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Research Article

Anti-Urolithiatic Potential of Catharanthus Roseus Aqueous and Ethanolic Leaf Extractions: An Invitro Study

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ABSTRACT

Urolithiasis is the formation of calcium oxalate crystals within the urinary tract and kidney. The use of plant-based (natural) therapies for the treatment of kidney stones that exhibit few or less severe side effects than standard therapies. Background: The aim of this study was to evaluate the anti-urolithiasis potential of the aqueous and ethanolic extract of Catharanthus roseus leaves compared to the standard drug, using an in-vitro calcium oxalate crystallisation method. Methods: Leaves of the Catharanthus roseus plant were shade dried, powdered, and then extracted using aqueous and ethanolic solvents according to standard extraction techniques documented in the published literature. Preliminary phytochemical screening of the extracts was conducted to determine the major bioactive components of the extracts. The ability of each extract to prevent the formation of calcium oxalate crystals was determined using the egg membrane pouch method, and then determined using a modified KMnO₄ titration method. Results: The aqueous extract of Catharanthus roseus inhibited the formation of calcium oxalate crystals by 64.5% while the ethanolic extract produced 61% inhibition, and the standard drug Neeri produced 70% inhibition of calcium oxalate crystal formation. These findings suggest that both aqueous and ethanolic extracts of Catharanthus roseus leaves exhibit significant anti-urolithiasis activity. Conclusions: The results of this study show that Catharanthus roseus leaf extract shows an effective anti-urolithiatic agent by reducing the formation of calcium oxalate crystals. There is a need for additional in-vivo and clinical research in order to determine its specific effectiveness in humans.

INTRODUCTION

1.1. Global Burden, Epidemiology and Pathophysiological Basis:

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Urolithiasis, commonly known as kidney stone disease, is a widespread and recurrent urological disorder affecting millions worldwide. The lifetime prevalence ranges from 1-20% globally, with India reporting approximately 7-8% [1]. Certain “stone belt” regions show even higher incidence due to hot climate, dehydration, and dietary habits rich in oxalate-containing foods. A major concern is recurrence, about 50% of patients develop another stone within 10 years [2].

The disease creates not only physical suffering but also economic burden due to hospital admissions, surgical procedures, and loss of productivity. Long-term complications include hypertension and chronic kidney disease. Risk factors include low water intake, high sodium consumption, excessive oxalate-rich foods, obesity, diabetes, and metabolic syndrome [3].

Stone formation is a complex physicochemical process. It begins with super-saturation of urine with calcium and oxalate ions, followed by nucleation, crystal growth, aggregation, and finally retention within renal tubules. Calcium oxalate stones account for nearly 75-80% of cases. Crystal adhesion to damaged renal epithelial cells plays a crucial role in stone development. An imbalance between promoters (calcium, oxalate, uric acid) and inhibitors (citrate, magnesium, glycoproteins) determines stone formation [3, 4].

1.2. Clinical Presentation and Current Treatment Limitations:

Clinically, urolithiasis presents with severe flank pain radiating to the groin (renal colic), hematuria, nausea, and vomiting. Diagnosis is mainly confirmed by non-contrast CT scan, ultrasonography, and urine biochemical analysis [5].

Current treatments include medical expulsive therapy using alpha-blockers, extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, and percutaneous nephrolithotomy (PCNL). Though effective in removing stones, these methods do not correct underlying metabolic abnormalities, leading to high recurrence rates [6].

Preventive pharmacotherapy, such as potassium citrate, thiazide diuretics, and allopurinol, is used, but long-term compliance is often poor due to cost and side effects. Hence, there is growing interest in herbal medicines that are cost-effective, safer, and suitable for long-term use [7, 8].

1.3. Phytotherapy and Rationale for *Catharanthus roseus*:

Medicinal plants have been traditionally used for kidney stone management in Ayurveda and other systems of medicine. Many herbs exhibit diuretic, antioxidant, anti-inflammatory, and crystal-inhibiting properties [9].

