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Assessment of In-Vitro Anti-Urolithiatic Activity of Selenicereus Undatus

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Abstract

The plant Selenicereus undatus, sometimes referred to as dragon fruit, has a variety of bioactive substances that have been shown to have pharmacological effects. Recurrence rates and related comorbidities continue to be major obstacles in spite of therapy improvements. Because of their possible therapeutic advantages in the management of urolithiasis, natural products have drawn attention. The purpose of this work was to assess, using experimental models, the anti-urolithiatic potential of S.undatus extract. In order to identify the bioactive compounds that make up the ethanolic extract of S. undatus, a phytochemical screening procedure was conducted. Following the administration of S. undatus extract to the animals, many parameters were examined, including urine pH, urinary biochemical components, kidney histology, and stone development.

Keywords - Kidney stone, urinary calculi, urolithiasis, nucleation assays.

INTRODUCTION

What Is Kidney Stone?

Medical terms for solid crystalline masses that develop in the kidneys or urinary tract include kidney stones, sometimes referred to as urolithiasis or nephrolithiasis. These crystals have the potential to combine and develop into bigger stones over time, which can result in pain, discomfort, and more serious issues including infection and blockage of the urinary system. An annual millions of individuals worldwide suffer from kidney stones, a prevalent urological ailment. Kidney stones are becoming more and more common, and dietary and lifestyle choices have a big impact on how they form. Dehydration, a high food consumption of specific chemicals (such as calcium, oxalate, and salt), certain medical conditions (Hyperthyroidism).[1]

Types of Kidney Stone

Calcium Stones

Calcium phosphate stones: It form when phosphate combine with the calcium in the urine. Calcium oxalate stones: These are a result of elevated oxalate levels in the urine, which may mix with calcium to form these stones.[2]

Uric Acid Stones

It happens if you eat a high-protein diet or have certain medical conditions that increase uric acid levels in the body. Forms due to too much uric acid in urine.[3]

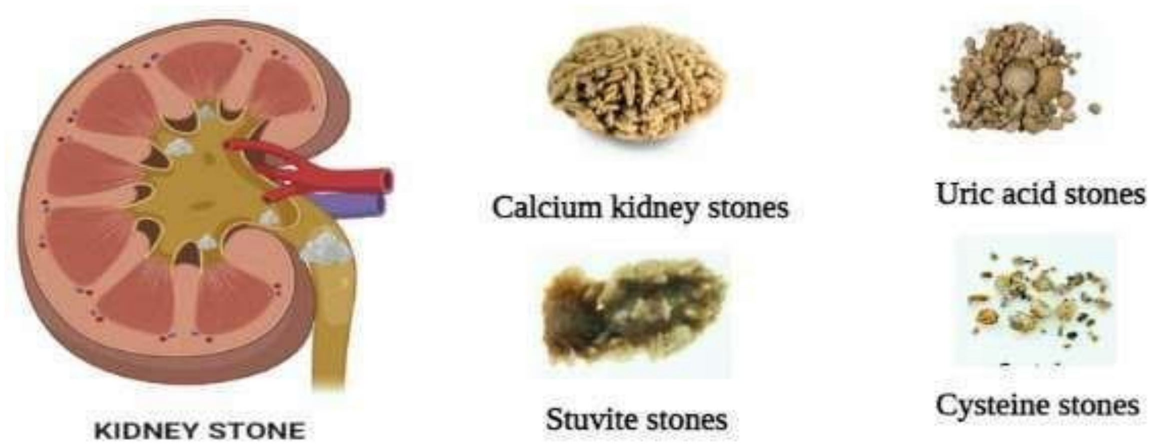


Figure 1*: Types of Kidneys Stones*

Struvite Stones

Struvite stones are less common and usually become a consequence of infections in the urinary system.[4] These stones grow and become large.

