIES

- Antiasthmatic Activity
- Antitussive Activity
- Expectorant Activity
- Antioxidant Activity
- Anti-inflammatory Activity
- Antimicrobial Activity
- Antidiabetic Activity
- Hepatoprotective Activity
- Nephroprotective Activity

- Anticancer Activity
- Immunomodulatory Activity
- Wound Healing Activity.[6]

Figure 2: Morphological characteristics of Solanum trilobatum plant.

4. MATERIALS AND METHODS

4.1. COLLECTION AND PROCESSING OF PLANT MATERIAL

The experimental plant material was collected from Kudiyanakuppam Village, located in the Jolarpet region of Tirupattur District, Tamil Nadu, India, during June 2026. The plant material was authenticated as Solanum trilobatum L., belonging to the family Solanaceae, by Dr. V. Ravi, M.Sc., Ph.D., Associate Professor and Head, PG & Research Department of Botany, Government Arts College for Men, Krishnagiri, Tamil Nadu, India. To eliminate surface contaminants, dust, and debris, the collected *Solanum trilobatum* leaves were thoroughly washed with tap water. Subsequently, the cleaned leaves were shade-dried at room temperature for two weeks and mechanically powdered. The powdered material was stored in clean, airtight containers until further extraction.

4.2. PREPARATION OF PLANT EXTRACT

The continuous hot extraction method utilizing a Soxhlet apparatus was employed to isolate bioactive constituents. Approximately 100 g of the coarsely pulverized Solanum trilobatum leaf powder was placed into the extraction thimble and systematically extracted with 500 mL of analytical grade ethanol. The extraction cycle was continued for 12 hrs until the solvent in the siphoning tube became completely translucent. The resulting liquid extract was filtered through Whatman No. 1 filter paper and concentrated. The obtained concentrated semi-solid ethanolic extract was dried completely, weighed, and stored in a sterile container at 4 °C for subsequent phytochemical screening and antiurolithiatic bioassays.

Figure 3: Preparation of Solanum trilobatum leaf extract by Soxhlet extraction.

4.3. PHYTOCHEMICAL SCREENING

a. PREPARATION OF STOCK SOLUTIONS

For the qualitative phytochemical screening, a total of 1g of solanum trilobatum extract was dissolved in 100mL of 80%(v/v) ethanol to prepare the stock solution according to standard established methodologies.

b. TEST FOR ALKALOIDS (MAYER'S TEST)

A total of 50mg of sample was dissolved in 5ml of distilled water, acidified with 2M hydrochloric acid, filtered, and approximately 2ml of the filtrate was treated with 1ml of Mayer's reagent along the side of the test tube to observe the formation of a white precipitate.

c. TEST FOR FLAVONOIDS (SHINODA'S TEST)

A piece of magnesium ribbon followed by the dropwise addition of concentrated hydrochloric acid was introduced to the stock solution and heated to monitor the appearance of a characteristic magenta coloration.

d. TEST FOR TANNINS (FERRIC CHLORIDE TEST)

A few drops of neutral 5% ferric chloride solution were added directly into the stock solution to evaluate the development of a dark green colour indicating the presence of phenolic hydroxyl group compounds.

e. TEST FOR SAPONINS (FOAM TEST)

A small amount of the stock solution was shaken thoroughly with a small quantity of water to detect the presence of a persisting foam suspension that remains stable for approximately 10 minutes.

f. TEST FOR STEROIDS (ACID-CHLOROFORM TEST)

Approximately 1mg of the plant extract was dissolved in 10ml of chloroform and 10ml of concentrated sulfuric acid in a test tube to observe if the upper layer transitions to red and the sulfuric acid layer exhibits a yellow hue with green fluorescence.

g. TEST FOR TRITERPENOIDS (SALKOWSKI TEST)

A total of 5ml of the stock solution was mixed with 2ml of chloroform followed by the cautious addition of 3ml of concentrated sulfuric acid to detect the formation of a positive reddish-brown coloration at the layer interface.

h. TEST FOR CARBOHYDRATES (MOLISCH'S TEST)