Catharanthus roseus (family Apocynaceae), commonly known as Madagascar periwinkle, is widely cultivated in India. It is rich in bioactive compounds such as indole alkaloids (vindoline, vincristine, vinblastine), flavonoids (quercetin, kaempferol), triterpenoids (ursolic acid, oleanolic acid), phenolic acids, and saponins [10].

These phytochemicals exhibit multiple pharmacological actions relevant to urolithiasis:

- Antioxidant activity protects renal epithelial cells from oxalate-induced damage.
- Anti-inflammatory effects reduce cytokine-mediated crystal adhesion.
- Diuretic action increases urine output, reducing supersaturation.

- Crystal growth and aggregation inhibition through calcium chelation and surface adsorption [10, 11].

In vitro studies have demonstrated significant inhibition of calcium oxalate nucleation and aggregation by *C. roseus* leaf extract, suggesting strong anti-urolithiatic potential. Additionally, its reported antidiabetic and antihypertensive properties may help manage metabolic conditions associated with stone formation [12, 13].

1.4. Research Rationale and Scientific Significance:

Despite promising preliminary evidence, systematic evaluation of *C. roseus* leaf extract in controlled experimental models remains limited. There is a need for standardised phytochemical profiling and mechanistic investigation under laboratory conditions [14].

The present study aims to evaluate the anti-urolithiatic activity of aqueous leaf extract of *Catharanthus roseus* using in-vitro calcium oxalate crystallisation models. The study focuses on assessing inhibition of nucleation and aggregation processes, which are critical stages in stone formation [15].

Scientific validation of this plant may provide a cost-effective, natural alternative or adjunct to conventional therapy. Given the high recurrence rate and economic burden of urolithiasis in India, the development of plant-based preventive strategies holds significant public health importance [16].

Thus, *Catharanthus roseus* represents a promising candidate for anti-urolithiatic research due to its rich phytochemical composition, multiple pharmacological mechanisms, and traditional therapeutic relevance [17].

2. Plant Profile:



Fig 1: *Catharanthus roseus*

2.1.1. Scientific Classification:

Kingdom: Plantae

Family: Apocynaceae

Genus: *Catharanthus*

Species: *C. roseus* [18].

2.1.2. Common Names:

Madagascar periwinkle

Sadabahar

Vinca

Nithya Kalyani [19].

2.1.3. Geographical Distribution:

Native to Madagascar

Widely cultivated in India, Africa, Southeast Asia, and tropical regions [20].

2.2. Morphological Characteristics:

2.2.1. Habit:

- Evergreen perennial herb
- Grows up to 30-100 cm tall [21].

2.2.2 Root:

- Tap root system [22].

2.2.3. Stem:

- Green, smooth, branched, and contains milky latex [23].

2.2.4. Leaves:

- Simple, opposite
- Elliptical or oblong
- Dark green, glossy surface [24].

2.2.5. Flowers:

- Pink, purple, or white
- Five petals
- Axillary position [25].

2.2.6. Fruit:

- Pair of cylindrical follicles
- Contains numerous small black seeds [26].

2.3. Chemical Constituents:

- Vincristine
- Vinblastine
- Ajmalicine
- Reserpine

- These are important anticancer alkaloids [27].

2.4. Medicinal Uses:

- Cancer treatment – Source of important anticancer drugs like vincristine and vinblastine.
- Diabetes management – Leaf extract helps in lowering blood sugar levels.
- Kidney stones – May help in reducing the formation of calcium oxalate crystals.
- High blood pressure – Shows a mild antihypertensive effect.
- Wound healing – Used traditionally for minor cuts and skin problems.
- Antimicrobial action – Active against some bacteria and fungi.
- Anti-inflammatory – Helps in reducing swelling and pain.
- Antioxidant property – Protects cells from damage by free radicals [28, 29, 30].

3. MATERIALS AND METHODS:

3.1. Chemicals used:

Calcium chloride, Sodium oxalate, Potassium permanganate [KMnO₄], Sulphuric acid [H₂SO₄], Tris buffer, Calcium chloride, Hydrochloric acid [HCl], Neeri [48, 49].

