

Antirolithiatic potential of *Caralluma adscendens* medicinal plant by the inhibition of nucleation and aggregation of calcium oxalate crystal *in vitro*.

Arshu P. Patel^{*1}, Nikita R. Kature², Manish Ahire³, Jagdish Kudnar⁴, Sagar Vaidya⁵, Sambodhi Patil⁶, Ravindra Jadhav⁷

^{1,2,4,6}Pravara Rural College of Pharmacy, Pravaranagar, Loni, Rahata, Ahmednagar-413736, Maharashtra, India

^{3,5}JES, SND institute of pharmacy Babhulgaon Yeola Nashik 423401

⁷Pravara Institute of pharmacy Pravaranagar, Loni, Rahata, Ahmednagar-413736, Maharashtra, India

Address for correspondence Author

E-mail: arshupatel59@gmail.com

ABSTRACT

Background: In several regions of India, *Caralluma adscendens* var. *fimbriata* is a species that is used in traditional medicine for its anti-inflammatory, analgesic, antifungal, antibacterial, anti-cancer, anti-diabetic, appetite suppression, antiobesity properties and antioxidant properties. The objective of this investigation was to assess the plant's ethanolic extract's antirolithiatic activity as well as phytochemical investigation. Kidney stones are treated with medicinal plants in ayurvedic medicine. The ethanolic extract of *Caralluma adscendens* leaves shown antirolithiatic efficacy against CaOx urolithiasis *in vitro*. **Methods:** Both CaOx monohydrate (COM) and dihydrate (COD) crystallised in a synthetic urine system. Spectrophotometric methods were used to measure the nucleation, growth, and aggregation of crystals. At the same dosages, the results were compared to those of the polyherbal drug cystone. Urine crystals were also visible under optical microscopy. Statistical differences and percentage inhibitions were calculated and compared using pre-established procedures. To identify any active phytoconstituents in the study plants, a preliminary phytochemical screening was also conducted. **Results:** Using HPLC analysis, the phytochemicals present in plant extracts were investigated and the results showed that the ethanolic extract of *Caralluma adscendens* demonstrated substantial antirolithiatic characteristics and could dissolve calcium oxalate crystals.

Keywords: *Caralluma adscendens*, phytochemical analysis, HPLC, Antirolithiatic activity