Cystine stones

Cystine stones are a type of kidney stone composed of the amino acid cystine. They are relatively rare, accounting for 1-2% of all kidney stones. These stones are typically larger and more difficult to treat compared to other types of kidney stones.[5]

Epidemiology of Kidney Stone

The epidemiology of kidney stones encompasses various factors contributing to the prevalence, incidence, and distribution of this condition within populations. Kidney stones, also known as nephrolithiasis, occur when certain substances in the urine form crystals that aggregate and develop into solid masses within the kidney The prevalence of kidney stones has been steadily increasing worldwide, affecting approximately 1 in 10 individuals at some point in their lives. Various demographic factors influence the risk of developing kidney stones, including age, sex, ethnicity, geography, and socioeconomic status.[6] Peak incidence typically observed between the ages of 30 and 50. However, the prevalence among women has been rising, narrowing the gender gap. Regions with hot climates and low water intake tend to have higher rates of kidney stone formation. Additionally, dietary habits rich in animal protein, sodium, and oxalate contribute to stone formation, while inadequate consumption of fluids reduces urine volume, leading to higher concentrations of stone-forming substances. Genetic predisposition is significant with certain metabolic disorders increasing the risk. These include hypercalciuria, hyperoxaluria, and cystinuria, among others.[7]

Urinary Tract Stone

Urinary calculi, sometimes referred to as stones in the urinary system or urolithiasis, are solid masses resulting from urine's crystalline components. These stones causing varying degrees of discomfort and complications. UTS reflects a complex interplay of genetic, environmental, and lifestyle factors. While the exact causes are multifactorial, several risk factors contribute to their formation.[8] These include dehydration, which leads to concentrated urine and increased likelihood of crystallization; high intake of salt, oxalate-rich foods; as well as certain medical conditions like hyperparathyroidism, gout, and urinary tract infections. Demographic factors are important in epidemiology of urinary tract stones. Men are more commonly affected than women, with a peak incidence typically observed in middle-aged individuals.[9]