3.2. Collecting and Identifying the Plant Material:

Fresh leaves of the plant *Catharanthus roseus* were collected from the local area of Nellore, Andhra Pradesh. They were authenticated as well as thoroughly cleaned using distilled water to

remove dust and impurities. The leaves were then shade-dried at ambient temperature for several days before being ground to a coarse powder suitable for extraction using a mechanical grinder.

3.3. Preparation of Plant Extract:

The leaves were shade-dried and powdered. The crude plant extract was prepared by the Soxhlet extraction method. 50g of powdered plant material was extracted with 500 ml of ethanol and water individually. The process of extraction was carried out up to 6 cycles, till the solvent in the siphon tube of the extractor became colourless. The two extracts were filtered separately. Further, the dried extracts were maintained in a refrigerator at 4 °C for further antiurolithiatic activity [49].



Fig 2: Preparation of plant extract using Soxhlet Apparatus.

3.4. Conducting Preliminary Phytochemical Screening:

The Aqueous and ethanolic Extract were screened qualitatively for the presence of major bioactive components, such as alkaloids; flavonoids; tannins; saponins; glycosides; and phenolic compounds through standard chemical tests.

3.5. Preparation of calcium oxalate crystals:

To prepare calcium oxalate, a 0.1 M calcium chloride solution is prepared by dissolving 1.47 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 100 mL of distilled water. Similarly, 0.1 M sodium oxalate solution is prepared by dissolving 1.26 g of $\text{Na}_2\text{C}_2\text{O}_4$ in 100 mL of distilled water. The pH of both solutions is maintained between 5.5 and 6.5 [50].

The calcium chloride solution is placed on a magnetic stirrer, and the sodium oxalate solution is added slowly dropwise while stirring. This reaction leads to the formation of calcium oxalate precipitate. The mixture is stirred for 15-20 minutes and then allowed to stand for 30 minutes for proper crystal formation [51].

The precipitate is filtered, washed with distilled water, and dried at 40-50 °C. The dried crystals are then stored in a desiccator for further experimental use [52].



Fig 3: Preparation of calcium oxalate precipitate.

3.6. Preparation of a Semi-Permeable Membrane from Eggs:

Eggshell decalcification is performed to obtain the inner semi-permeable membrane, which can be

used in in-vitro anti-urolithiatic studies. Eggshells are mainly composed of calcium carbonate (CaCO_3). When exposed to white vinegar, which contains about 4-5% acetic acid, the calcium carbonate reacts with the acid to form soluble calcium acetate, carbon dioxide, and water. This reaction gradually dissolves the hard shell and leaves behind a thin membrane [53].



Fig 4: Decalcification of egg shells.

For the procedure, fresh chicken eggs are first washed with distilled water to remove impurities. The clean eggs are then placed in a glass container and completely submerged in white vinegar. They are left undisturbed for about 24-48 hours at room temperature. During this period, bubbles appear on the shell surface due to carbon dioxide release. After the shell dissolves, the soft decalcified egg is removed and washed with distilled water. A small opening is made to remove the egg contents, and the remaining membrane is rinsed and used for further experimental use [54].



Fig 5: Decalcified eggs.

3.7. Preparation of 0.1M Tris Buffer:

Tris buffer is used to maintain a stable pH in biochemical experiments. To prepare a 0.1 M Tris buffer, 12.1 g of Tris (Tris (hydroxymethyl) aminomethane) is dissolved in about 800 mL of distilled water with continuous stirring [55]. The pH of the solution is checked and adjusted to pH 7.0-7.4 by adding dilute hydrochloric acid dropwise. Finally, the volume is made up to 1000 mL with distilled water, mixed well, and stored in a clean container for laboratory use [56].

3.8. Preparation of Egg Membrane Pouches Containing Calcium Oxalate and Extracts:

In this experiment, an egg membrane pouch model was used to evaluate the anti-urolithiatic activity of plant extracts. Previously prepared decalcified eggshell membranes were used as semi-permeable pouches. A total of four egg membranes were selected and carefully cleaned with distilled water to remove any remaining impurities [57].