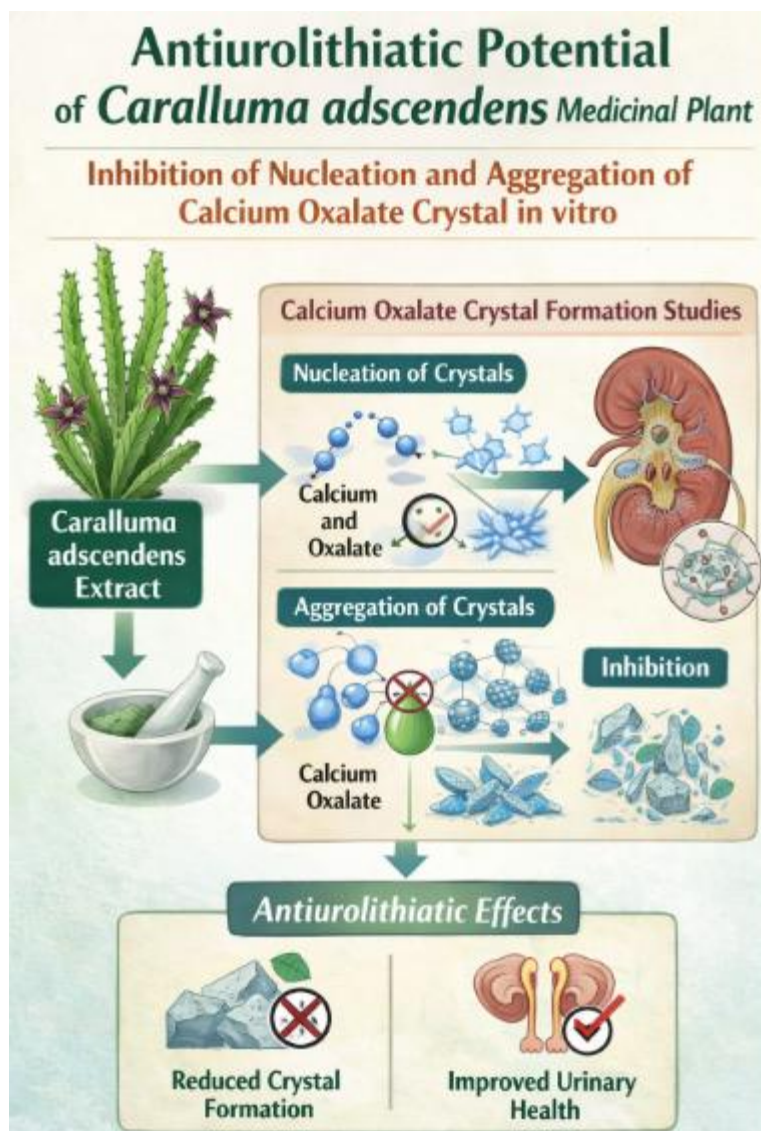


Fig no 1 :Graphical abstract of Antiuro lithiatic potential of *Caralluma adscendens* medicinal plant by the inhibition of nucleation and aggregation of calcium oxalate crystal *in vitro*

1. Introduction

Plants have been used for their medicinal properties for centuries. Across the world, therapeutic products derived from plants have been passed down through generations, leading to the development of various medicines. The healing qualities of plants stem from their phytochemicals and bioactive compounds such as phenols, flavonoids, alkaloids, and tannins, which enable them to treat infectious diseases effectively. *Caralluma adscendens* var. *fimbriata*, also known as *Yugmaphallottatna* in Sanskrit; is a perennial, edible, thick succulent herb resembling a cactus. It grows extensively in certain Indian villages and the arid hill areas of the Satara district in Maharashtra. This plant, which belongs to the Asclepiadaceae family, is locally known in Maharashtra as "*Makadshenguli*" or "*Shengulmakad*" ^[1]. The process by which calculi or stone develop or build up in the urinary tract is known as urolithiasis. About 12% of people worldwide suffer with urolithiasis, which is currently the third most common urinary ailment. Males are more likely than females to experience recurrences of this condition. Based on the

size, nature, and location of the stones, over 90% of patients with upper urinary tract stones are currently receiving treatment, with success rates ranging from 68% to 86%. Increased dietary protein intake has been linked to an increased incidence of renal stones ^[2]. Calcium phosphate or calcium oxalate make up about 75% of kidney stones.

Medicinal plants have been integral to ancient traditional practices for treating kidney stones. They offer an inexpensive source of medication, are considered relatively safe with little to no side effects, and are easily accessible and affordable ^[3]. Researchers have used in vitro techniques to examine *Caralluma adscendens* anti-urolithiasis qualities in light of the difficulties involved in the medical treatment of kidney stones and the plant's considerable therapeutic value owing to its high levels of polyphenols and flavonoids. *Caralluma adscendens* has been used traditionally to treat kidney stone development and other chronic renal disorders, according to ethnobotanical research. Despite these assertions, there is a dearth of scientific evidence supporting this plant's ability to prevent kidney stones. Thus, the current study used the litholytic-activity and anti-crystallization methods to identify the chemical components in *Caralluma adscendens* extracts and evaluate their possible anti-urolithiasis activity ^[4].



Fig. 2. Images of *Caralluma adscendens*

2. Materials and methods

2.1 Sample collection & Authentication

Rahuri Vidyapeeth, Rahuri's Medicinal Garden, is where *Caralluma adscendens* was gathered. MPKV, Rahuri 413722, Medicinal and Aromatic Plants Project. Plant leaves are picked off and given a water wash. The plant materials were ground into powder after being shade-dried. Dr. S. P. Giri, Research Guide, Department of Botany and Research Centre, PADMASHRI VIKHE PATIL COLLEGE OF ARTS, SCIENCE AND COMMERCE, Loni, Maharashtra, India, physically removed the complete plant species growing alongside the soil for collecting and authentication.

2.2 Drugs and Chemicals

The Himalaya Drug Company provided the polyherbal medication cystone, and all of the chemicals and solvents used in the experiment were of reagent grade and available for purchase.