A total of 1ml of the stock solution was mixed with a few drops of 1% alpha-naphthol and 2-3ml of concentrated sulfuric acid along the side of the test tube to monitor the formation of a diagnostic reddish-violet or purple ring at the junction of the two liquids.

i. TEST FOR PROTEINS (BIURET TEST)

To 2ml of the test solution, 5 drops of 1% copper sulphate solution and 2 ml of 10% sodium hydroxide were added and mixed thoroughly to confirm the presence of proteins via the development of a distinct purple or violet hue.

j. TEST FOR GLYCOSIDES

To 2 ml of extract add 4ml glacial acetic acid and few drops of ferric chloride and 2ml conc. Sulphuric acid along the sides of the test tube. Brown ring at interface indicates presence of glycosides.

Table 1: Phytochemical analysis of *solanum trilobatum* leaf extract.

S.NO	TARGET METABOLITE CLASS	NAME OF TEST	ENDPOINT OBSERVED	RESULT
1.	Alkaloids	Mayer test	Cream white precipitate	+
2.	Flavonoids	Shinoda test	Pink colour developed	+
3.	Tannins	Ferric chloride test	Bluish black colour	+
4.	Saponins	Foam test	Stable foam formed	+
5.	Steroids	Acid-chloroform test	Yellow hue with green fluorescence	+
6.	Triterpenoids	Salkowski test	Reddish-brown colour	+
7.	Carbohydrate	Molisch test	Violet ring at junction	+
8.	Protein	Biuret test	Violet colour	+
9.	Glycosides	Keller-Killiani test	Brown ring +greenish purple layer	+

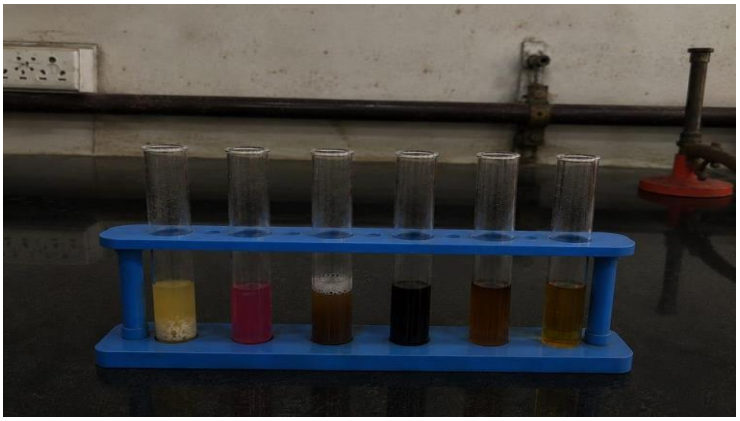

Figure 4: Phytochemical screening of Solanum trilobatum leaf extract.

4.4. EVALUATION FOR ANTI-UROLITHIATIC ACTIVITY

4.4.1. PREPARATION OF CALCIUM OXALATE CRYSTALS

Calcium oxalate crystal was prepared by dissolving 1.47 g of calcium chloride dihydrate in 100 mL of distilled water and 1.34 g of sodium oxalate in 100 mL of 2N sulphuric acid. Both solutions were mixed with continuous stirring to obtain a calcium oxalate precipitate. The precipitate was washed with ammonia solution followed by distilled water to remove impurities and then dried at 60 °C. The prepared calcium oxalate crystals were used for nucleation, aggregation and dissolution studies.

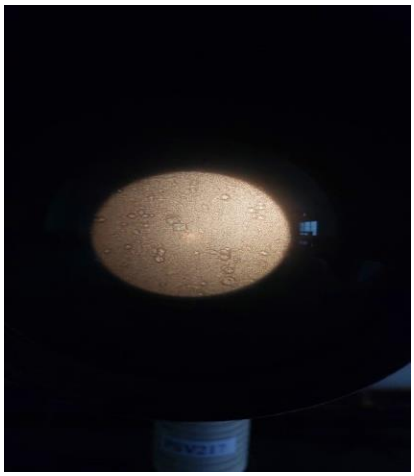

Figure 5: Microscopic view of calcium oxalate crystals.