Each egg membrane was filled with different contents to study their effect on calcium oxalate crystals. The first egg pouch contained only 1 g of calcium oxalate crystals and served as the control. The second pouch contained a mixture of 1 g of calcium oxalate and 1ml of ethanolic extract of the plant sample [58]. The third pouch was filled with 1ml of calcium oxalate along with the 1ml of aqueous extract of the plant material. The fourth pouch contained 1 g of calcium oxalate combined with the standard anti-urolithiatic drug Neeri, which was used for comparison with the plant extracts [59].

After filling, the open ends of the egg membranes were carefully tied to form sealed pouches. These prepared egg pouches were then placed in separate containers, each containing 100ml of Tris buffer solution [60]. The samples were incubated under controlled conditions for a specific period. This setup allowed interaction between the extracts and

calcium oxalate crystals through the semipermeable membrane, which helps in evaluating the potential anti-urolithiatic activity [61].

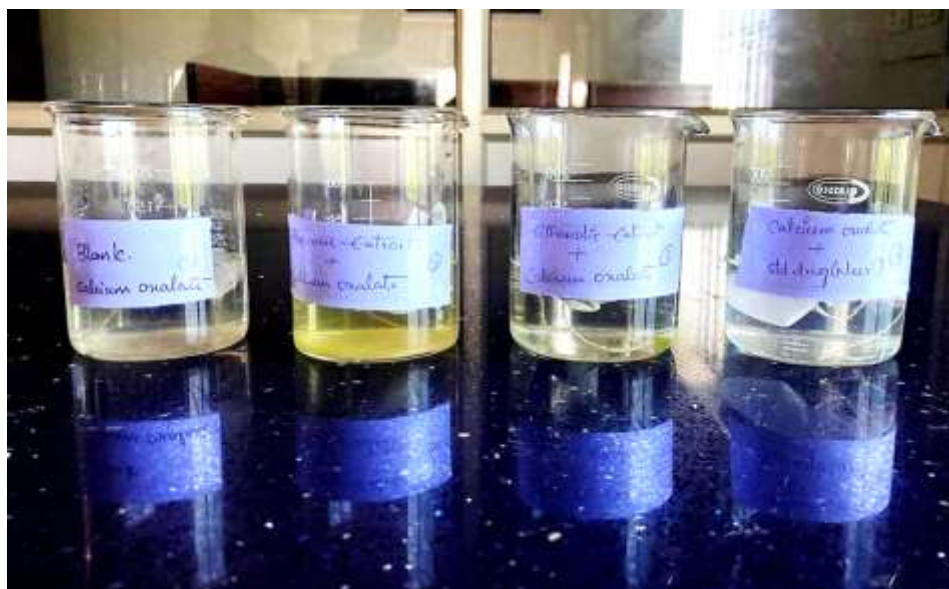


Fig 6: Immersion of egg pouches in Tris buffer solution

3.9. Preparation of 0.9494 N KMnO_4 Solution:

To prepare a 0.9494 N KMnO_4 solution, accurately weigh 15 g of potassium permanganate and dissolve it in about 800 mL of distilled water with continuous stirring [62]. The solution is then transferred to a 1000 mL volumetric flask, and the final volume is made up to 1 litre with distilled water. The solution is mixed well and stored in a dark-colored bottle to protect it from light and maintain stability [63].

3.10 Study of In Vitro Anti Urolithiatic Activity Through Titrimetric Method:

After incubation of the egg membrane pouches in Tris buffer, the remaining calcium oxalate content was estimated using the potassium permanganate titrimetric method (permanganometry). This method is commonly used to determine the amount of oxalate present in a sample based on oxidation-reduction reaction [64].

After the incubation period, the contents present inside each egg pouch were carefully transferred

into separate conical flasks. Each pouch sample represented different experimental conditions, such as control, plant extracts, and standard drug treatment. To ensure proper acidic conditions for the reaction, 2 mL of 1 N sulphuric acid (H_2SO_4) was added to each conical flask containing the sample [65].

The titration was then carried out using 0.9494 N potassium permanganate (KMnO_4) solution taken in the burette. During the titration process, the potassium permanganate acts as a strong oxidising agent and reacts with oxalate ions present in the sample. As the KMnO_4 solution was added gradually from the burette, it oxidised the oxalate ions present in the conical flask under acidic conditions [66].