2.3 Preparation of Plant Extract

The entire *Caralluma adscendens* plant was cleaned dried in the shade, and ground to powder in a mechanical grinder. Separately, the required number of powder samples was weighed and transferred to a round bottom flask and added ethanol as a solvent into the flask. Ethanolic extract was prepared using reflux condensation apparatus. The grounded coarse powders (70 g) were immersed in Ethanol (350 mL) for 6 hrs at room temperature with frequent agitation. After filtering the extract through Whatman No. 1 filter paper, the filtrate was dried at 60° and evaporated. The finished dried sample was maintained at -20° and labelled sterile vials. At the time of the experiment, the final dried sample—known as the ethanolic extract of *Caralluma adscendens* was reconstituted in water to 1000 µg/ml.

2.4 Phytochemical Analysis

Using qualitative analytical techniques, a preliminary phytochemical screening was carried out on the plants being studied in order to determine the presence of different phytoconstituents. To identify the main classes of phytoconstituents, including reducing sugars, proteins, flavonoids, tannins, saponins, anthracene glycosides, polyphenol compounds, cyanogenic glycosides, alkaloids, etc., extracts were made in accordance with predetermined test procedures and qualitative analysis was carried out. The presence of biological components in the freshly made crude extract was assessed qualitatively^[5,6].

2.5 High performance liquid chromatography (HPLC) analysis

Five millilitres of acetonitrile-methanol-water (2:2:1 v/v) were used to dissolve precisely 200 milligrammes of *Caralluma adscendens* powder, which had been weighed. It was stored in an airtight HPLC-grade container at room temperature for 12 hours, and then it was placed in a water bath set at 55°C for 10 minutes. Additionally, to speed up the solid-liquid extraction, the sample was ultrasonically sonicated for ten minutes before being filtered through a 0.45 µm nylon filter. 20 µl of a freshly made sample was introduced into the HPLC-DAD device in order to examine the therapeutic phytoconstituents found in powdered *Caralluma adscendens*.

3. Pharmacological activity (*in-vitro*)

Experimental protocol

By tracking changes in turbidity over time after adding a 0.01 M sodium oxalate solution to fake urine, the extract's effect on calcium oxalate (CaOx) crystallisation was evaluated. A UV/Visible Spectrophotometer was used to measure absorbance at 620 nm in order to assess the production of calcium oxalate precipitates at 37°C and a pH of 6.8^[7, 8].

3.1 Aggregation assay

The steps are broken down as follows:

- (a) Solution Preparation: CaCl₂ and Na₂C₂O₃ are combined to create a solution. After that, this solution is heated for an hour at 60°C in a water bath. The solution is then incubated at 37°C for the entire night [9].
- (b) CaOX Crystal Solution Preparation: A buffer of 0.5 mol/lit Tris HCl and 0.15 mol/lit NaCl is used to prepare the CaOX crystal solution at a concentration of 0.8 mg/ml^[10].

- (c) Extract solution preparation: 200, 400, 600, 800, and 1000 µg/ml concentrations of extract solutions are made ^[11].
- (d) Extract Addition to CaOX Solution: Three millilitres of the CaOX crystal solution are mixed with one millilitre of each prepared extract solution.
- (e) Incubating: The mixture is thereafter incubated for 30 minutes at 37°C.
- (f) Measurement: Following incubation, the solution's optical density is determined at 620 nm ^[12, 13].

This experiment likely aims to determine if the extract has any inhibitory effects on the aggregation of CaOX crystals. The optical density at 620nm can provide insights into the extent of aggregation, with lower optical density potentially indicating inhibition of aggregation.

3.2 Nucleation assay

- (a) Solution Preparation: Final concentrations of sodium oxalate (Na₂C₂O₄) and calcium chloride (CaCl₂) are made at 7.5 mmol/L and 5mmol/L, respectively. These solutions are made in a pH 6.5 buffer with 0.05 mol/L Tris and 0.15 mol/L NaCl.
- (b) Combining Different Solutions: One millilitre of each CaCl₂ and Na₂C₂O₄ concentration is combined. The finished mixes are mixed and then incubated at 37°C for 30 minutes.
- (c) Optical Density (OD) Measurement: A UV-visible spectrophotometer is used to quantify the mixes' optical density at 620 nm ^[14].
- (d) Percentage Inhibition of Nucleation Calculation: The following formula is used to determine the percentage inhibition of nucleation:

$$\% \text{ Inhibition} = [(\text{OD control} / \text{OD test}) - 1] \times 100$$

Where:

OD Test is the Optical density of the test mixture.