4.4.2. TURBIDIMETRIC METHOD

The capacity of the plant extracts to prevent calcium oxalate nucleation and aggregation both in the presence and absence of inhibitors (standard medication and extracts) was used to assess their in vitro anti-urolithiatic efficiency. The turbidity fluctuations in each experiment were measured with a Jasco V-630 UV/ Visible spectrophotometer. Three different concentrations of

the plant extracts (10 mg/ml, 20 mg/ml, and 30 mg/ml) were assessed in each experiment.

4.4.3. NUCLEATION ASSAY

Calcium chloride and sodium oxalate solutions were prepared at final concentrations of 5 mmol/L and 7.5 mmol/L, respectively, using 0.05 mol/L NaCl buffer (pH 6.5). The plant extract was tested at three different concentrations of 10, 20, and 30 mg/mL. For the assay, 100 µL of each concentration of the plant extract was mixed with 950 µL of calcium chloride solution and incubated at 37 °C. Subsequently, 950 µL of sodium oxalate solution was added to initiate calcium oxalate crystallization. The optical density of the reaction mixture was measured at 620 nm. The inhibitory effect of the extract on calcium oxalate nucleation was determined by comparing the turbidity of the extract-treated samples with that of the control. Cystone was used as the standard drug for comparison.[6]

The following formula was used to determine the extract's ability to inhibit nucleation

$$\% \text{ Inhibition of nucleation} = [(C-S)/C] \times 100$$

Where, S stands for the turbidity measured for the plant extract and C stands for the baseline turbidity measured in the absence of the plant extract.

4.4.4. AGGREGATION ASSAY

Solutions containing 50 mmol/L calcium chloride and 50 mmol/L sodium oxalate were combined to create calcium oxalate monohydrate crystal (COM) crystals. To promote crystal formation, the mixture was kept at 37 °C for the whole night after being equilibrated at 60 °C for one hour in a water bath. Centrifugation was used to collect the produced crystals. The crystals were then dried at 37 °C. To obtain a final concentration of 0.8 mg/mL, the crystals were subsequently suspended in a buffer containing 0.05 mol/L Tris and 0.15 mol/L NaCl (pH 6.5).

The produced COM crystals were used for the aggregation test both with and without Solanum trilobatum extract. The turbidity of the reaction mixture was measured to track crystal aggregation after the stirring was stopped. Cystone solution was used as the positive control.[13]

The following formula was used to determine the percentage inhibition of calcium oxalate crystal aggregation (Ir).

$$Ir = (1 - \text{Turbidity}_{sample}/\text{Turbidity control}) \times 100.$$

Where Ir represent the percentage inhibition of calcium oxalate crystal aggregation.

4.4.5. TITRIMETRIC METHOD

The titrimetric method was employed to determine the antiurolithiatic activity of plant extracts by assessing their ability to dissolve preformed calcium oxalate crystals. The procedure consisted of the following steps.

a. STEP 1: PREPARATION OF EXPERIMENTAL KIDNEY STONES (CALCIUM OXALATE STONES) BY HOMOGENEOUS PRECIPITATION

Calcium oxalate were prepared by a homogeneous precipitation technique. Calcium chloride dihydrate (1.47 g) was dissolved in 100 mL of distilled water, while sodium oxalate (1.34 g) was dissolved separately in 100 mL of 2 N sulfuric acid. Equal volumes of both solutions were mixed with continuous stirring, resulting in the formation of calcium oxalate precipitate. The precipitate was treated with ammonia solution to remove any residual sulfuric acid, followed by repeated washing with distilled water. Finally, the purified calcium oxalate crystals were dried in a hot air oven at 60 °C until complete removal of moisture.[12]

b. STEP 2: PREPARATION OF EGGSHELL SEMI-PERMEABLE MEMBRANE

Fresh farm eggs were used for the preparation of the semi-permeable membrane. A small opening was made at the apex of each egg to remove the albumin and yolk completely. The empty eggshells were thoroughly rinsed with distilled water and immersed in 2 M HCL overnight to dissolve the calcified shell. After complete decalcification, the membranes were carefully washed with distilled water and treated with ammonia solution to neutralize any remaining acid. The membranes were again rinsed with distilled water and preserved in 2% ammonia solution until further use.[13]

c. STEP 3: ESTIMATION OF CALCIUM OXALATE BY TITRIMETRY

Approximately 5 mg of preformed calcium oxalate crystals were enclosed in the prepared eggshell membrane along with the plant extract at concentrations of 10, 20, and 30 mg/mL.