The solution was continuously swirled during titration to ensure uniform mixing and complete reaction. The endpoint of the titration was indicated by the appearance of a light pink colour that persisted for about 30 seconds. This colour change confirmed that the reaction was complete

and excess potassium permanganate was present [67, 68].

Each egg pouch sample, including the control pouch containing only calcium oxalate, the pouch with ethanolic extract, the pouch with aqueous extract, and the pouch with the standard drug Neeri, was titrated separately under the same experimental conditions. The volume of potassium permanganate solution consumed during titration was recorded for each sample [69].

The obtained titration values were further used to calculate the amount of calcium oxalate present and to evaluate the anti-urolithiatic activity of the plant extracts by comparing them with the control and standard drug [70].

4. RESULTS

4.1. Phytochemical Study results For Leaves of Catharanthus Roseus

Table 1: Phytochemical Study results For Leaves of Catharanthus Roseus

Sr. No	Phytochemical Test	Reagents	Procedure	Observation	Result
1	Glycosides (Sodium Nitroprusside Test)	Vinca leaf extract, Sodium nitroprusside solution, Pyridine, Sodium hydroxide solution	About 2 ml of Vinca leaf extract was taken in a test tube. A few drops of sodium nitroprusside solution were added followed by pyridine. The mixture was shaken and made alkaline by adding sodium hydroxide dropwise and allowed to stand for a few minutes.	Reddish colour developed.	Indicates presence of glycosides.  Fig 7: Test for Glycosides.
2	Phenolic Compounds (Ferric Chloride Test)	Vinca leaf extract, 5% Ferric chloride solution, Distilled water	About 2 ml of extract was taken in a test tube and a few drops of freshly prepared 5% ferric chloride solution were added and mixed well.	Deep blue, green, or bluish-black colour appeared.	Confirms presence of phenolic compounds.  Fig 8: Test for Phenolic compounds.
3	Saponins (Foam Test)	Vinca leaf extract, Distilled water	About 2 ml of extract was diluted with 5 ml distilled water and shaken	Formation of stable persistent foam.	Indicates presence of saponins.

			vigorously for 10–15 seconds, then allowed to stand for 10 minutes.		 Fig 9: Test for Saponins. Confirms presence of saponins.
4	Tannins (Ferric Chloride Test)	Vinca leaf extract, 5% Ferric chloride solution, Distilled water	2 ml of extract was taken in a test tube and a few drops of ferric chloride solution were added and mixed gently.	Bluish-black or greenish-black colour formed.	 Fig 10: Test for Tannins. Indicates presence of alkaloids.
5	Alkaloids (Tannic Acid Test)	Vinca leaf extract, Tannic acid solution	2 ml of extract was taken and a few drops of tannic acid solution were added. The mixture was shaken and allowed to stand for a few minutes.	Buff or pale-yellow precipitate formed.	 Fig 11: Test for Alkaloids. Indicates presence of terpenoids.
6	Terpenoids (Salkowski Test)	Vinca leaf extract, Chloroform, Concentrated sulfuric acid	2 ml of extract was mixed with 2 ml chloroform. Concentrated sulfuric acid was carefully added along the side of the test tube to form a layer.	Reddish-brown colour at the interface of layers.	

					 Fig 12: Test for Terpenoids.
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4.2. Titrimetric Method Results:

After the incubation period, the remaining oxalate in the mixture was determined by potassium permanganate (KMnO_4) titration, which allowed for an assessment of the ability of the extract to inhibit the crystallization of calcium oxalate. The results of this study were then compared against similar control samples to assess the anti-urolithic effects of the plant extract.

Table 2: Effect of Sample Volumes on Calcium Oxalate Inhibition.

Sr. No	Sample	Volume of KMnO_4 used (ml)	% Inhibition of calcium oxalate
1.	Blank (Calcium Oxalate)	2.00	0%
2.	Ethanollic Extract + Calcium Oxalate	0.78	61%
3.	Aqueous Extract + Calcium Oxalate	0.71	64.5%
4.	Standard Drug (Neeri) + Calcium Oxalate	0.60	70%

5. DISCUSSION:

The anti-urolithiatic activity of *Catharanthus roseus* leaf extracts was evaluated using the egg membrane pouch model, followed by estimation of calcium oxalate through the permanganometric titration method. In this study, both aqueous and ethanolic extracts were tested for their ability to dissolve calcium oxalate crystals, and the results were compared with the standard drug Neeri [71].