OD Control is the optical density of the control mixture.

This calculation provides a measure of the extent to which the test mixture inhibits nucleation compared to the control mixture ^[15].

4. Statistical analysis

"The data were presented as mean ± SEM. Statistical significance between means was evaluated using one-way ANOVA followed by Dunnett's test. A P value ≤ 0.05 was considered statistically significant."

5. Results

5.1 Qualitative assays used to identify phytoconstituents in the ethanolic extract of *Caralluma adscendens*

Table 1: Phytochemical tests of *Caralluma adscendens* extract

Sr. no	Phytochemicals	<i>Caralluma adscendens</i> extract
1.	Alkaloids	Absent
2.	Carbohydrates	Present
3.	Glycosides	Present
4.	Steroids	Absent
5.	Flavonoids	Present
7.	Tannins	Present
8.	Terpenoids	Present
9.	Protein	Present

5.2 Phytochemical analysis of *Caralluma adscendens* extract by HPLC

The initial phytochemical analysis indicated the existence of both reducing sugars and proteins within the plant extracts. "Steroids, coumarin, proteins, carbohydrates, diterpenes, phytosterol, flavonoids, saponins, and Glycosides were all confirmed to be present, along with a significant concentration of minerals and elemental compounds." No any polyphenolic/phenolic acids and alkaloids have been detected. Flavonoids; Quercetin and Chrysin have been detected. Including Pregnane and Megastigmane glycosides, *Caralluma adscendens* shows several important glycosides and terpenoid's which might responsible for the various pharmacological activities of the plant, such as its appetite suppressing and antioxidant effects. Importantly, two major non reported glycosides (presumably not much known) have been detected and need further study to characterize them by LC-MS.

Table 2: HPLC analysis data of ethanolic extract of *Caralluma adscendens*

Test for determination	Name of compounds identified	Number of compound s identified	Contribution in %	Any remarks
Aliphatic/Phenolic/Polyphenolic acids.	Not Found			
Phyto-amines/Alkaloids	Not Identified			
Polyphenols/Flavonoids/Antioxidants	Quercetin, Chrysin		12.5%	
Glycosides/sugar-Terpenoids/Steroids	Pregnane Megastigmane	5	13.15%	Quercetin, Chrysin
Terpenoids/tocopherols/Antioxidants	Two unknown Tocopherol identified	6	3.37%	Pregnane, megastigmane
Phytosterols/Steroids	Not observed	8		

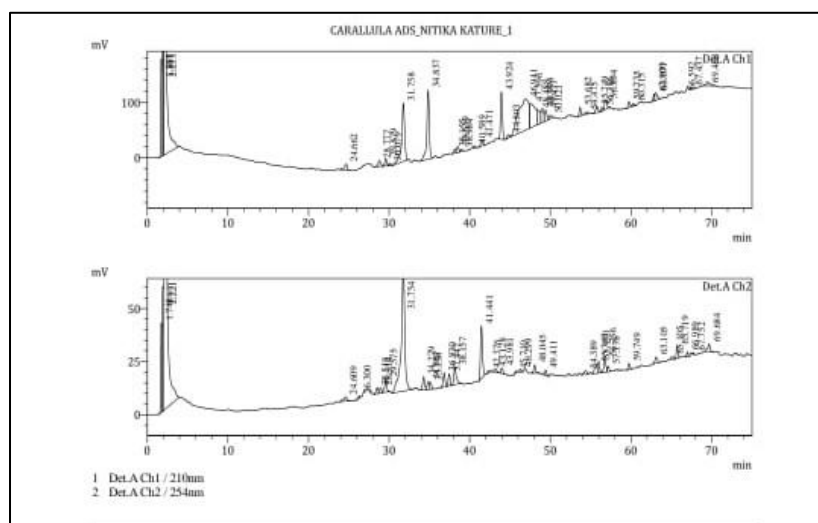


Fig. 3. HPLC Chromatogram of *Caralluma adscendens* extract

5.3 Pharmacological Activity (in vitro)

The *Caralluma adscendens roxb.* Leaf extract was screen for the Anti-urolithic activity.