The membrane sacs were then suspended in conical flasks containing 100 mL of 0.1 M Tris buffer and incubated in a water bath at 37 °C overnight.

Following incubation, the membrane sacs were removed, and 2 mL of 1 N sulfuric acid was added to the contents of each flask. The solution was then titrated against 0.1356 M potassium permanganate ($KMnO_4$) until a faint permanent pink colour appeared, indicating the

endpoint.

A flask containing only calcium oxalate served as the blank, while flasks containing calcium oxalate with Cystone (10, 20, and 30 mg/mL) acted as the positive control. The test groups contained calcium oxalate with ethanolic plant extracts at the same concentrations. All experiments were carried out in duplicate, and the amount of calcium oxalate dissolved was calculated using the mole concept.

FORMULA

% Dissolution = [(Blank titre- Sample titre) /Blank titre] × 100

Figure 6: Eggshell membrane preparation for the dissolution assay.

5. RESULTS AND DISCUSSION

5.1. RESULTS

The antiurolithiatic activity of Solanum trilobatum extract was evaluated against calcium oxalate crystal formation using nucleation, aggregation, and crystal dissolution assay at concentrations of 10, 20, and 30mg/ml. The extract exhibited a concentration-dependent increasing activity across the tested concentrations.

In the nucleation assay, the extract showed 20.0%, 39.0% and 50.4% inhibition at 10, 20, and 30mg/ml, respectively. In comparison, Cystone showed 28%, 42% and 57% inhibition, respectively.

Table 2: Percentage inhibition of calcium oxalate nucleation by Solanum trilobatum extract.

SAMPLE	CONCENTRATION	OPTICAL DENSITY OF CONTROL	OPTICAL DENSITY OF SAMPLE	% INHIBITION
CONTROL	-	0.350	0.350	0.00
TEST 1 S. trilobatum	10 mg/mL	0.350	0.280	20.0
TEST 2 S. trilobatum	20 mg/mL	0.350	0.214	39.0
TEST 3 S. trilobatum	30 mg/mL	0.350	0.174	50.4
TEST 4 Cystone	10 mg/mL	0.350	0.252	28.0
TEST 5 Cystone	20 mg/mL	0.350	0.200	42.80
TEST 6 Cystone	30 mg/mL	0.350	0.151	57.0

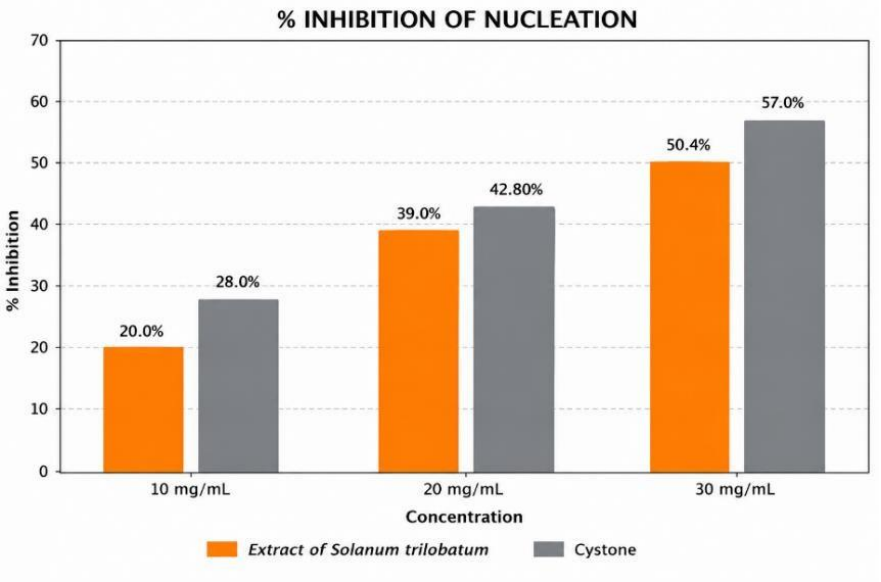

Figure 7: Comparison of the Percentage Nucleation Inhibition of Calcium Oxalate by Solanum trilobatum with Cystone.