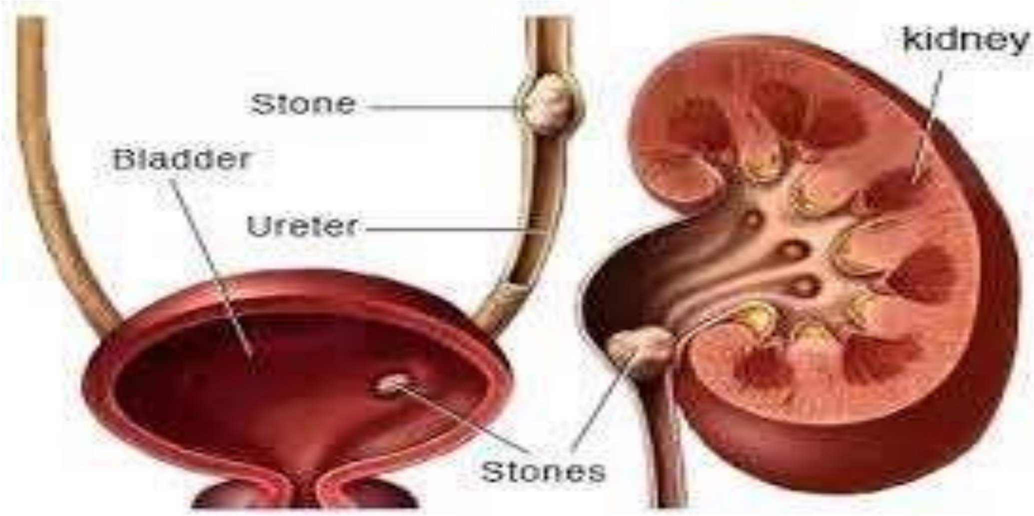
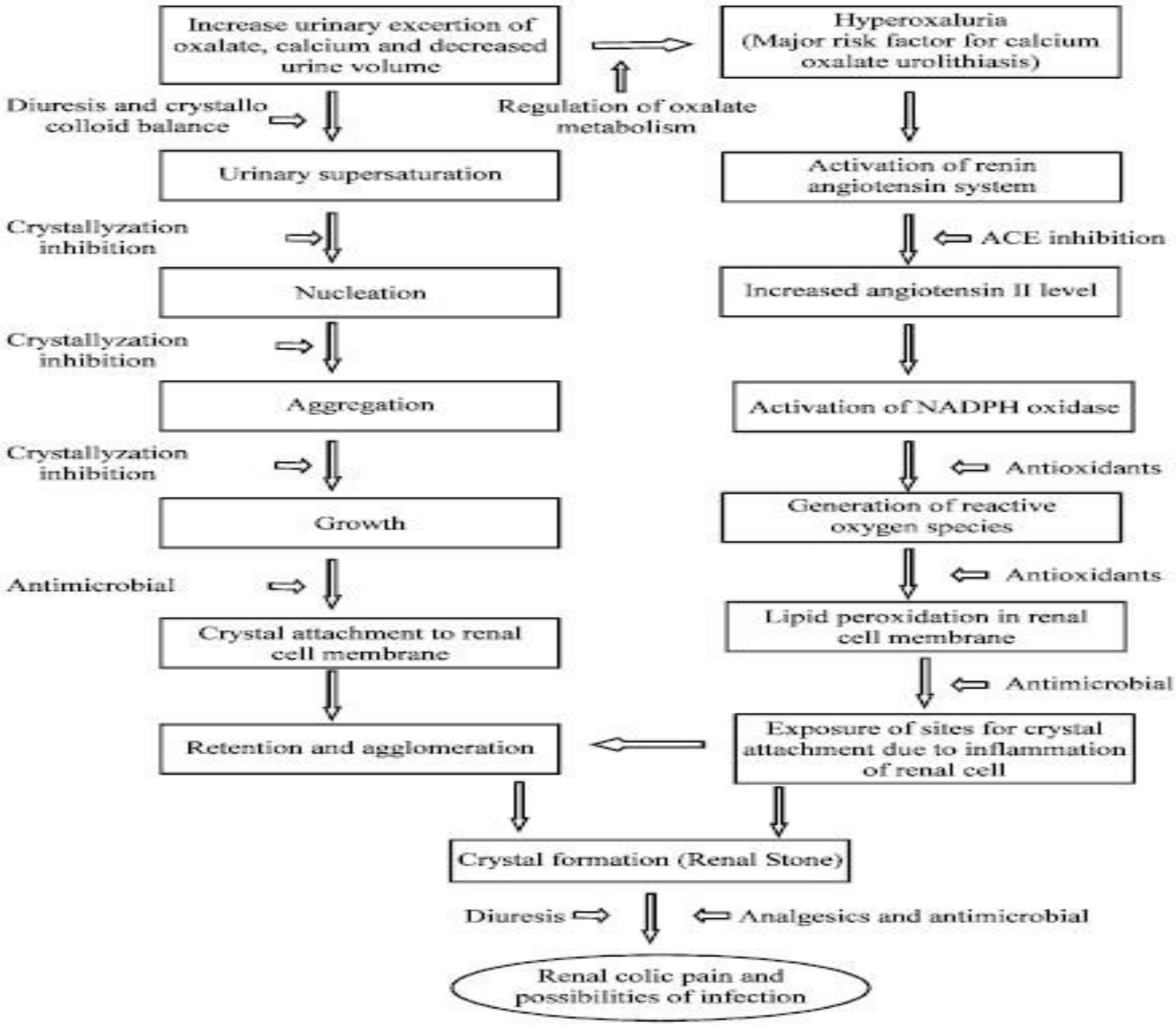


***Figure 2**: Kidneys Stone*

Mechanism of Action of Phyto Therapeutic Agents



Plant Profile

Figure 3: *Selenicereus Undatus Plant*

Selenicereus undatus, commonly known as pitaya or dragon fruit, is a tropical fruit native to Central America and Mexico. It belongs to the family Cactaceae and is characterized by its vibrant appearance and unique flavor. The fruit has gained popularity worldwide due to its exotic appearance, mild sweetness, and potential health benefits. Pitaya typically has a vibrant pink or yellow peel with green scales, and its flesh can be either white or red, dotted with tiny black seeds.[10]

Scientific Classification

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Order: Caryophyllales
Family: Cactaceae
Subfamily: Cactoideae
Tribe: Hylocereeae
Genus: Selenicereus
Species: Selenicereus undatus

Vernacular Names of Plant

Pitahaya

This is the Spanish name for the fruit and is widely used in Latin American countries.[11]

Dragon fruit

This is the English name commonly used in many English-speaking countries due to the fruit's resemblance to dragon scales.