Table 3: Pharmacological data (*in vitro*) of Anti-urolithic activity of *Caralluma adscendens* extract

Treatment	Control	Standard	Ethanollic extract	Ethanollic extract	Ethanollic extract	% Inhibition
% Inhibition at time in sec	Absorbance (200µg/ml)	Absorbance (400µg/ml)	Absorbance (600µg/ml)	Absorbance (800µg/ml)	Absorbance (1000µg/ml)	
0	0.1	0.1	0.1	0.1	0.1	-
120	0.15	0.14	0.11	0.12	0.13	42.86%
240	0.2	0.18	0.12	0.14	0.16	44.44%
360	0.25	0.22	0.13	0.16	0.19	45.45%
480	0.3	0.26	0.14	0.18	0.22	46.15%
600	0.35	0.3	0.15	0.2	0.25	46.67%

The aggregation assay of Standard antiurolithic cystone and Ethanollic extract of leaf parts of *Caralluma adscendens roxb.* (200, 400, 600, 800, 1000µg/ml) was observed *in vitro*. The data represent mean of total % inhibition of aggregation assay.

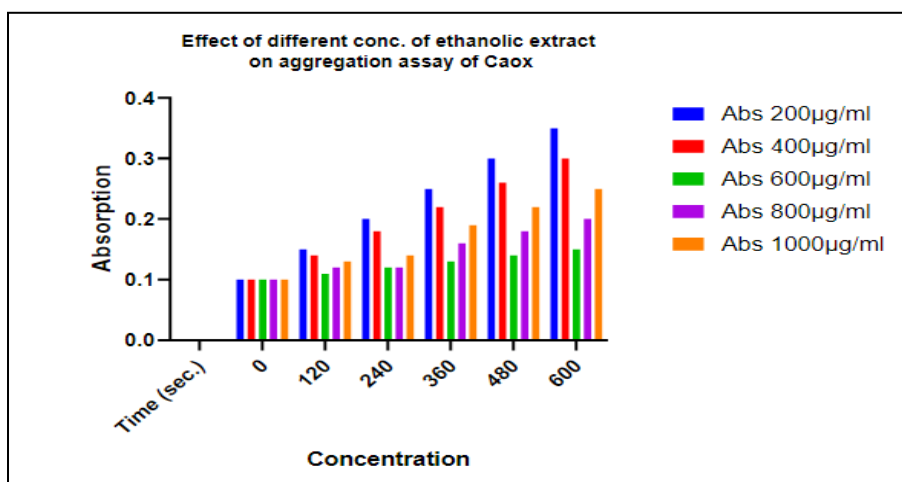


Fig. 4. Graph showing effect of concentrations of *Caralluma adscendens* extract on aggregation assay of CaOx

Table 4: Data of Nucleation Assay of *Caralluma adscendens*

Treatment	Control	Standard Cystone	Ethanolic extract	Ethanolic extract	Ethanolic extract	% Inhibition
% Inhibition at time in sec	Absorbance (10mg/ml)	Absorbance (25mg/ml)	Absorbance (50mg/ml)	Absorbance (75mg/ml)	Absorbance (100mg/ml)	
0	0.10	0.09	0.08	0.07	0.06	-
120	0.14	0.13	0.1	0.09	0.08	42.86%
240	0.18	0.16	0.12	0.11	0.1	44.44%
360	0.22	0.18	0.14	0.13	0.12	45.45%
480	0.26	0.2	0.16	0.15	0.14	46.15%
600	0.30	0.22	0.18	0.17	0.16	46.67%

The Antirolithic Capacity of Standard antirolithic mixture and ethanolic extract of leaf parts of *Caralluma adscendens* roxb (10, 25, 50, 75, 100 mg/ml) was observed *in vitro*.