In the aggregation assay, the extract demonstrated 7.65%, 44.59% and 61.74% inhibition at 10, 20, and 30mg/ml, respectively. At 30mg/ml, the aggregation inhibition of the extract (61.74%) was comparable to that of Cystone (63.00%).

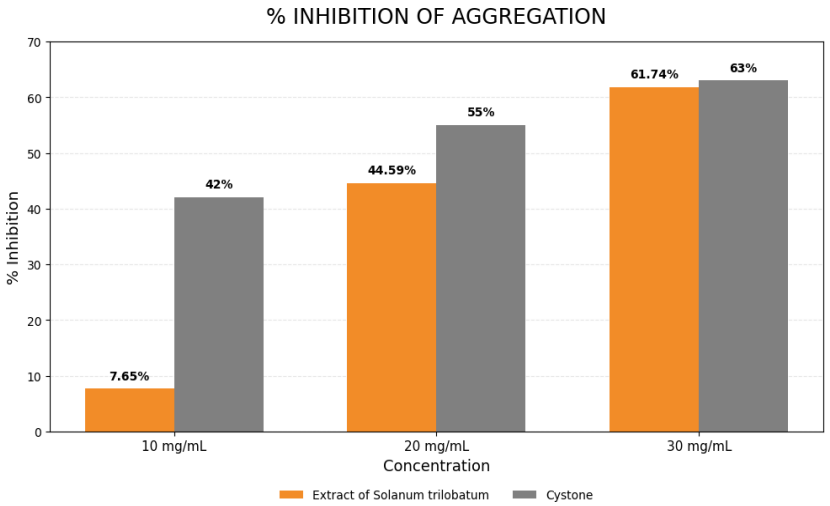

Figure 8: Comparison of the Percentage Aggregation Inhibition of Calcium Oxalate by Solanum trilobatum with Cystone.

Table 3: Percentage aggregation of calcium oxalate crystals by Solanum trilobatum extract.

SAMPLE	CONCENTRATION	TURBIDITY OF CONTROL	TURBIDITY OF SAMPLE	% INHIBITION (Ir)
CONTROL	-	75.8	75.8	0.00
TEST 1 S.trilobatum	10 mg/mL	75.8	70.0	7.65
TEST 2 S.trilobatum	20 mg/mL	75.8	42.0	44.59
TEST 3 S.trilobatum	30 mg/mL	75.8	29.0	61.74
TEST 4 Cystone	10 mg/mL	75.8	43.96	42.00
TEST 5 Cystone	20 mg/mL	75.8	34.11	55.00
TEST 6 Cystone	30 mg/mL	75.8	28.05	63.00

For crystal dissolution, the extract exhibited 45.36%, 50.82%, and 55.73%dissolution at 10, 20, and 30mg/ml respectively, whereas Cystone showed 50.0%, 60.0%, and 78.0% dissolution, respectively.

Table 4: Percentage dissolution of calcium oxalate crystals by Solanum trilobatum extract.

SAMPLE	CONCENTRATION	BLANK TITRE (ml)	SAMPLE TITRE (ML)	% DISSOLUTION
CONTROL	-	10.00	10.0	0.00
TEST 1 S.trilobatum	10 mg/mL	10.00	5.46	45.36
TEST 2 S.trilobatum	20 mg/mL	10.00	4.92	50.82
TEST 3 S.trilobatum	30 mg/mL	10.00	4.43	55.73
TEST 4 Cystone	10 mg/mL	10.00	5.00	50.00
TEST 5 Cystone	20 mg/mL	10.00	4.00	60.00
TEST 6 Cystone	30 mg/mL	10.00	2.20	78.00