Buah naga

This is the Indonesian and Malaysian name for the fruit, which translates to "dragon fruit."

Thanh long

This is the Vietnamese name for the fruit, which also translates to "dragon fruit." [12]

Materials & Methodology

Plant collection & Authentication

In March 2024, leaves of selenicereus undatus werw gathered from Sangola, located in Solapur district, India. Department of Botany & Physiology's Dr. R.R Tembhurne verified the authenticity of plant.

Processing & Extraction

Powder is prepared from fresh leaves, dried, and cleaned under running water.[13]

Preparation of Ethanolic extract

A few changes made to the extract preparation techniques from those outlined in after being cleansed with ordinary water and dried, the leaf sample was placed in blender to be ground into powder. Ethanol is utilised in range of ratios as a solvent for the Soxhlet extraction procedure. The extract should bew mixed with muslin cloth, transferred to 50 ml tubes, and centrifuged for 15 minutes at 4000RPM and 25 degrees after it has been collected for 6 to 8 hours. Once collected, the supernant was set aside for drying.[14]

Preliminary Phytochemical Analysis

Sr No.	Name of Test	Observation	Inference
1.	**Test for Alkaloids** **Dragandroff's test** To a few mL of extract, 1 or 2 ml of Dragendroff reagent (potassium bismuth iodide) were added. **Mayers test** To a few ml of extract, two drops of Mayers reagent (potassium mercuric iodide solution) were added. **Hager's test** To a few ml of extract, 1 to 2 ml of Hagers reagent (saturated solution of picric acid) were added. **Wagner's test:** To a few ml of extract, few drops of Wagners reagent (iodine in potassium iodide) were added.	Orange-brown coloured ppt. Cream coloured ppt. Yellow coloured ppt. Reddish Brown Coloured ppt.	Alkaloid Present. Alkaloid Present. Alkaloid Present. Alkaloid Present.
2.	**Test for Saponins** The extract was taken in a test tube and shaken vigorously.	Formation of stable foam.	Saponin Present
3.	**Test for Phenols** Extract mixed with 2 ml of 2% solution of FeCl3.	Blue / Green colour	Phenols Absent
4.	**Test for Tannins** Extract mixed with 2 ml of 2% solution of FeCl3.	No black colour.	Tannins Present
5.	**Test for Flavonoids** Extract was treated with few drops of Sodium hydroxide solution.	Forms the intense yellow colour. colourless on the addition of dilute acid.	Flavonoids Present

6.	**Test for Glycosides:** **Keller Killani test:** A solution of 0.5 ml, containing glacial acetic acid & 2-3 drops of ferric chloride was mixed with 2 ml of extract. Later, 1 ml of conc.H2SO4 was added along the walls of test tube.	The appearance of deep blue colour at the junction of two liquids	Glycosides Present.
7.	**Test for Carbohydrates** **Molish test:** Few drops of alcoholic a-naphthol solution were added to 2 mL of extract. Later, few drops of concentrated H2SO4 were added along the walls of test tube. **Benedict's test** To 5 ml of Benedict's reagent, 8-10 drops of extract were added, then heated for 5 minutes.	At the junction of two liquids, a violet colour ring appeared. Dark – Red ppt.	Carbohydrate Present. Carbohydrate Present.

***Table 1**: Preliminary Phytochemical Analysis*

Nucleation Assay

In a beaker, mix equal volumes (10 ml each) of the 5 mM calcium chloride solution and the 5 mM sodium oxalate solution to initiate nucleation as a control. In separate beakers, add varying concentrations of the test substance to 10 ml of the 5 mM calcium chloride solution each beaker, add 10 ml of the 5 mM sodium oxalate solution to initiate nucleation in the presence of the test substance. After incubation 30 min, measure the absorbance of the solutions at 620 nm using a spectrophotometer.[15]

Percent inhibition: (1- absorbance of test solution / absorbance of control) x 100