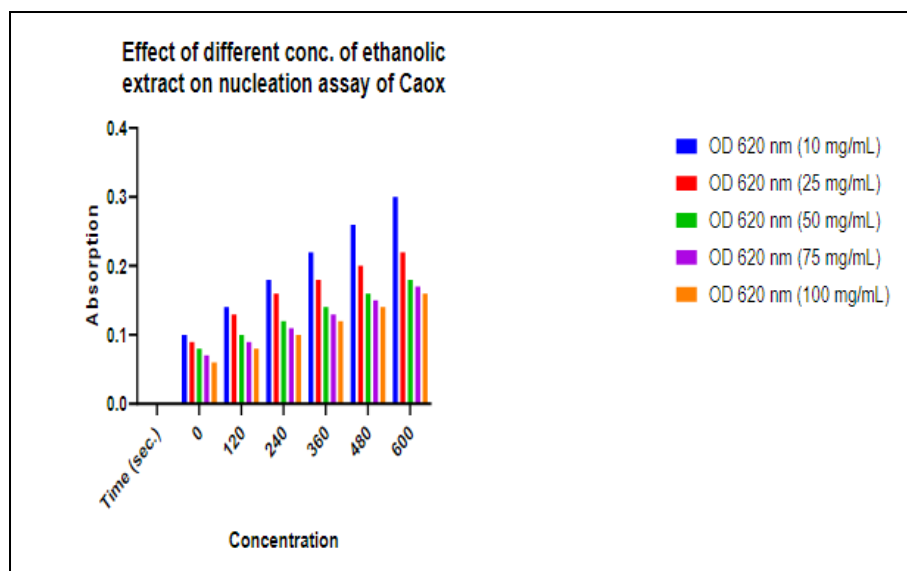


Figure 5: Graph illustrating how *Caralluma adscendens* extract concentrations affect the CaOx nucleation assay. Table 5 shows how varied extract concentrations affect the inhibition of CaOx crystals.

Concentration	CaOx nucleation	Concentration	CaOx aggregation
200µg/ml	0.2000±0.03055	10mg/ml	0.2250±0.03819
400µg/ml	0.1633±0.01944	25mg/ml	0.2000±0.03055
600µg/ml	0.1300±0.01528	50mg/ml	0.1250±0.007638
800µg/ml	0.1200±0.01528	75mg/ml	0.1467±0.01606
1000µg/ml	0.1100±0.01528	100mg/ml	0.1717±0.02358

4. Discussion

Stones in the kidney or urinary system are a symptom of urolithiasis. Nucleation, crystal development, crystal aggregation, and crystal retention are the steps in the multi-step pathophysiology of calcium oxalate stones [16]. There have been several advancements in the pathogenesis and treatment of urolithiasis, however no medication has shown complete clinical efficacy. Extracorporeal shock-wave lithotripsy (ESWL) and endoscopic stone removal are very costly procedures that frequently result in recurrence (Galani and Panchal, 2014) [17].

It is therefore very desirable to create a medication to stop this sickness or its recurrence. Long before modern medicine, antilithiatic plants and their formulations were utilised to treat kidney stones. Numerous Indian herbs have been discovered to be low-cost, efficient antilithiatic medicines with few adverse effects. The possible antiurolithiatic effects of these herbs are routinely assessed [18].

A diet high in fruits and vegetables may help reduce urolithiasis, according to recent research on both humans and animals [19]. Regularly eating a natural diet rich in phytochemicals has been shown to raise urine volume and pH while also increasing levels of stone inhibitors such as magnesium, potassium, citrate, and phytate, which are linked to decreased supersaturation of uric acid and calcium oxalate [20]. About 100 different species make up the genus, which is well-known for its antibacterial, antioxidant, hypolipidemic, and appetite-suppressing qualities [21,

23]. Pregnane glycosides, flavone glycosides, megastigmane glycosides, bitter principles, saponins, alkaloids, sterols, and the hydrocarbon n-pentatriacontane are among the herb's main phytochemical elements [24].

All of the mixture extracts in the current investigation readily inhibited crystal nucleation, growth, and aggregation, according to the results of the *in vitro* tests. Significant inhibitory power against the crystallisation process was demonstrated by the mixture's aqueous extract. Consequently, it is clear that the mixture's aqueous extract successfully inhibits CaOx crystallisation, most likely as a result of the mixture extracts varied chemical contents. These substances might act as catalysts for the crystallisation process. Furthermore, the nucleation and aggregation experiment results indicate that the combination extract significantly inhibited the CaOx crystal formation beginning stage by causing morphological alterations. The combination extract's effectiveness is comparable to that of the brand-name medication cystone, suggesting that it may be a powerful herb for lithiasis prevention.