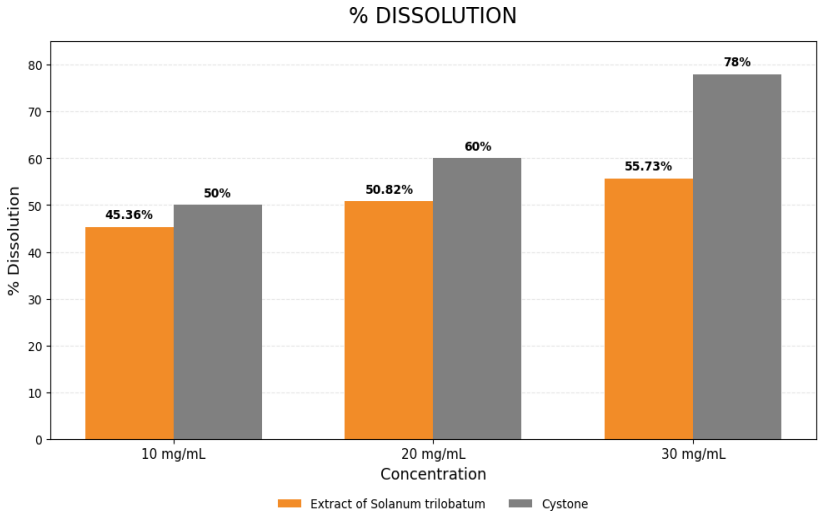

Figure 9: Comparison of the Percentage dissolution of Calcium Oxalate by Solanum trilobatum with Cystone.

5.2. DISCUSSION

The present findings demonstrate that the Solanum trilobatum extract possesses promising in vitro antiurolithiatic activity, with a concentration-dependent response across the evaluated parameters. The progressive increase in nucleation inhibition from 20.0% to 50.4% suggests that the extract may interfere with the initial formation of calcium oxalate crystals. Although the nucleation inhibitory activity of the extract was lower than Cystone at all tested concentrations, the increase in response with concentration indicates its potential to inhibit crystal formation.

The marked increase in aggregation inhibition, particularly from 7.65% at 10mg/mL to 61.74% at 30 mg /mL, suggests that the extract may interfere with crystal-crystal interaction and subsequent enlargement of calcium oxalate crystals. The activity at 30mg/mL was almost comparable to Cystone (63%), indicating appreciable inhibition of crystal aggregation.

The extract also exhibited a concentration-dependent increase in crystal dissolution, reaching 55.73% at 30mg/ml. However, its dissolution activity was lower than that of Cystone (78% at 30mg/ml), suggesting a comparatively moderate effect on preformed calcium oxalate crystals. The observed antiurolithiatic activity may be attributed to the combined action of bioactive phytoconstituents such as flavonoids, phenolic compounds, saponins, glycosides and steroidal alkaloids, which may interfere with different stages of calcium oxalate crystallization and crystal stability. Overall, the findings indicate that S. trilobatum has promising potential, particularly in inhibiting calcium oxalate crystal aggregation, and supports its further investigation as a potential plant source for antiurolithiatic activity.

6. CONCLUSION

The present study demonstrated that the ethanolic extract of Solanum trilobatum possesses promising in vitro antiurolithiatic activity against calcium oxalate crystals. The extract exhibited concentration-dependent inhibition of crystal nucleation and aggregation with the highest inhibition observed at 30 mg/ml (50.4% and 61.74% respectively). In the titrimetric dissolution assay, the extract effectively dissolved calcium oxalate crystals, showing 55.73% dissolution at the higher concentration, although the activity was lower than that of the standard drug Cystone (78.0%). The antiurolithiatic activity may be attributed to phytoconstituents present in Solanum trilobatum, including flavonoids, phenolic compounds, alkaloids, saponins, and glycosides, which are known to inhibit crystal formation and provide antioxidant and nephroprotective effects. Overall, the findings provide preliminary scientific support for the traditional medicinal use of Solanum trilobatum and suggest that it has potential as a natural antiurolithiatic agent. Further in vivo and clinical studies are recommended to establish its therapeutic efficacy and safety.

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