Aggregation Assay

Mix equal volumes of 10 mM calcium chloride solution and 10 mM sodium oxalate solution to form calcium oxalate crystals. This will result in a 5 mM solution of calcium oxalate. Incubate the mixture at 37°C for 30 minutes to allow for crystal formation. Take 1 mL of the calcium oxalate crystal suspension in a centrifuge tube. Add 1 mL of Tris buffer (0.05 M, pH 7.4) to the tube. Add different concentrations of the test compound to separate tubes. Make up the final volume to 3 ml. After incubation 30min, measure the absorbance of each solution at 620 nm using a spectrophotometer. Use distilled water as the blank.[16]

Percent inhibition:(Absorbance of control-Absorbance of test/Absorbance of control) x 100

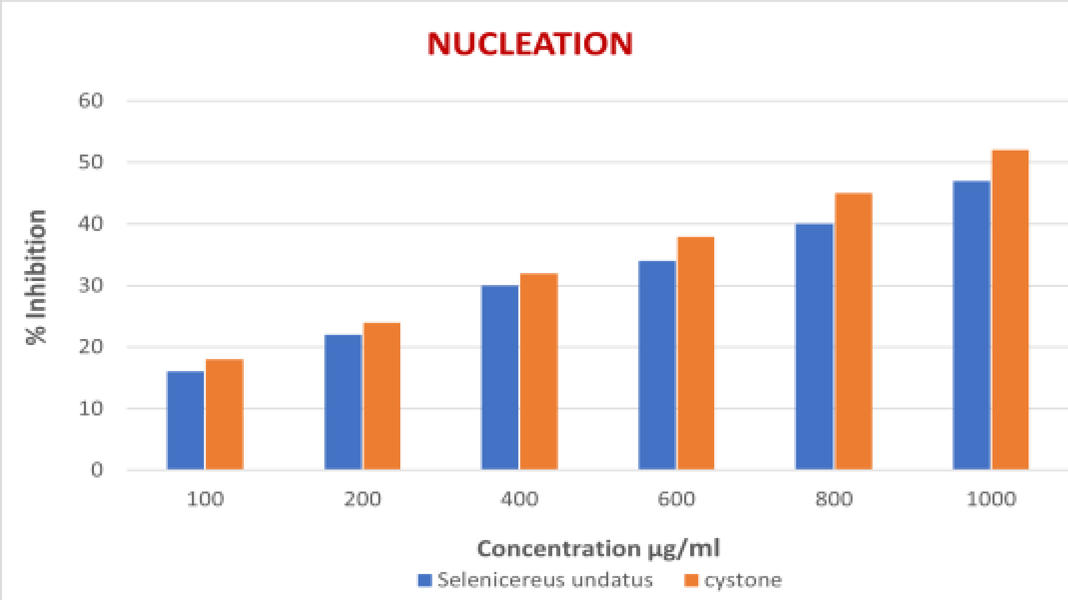
Result

Sr No.	Constituent present in Ethanolic extract	Observations
1	Alkaloids	+
2	Saponins	+
3	Phenols	**-**
4	Tannins	+
5	Glycosides	+
6	Flavonoids	+
7	Carbohydrates	+