A light microscope was used to study the beginning, aggregation, maturation, and prevention of stone formation. When compared to the common medication cystone, mixture extracts at their maximum concentration (1000 µg/mL) demonstrated notable inhibition. The extract's increased concentration of phytochemicals that prevent crystal formation is what causes this effect. These results support the earlier Wesson et al. [25].

Tamarindus indica pulp also showed a similar prevention of stone formation, and the findings align with those of *Rotula aquatic* [26]. The findings showed that, in comparison to the common medication cystone, the mixture extract dramatically decreased the start, aggregation, maturation, and inhibition of CaOx crystal formation. The combination extracts decreased the quantity of crystals in a manner comparable to that of the common medication cystone, according to the unique microscopy technology employed in this study. This suggests that the phytochemicals in the plant directly affect the crystals that are generated.

Compared to the common drug cystone, the extract mixture exhibits improved demineralisation and a greater ability to dissolve calcium oxalate, the primary component of kidney stones. Kidney stones are created when calcium phosphate, calcium oxalate, and other urine chemicals crystallise on the inside of the kidney. The initial mineral phase formation is the name given to this process. Crystal growth is the process by which these crystals can combine over time to create a tiny, hard mass known as a stone. Calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) are the two forms of calcium oxalate stones [27].

The study's results were consistent with previous research on *Kalanchoe pinnata* extract by Phatak and Hendre (2015)[28] and *Peltophorum pterocarpum* leaves by Jha et al. (2016). According to these reports, phytochemicals are responsible for the anti-urolithiatic actions.

5. Conclusion

This study is the first to evaluate how *Caralluma adscendens* ethanolic extract affects calcium oxalate crystal reduction. The extract showed promise in reducing crystal deposition, increasing the rate at which oxalate crystals dissolve, and preventing their growth *in vitro*. The results suggest that urolithiasis research and treatment may benefit from this ethanolic extract, which is enhanced with potent phytochemicals based on our laboratory findings and the traditional wisdom of South Indian healers. Kidney stone development and recurrence may be avoided with the help of the phytoconstituents in this extract.

REFERENCE :

- [1] Hewagama SP, Hewawasam RP. Antiurolithiatic potential of three Sri Lankan medicinal plants by the inhibition of nucleation, growth, and aggregation of calcium oxalate crystals in
- [2] Ghogare PB, Aher RK. Evaluation of Antimicrobial Efficacy of *Caralluma adscendens* var. *fimbriata* Extracts: A Molecular Docking Study.9, September 2022, Vol. 10.
- [3] Aryal S, Kuwar P, Thapa C. Antiurolithiatic activity of selected plants extracts against calcium oxalate crystals. *Journal of Medicinal Plants Research*.2021 Apr 30; 15(4):172-7.
- [4] Dutt HC, Singh S, Avula B, Khan IA, Bedi YS. Pharmacological review of *Caralluma R. Br.* with special reference to appetite suppression and anti-obesity.*Journal of medicinal food*.2012 Feb 1; 15(2):108-19.
- [5] Packialakshmi N, Naziya. Screening of antibacterial and phytochemical analysis.2014,Vol. 3. ISSN: 2277- 7695.
- [6] Padwal AD, Varpe SN, Waman MB. Phytochemical and nutritional analysis of *Caralluma fimbriata* L. *Int J Res BiosciAgric Technol*. 2016; 1: 193-5.
- [7] Evans WC. *Trease and Evans Pharmacognosy*.15th ed. London: WB Saunders; 2002.
- [8] Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention.*African journal of traditional, complementary and alternative medicines*.2013 Aug 14; 10(5):210-29.
- [9] Costa-Bauzá A, Perelló J, Isern B, Sanchis P, Grases F. Anticalcifying effect of phytate in biological fluids and osteoblastic cell culture. *Urol Res*.2006; 34(3):150-158.
- [10] Opalko W, Tiselius HG, Albrechtsson E, Larsson L. Effects of urine dilution on the crystallization of calcium oxalate in urine: an in vitro study. *Scand J Urol Nephrol*.1997; 31(2):137-141.
- [11] Thakur M, Vasudeva N, Sharma S, Datusalia AK. Plants and their bioactive compounds as a possible treatment for traumatic brain injury-induced multi-organ dysfunction syndrome.*CNS & Neurological Disorders- Drug Targets-CNS & Neurological Disorders*.2023 Nov 1; 22(9):1313-34.
- [12] Atmani F, Slimani Y, Mimouni M, Hacht B. Prophylaxis of calcium oxalate stones by *Herniariahirsuta* on experimentally induced nephrolithiasis in rats. *BJUinternational*. 2003 Jul; 92(1):137-40.