The blank sample containing only calcium oxalate showed the highest titration value of 2 mL, indicating the presence of maximum undissolved calcium oxalate crystals. When plant extracts and the standard drug were added, the titration values were significantly reduced, which suggests dissolution or inhibition of calcium oxalate crystals [72].

The ethanolic extract showed a burette reading of 0.78 mL, which corresponds to 61% inhibition of calcium oxalate. The aqueous extract showed a titration value of 0.71 mL, corresponding to 64.5% inhibition. The standard drug Neeri showed the lowest titration value of 0.6 mL, indicating 70% inhibition of calcium oxalate crystals [73].

From these results, it is evident that both extracts of *Catharanthus roseus* possess considerable anti-urolithiatic activity. The aqueous extract showed slightly better activity than the ethanolic extract,

which may be due to the presence of water-soluble phytoconstituents such as flavonoids, alkaloids, and phenolic compounds that help in dissolving or inhibiting calcium oxalate crystal formation [74].

However, the standard drug Neeri demonstrated the highest activity among the tested samples. Even though the plant extracts showed slightly lower activity compared to the standard drug, their significant inhibitory effect indicates that *Catharanthus roseus* leaves contain bioactive compounds with potential lithotriptic properties [75].

Overall, this in-vitro study suggests that both aqueous and ethanolic extracts of *Catharanthus roseus* leaves possess promising anti-urolithiatic activity. These findings provide preliminary evidence supporting the traditional use of plant-based medicines in the management of kidney stones. Further in-vivo and clinical studies are required to confirm their therapeutic effectiveness and to identify the active phytochemical constituents responsible for this activity [76].

6. CONCLUSION:

The present study demonstrated that *Vinca* leaf extracts possess noticeable anti-urolithiatic activity in an in-vitro model of calcium oxalate crystal formation. Both aqueous and ethanolic extracts showed the ability to reduce oxalate levels when compared with the blank sample, indicating their inhibitory effect on crystal formation. Among the tested samples, the aqueous extract showed relatively higher inhibition, suggesting the presence of water-soluble bioactive constituents responsible for the activity. These findings support the traditional medicinal importance of *Vinca* leaves and indicate their potential role in the prevention or management of kidney stone formation. Further pharmacological and clinical studies are required to confirm these effects.

7. AUTHOR CONTRIBUTIONS

Kongi Kavyasudha was responsible for guiding the project and being a primary investigator for the project by being involved in developing the research, designing the study, overseeing the experiments, analyzing the data, and providing a thorough revision to the manuscript. Shaik Afrin, Venumbaka Sravya Sree, Gothala Sushma Chandrika, Cheerla Susmitha, and Vakani Jahnavi were undergraduate pharmacy degree students conducting all experimental aspects of the research as part of their course project and under the direction of the project supervisor. They assisted by processing the plant materials, performing laboratory experiments, collecting data, performing a preliminary analysis of the data, and writing the initial manuscript draft. Dr. Yadala Prapurna Chandra assisted with institutional support for this research by providing laboratory resources, equipment, and administrative approval to conduct the research at the Rathnam Institute of Pharmacy. All authors provided input on the analysis of the data, reviewed the manuscript, and approved the final manuscript for publication.

8. Funding:

“This research received no external funding”.

9. Institutional Review Board Statement

The present study involved in-vitro experimental work using plant extracts and did not involve human participants or animal subjects. Therefore, ethical approval from an Institutional Review Board (IRB) was not required for this research.

10. Informed Consent Statement

Not applicable. The study did not involve human participants or personal data requiring informed consent.



11. Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

12. Acknowledgments

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13. Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this research work.

14. Abbreviations

- KMnO₄ – Potassium Permanganate
- CaOx – Calcium Oxalate
- IRB – Institutional Review Board
- SD – Standard Deviation
- UV – Ultraviolet

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