***Table 3:** Result of preliminary phytochemical analysis of Selenicereus undatus extraxt*

(+) Indicates the presence of compound (-) Indicates the absence of compound

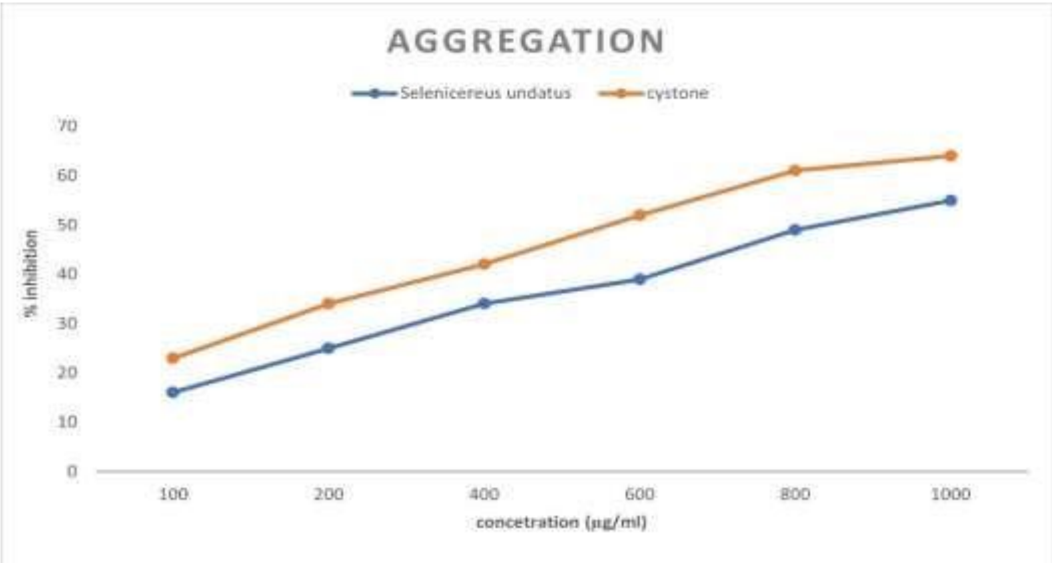
Nucleation Assay



Graph 1: *Effect of SULE and Cystone on Nucleation of CaOx crystals*

Addition of sodium oxalate solution to the reaction mixture containing calcium chloride resulted in the formation of numerous Ca0x crystals. Presence of selenicreus undatus leaves extract (1000 µg/ml) in the reaction mixture produced a percent reduction in nucleaction of 46.2±0.45% which was significantly lower (*P*<0.01) that produced by Cystone. (50.99±1.01%).