- [13] Hennequin C, Lalanne V, Daudon M, Lacour B, Druke T. A new approach to studying inhibitors of calcium oxalate crystal growth. *Urological research*.1993 Mar; 21:101-8.
- [14] Samuelsson G. *Drugs of natural origin*. Apotekarsocieteten; 1999.
- [15] Sharma N, Tiwari V, Tiwari M, Singh VK, Singh SK. Pharmacological evaluation of anti-urolithiatic activity of *Bergenia ligulata* rhizome extract in experimentally induced urolithiasis in rats. *J Ethnopharmacol*.2016; 194: 873-879.
- [16] Aggarwal KP, Narula S, Kakkar M, Tandon C. Nephrolithiasis: molecular mechanism of renal stone formation and the critical role played by modulators. *BioMed research international*.2013; 2013(1):292953.
- [17] Galani VJ, Panchal CJ. Pharmacognostic and phytochemical investigation of the leaves of *Ziziphus jujuba* Mill. *Int J Pharm Pharm Sci*.2014; 6(5):162-167.
- [18] Segura JW, Preminger GM, Assimos DG, Dretler SP, Kahn RI, Lingeman JE, Macaluso Jr JN, McCullough DL. Nephrolithiasis clinical guidelines panel summary report on the management of staghorn calculi. *The Journal of urology*.1994 Jun 1; 151(6):1648-51.
- [19] Taylor EN, Curhan GC. Diet and fluid prescription in stone disease. *Kidney international*. 2006 Sep 1; 70(5):835-9.
- [20] Meschi T, Nouvenne A, Ticinesi A, Prati B, Guerra A, Allegri F, Pigna F, Soldati L, Vezzoli G, Gambaro G, Lauretani F. Dietary habits in women with recurrent idiopathic calcium nephrolithiasis. *Journal of translational medicine*.2012 Dec; 10: 1-8.
- [21] VAR RA. EVALUATION OF ANTHELMINTIC ACT.
- [22] Tatiya AU, Kulkarni AS, Surana SJ, Bari ND. Antioxidant and hypolipidemic effect of *Caralluma adscendens* Roxb. in alloxanized diabetic rats.
- [23] Kuriyan R, Raj T, Srinivas SK, Vaz M, Rajendran R, Kurpad AV. Effect of *Caralluma fimbriata* extract on appetite, food intake and anthropometry in adult Indian men and women. *Appetite*.2007 May 1; 48(3):338-44.
- [24] Albers F, Meve U. *Asclepiadaceae Illustrated Handbook of Succulent Plants* Springer-Verlag Page 164. Includes a picture.2002.
- [25] Wesson JA, Johnson RJ, Mazzali M, Beshensky AM, Stietz S, Giachelli C, Liaw L, Alpers CE, Couser WG, Kleinman JG, Hughes J. Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. *Journal of the American Society of Nephrology*.2003 Jan 1; 14(1):139-47.

- [26] Sasikala V, Radha SR, Vijayakumari B. In vitro evaluation of *Rotula aquatica* Lour. for antiurolithiatic activity. *Journal of Pharmacy Research*. 2013 Mar 1; 6(3):378-82.
- [27] Saha S, Verma RJ. Inhibition of calcium oxalate crystallisation in vitro by an extract of *Bergenia ciliata*. *Arab journal of urology*. 2013 Jun 1; 11(2):187-92.
- [28] Phatak RS, Hendre AS. In-vitro antiurolithiatic activity of *Kalanchoe pinnata* extract. *International Journal of Pharmacognosy and Phytochemical Research*. 2015 May 25; 7(2):275-9.