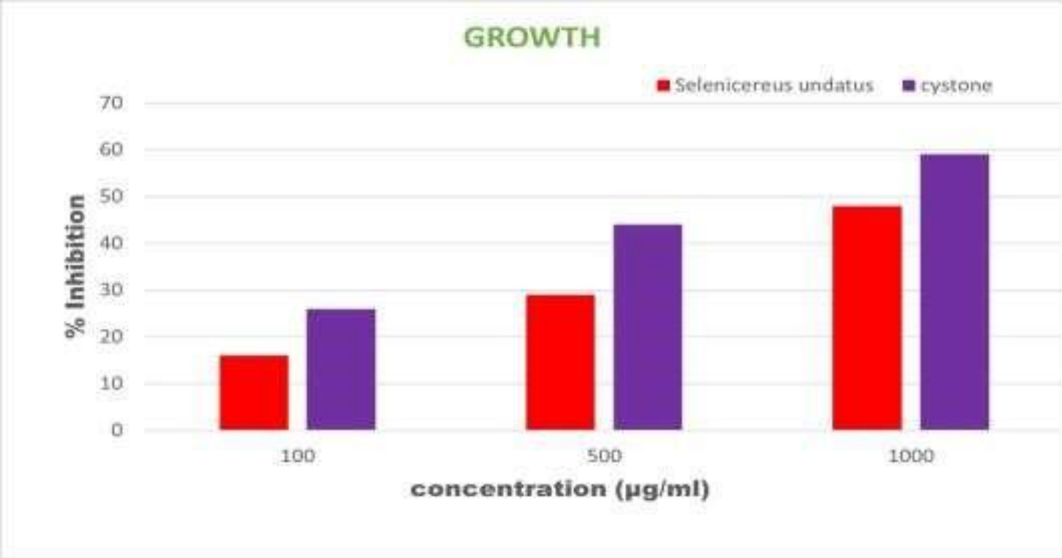
Aggregation Assay



Graph 2: *Effect of SULE and Cystone on Aggregation of CaOx crystals*

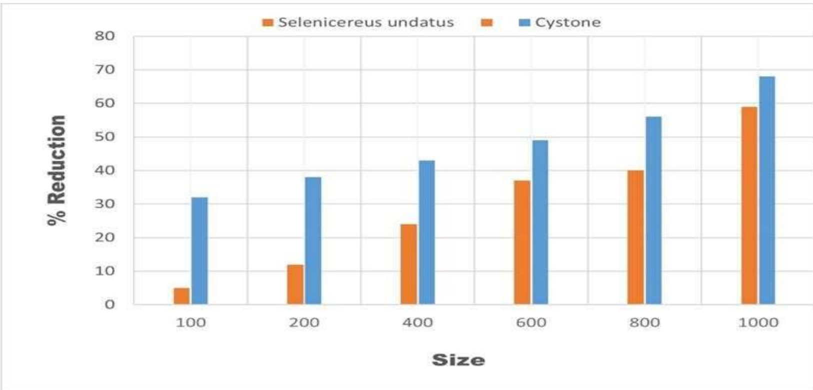
The Agglomeration of the CaOx crystals was significantly reduced (P<0.0001) by the extract of Selenicereus undatus. The percentage decrease in aggregation caused by SULE was discovered to be 53.02±2.09%, which is similar to cystone's (64±2.30%) at 1000 µg/ml concentration.

Oxalate Depletion Assay

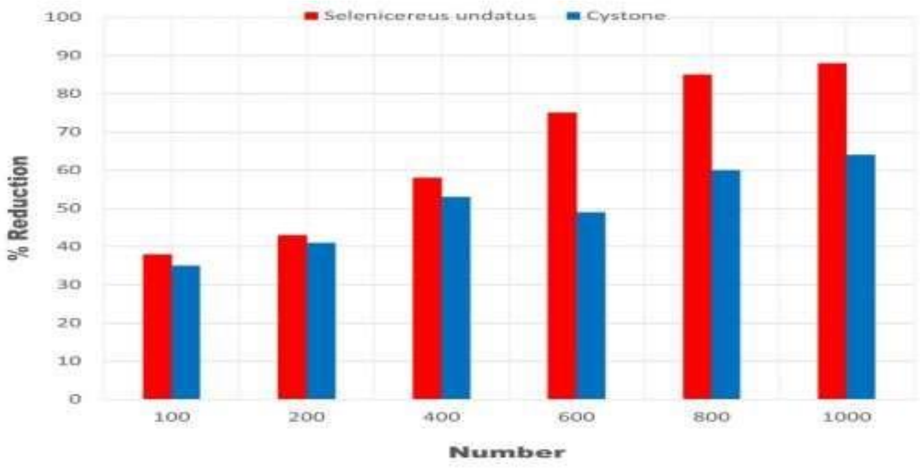


Graph 3: *Effect of SULE and Cystone on growth of CaOx crystals*

The growth was observed to be reduced by 47± 0.90% when SULE was present, and by 58.9±0.10% when cystone was present at 1000 µg/ml. At lower doses, SULE's CaOx crystal growth inhibitory impact was much less than that of cystone ($P<0.001$), but at higher concentrations, it was equivalent to crystals.



Graph 4: *Effect of SULE and Cystone on Size of CaOx crystals*



Graph 5: *Effect of SULE and Cystone on Number of CaOx crystals*

Discussion

This activity is crucial in addressing urolithiasis, a common urological disorder of urinary tract. Selenicereus undatus has been selected for this study due to its rich phytochemical profile, which includes flavonoids, saponins, Carbohydrates and phenolic compounds known for their medicinal properties. Results from such studies have shown promising anti-urolithiatic activity of Selenicereus undatus extracts. For instance, the flavonoids and saponins in the plant may bind to calcium oxalate crystals, preventing their growth and facilitating their dissolution.

Conclusion

The assessment of in vitro anti-urolithiatic activity of Selenicereus undatus demonstrates promising potential in the prevention and management of urolithiasis. The study's findings suggest that the extracts of Selenicereus undatus exhibit significant inhibitory effects on the formation and growth of calcium oxalate crystals, which are the primary components of most kidney stones. The bioactive compounds present in the fruit, including antioxidants, flavonoids, and polyphenols, likely contribute to these beneficial effects by altering the crystallization process and promoting the dissolution of existing stones. Further research, including in vivo studies and clinical trials, is essential to fully elucidate the mechanisms of action and to establish the efficacy and safety of Selenicereus undatus as a natural therapeutic agent for urolithiasis. However, these initial in vitro results are encouraging and suggest that Selenicereus undatus could be a valuable addition to the repertoire of natural remedies for preventing and treating kidney stones.

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