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Research Article

IN VITRO COMPARATIVE STUDY OF ANTI- UROLITHIATIC ACTIVITY OF T*RIGONELLA- FOENUM-GRAECUM, CORIANDRUM SATIVUM, TRIBULUS TERRESTRIS & STERCULIA FOETIDA*

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ABSTRACT

Background: Urolithiasis, or kidney stone formation, is a common disorder with a high recurrence rate. Conventional treatments may lead to side effects, necessitating exploration of alternative herbal therapies. Objective: To evaluate and compare the in vitro anti-urolithiatic activity of aqueous extracts of *Trigonella foenum-graecum, Coriandrum sativum, Tribulus terrestris, and Sterculia foetida* using nucleation and aggregation assays. Methods: Leaves were collected, authenticated, and extracted using the maceration method. Phytochemical analysis was conducted to determine total phenolic, alkaloid, and flavonoid content. Anti-urolithiatic activity was evaluated using calcium oxalate crystallization-based nucleation and aggregation assays. Results: All plant extracts exhibited dose-dependent inhibition of calcium oxalate nucleation and aggregation. Sterculia foetida showed the highest phenolic and alkaloid content and demonstrated the strongest inhibitory activity. Conclusion: The study indicates significant anti-urolithiatic potential of these plants, especially Sterculia foetida, suggesting their possible use in herbal formulations for kidney stone prevention.

Keywords: Urolithiasis, Calcium oxalate, Herbal medicine, Nucleation assay, Aggregation assay.

INTRODUCTION

The urinary system is the primary excretory system of the human body and plays a critical role in the elimination of metabolic waste products. It consists of two kidneys, two ureters, a urinary bladder, and a urethra. Of these organs, the kidneys serve as the principal site of filtration and excretion. Anatomically, the right kidney is situated slightly lower than the left to accommodate the space occupied by the liver [1].

The kidneys are a pair of bean-shaped, reddish-brown excretory organs located retroperitoneally on either side of the vertebral column. They extend vertically from the upper border of the twelfth thoracic vertebra to the mid-body of the third lumbar vertebra. Functionally, kidneys regulate the body's fluid balance, electrolyte concentration, and acid-base equilibrium, while also removing metabolic waste such as urea from the bloodstream.

Each kidney is approximately 11 cm in length, 6 cm in width, and 3 cm in thickness, with an average weight of 150 grams in males and 135 grams in females. Internally, the kidney is divided into three regions: the cortex, medulla, and renal pelvis. The cortex contains renal corpuscles and convoluted tubules, while the medulla is composed of renal pyramids that terminate in renal papillae, which drain into minor calyces. These calyces eventually converge to form the renal pelvis, a funnel-like structure that leads into the ureters.

The kidneys receive blood from the renal arteries, which branch off from the abdominal aorta. After filtration, the deoxygenated blood returns via the renal veins to the inferior vena cava. The vascular network within the kidney is highly specialized to facilitate the processes of filtration, reabsorption, and secretion.

One of the major clinical conditions affecting the kidneys is urolithiasis, a painful and recurrent disorder characterized by the formation of stones within the renal system—including the renal pelvis, ureter, bladder, and urethra. Derived from the Greek words ouron

(urine) and lithos (stone), urolithiasis has multifactorial etiologies including reduced urine output, urinary tract infections, high dietary intake of oxalates and calcium, metabolic disorders such as hyperparathyroidism, and genetic predisposition.

Approximately 60% of the population is affected by kidney stones at some point in life, with calcium oxalate stones being the most common type, accounting for nearly 80% of cases. The pathogenesis of stone formation involves a complex interplay between urine supersaturation, nucleation, crystal growth, aggregation, and crystal–cell interactions within renal tubular structures. Despite advancements in allopathic treatments, recurrence rates remain high, which has led to growing interest in exploring herbal and alternative therapeutic strategies. [2-7]

2.0 MATERIALS AND METHODS

2.1 Collection and authentication of Plant materials

Fresh leaves of *Trigonella foenum-graecum., Coriandrum sativum L., Tribulus terrestris., Sterculia foetida L* were collected locally form Tamil Nadu, India and authentication by DR.

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2.2 Extraction of *Trigonella foenum-graecum., Coriandrum sativum L., Tribulus terrestris & Sterculia foetida L*

The plant extract will be prepared by blending and macerating 500g of the fresh leaves (*Trigonella foenum-graecum., Coriandrum sativumL.,Tribulus terrestris., Sterculia foetida L)* with 100ml of distilled water and was kept at 40º C for 24 hours for extraction to take place. The resulting mixture will be filtered. The concentration of the extract recovered from the filtrations using the expression

Concentration = [X-Y] /Z g/ml

Where X = Weight of fresh leaves before blending

Y = Weight of leaves after filtration

Z = volume of water after filtration

Finally, the color consistency of aqueous extract Will noted and the fresh preparation was used for experimental run [8,9]

2.3 QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS

2.3.1 Determination of total Phenols

The total phenolic content in the samples were estimated by Folin Ciocalteau reagent method. The calibration curve was plotted by mixing 1 ml aliquots of 5, 10, 20, 40 and 80 mg/ml Gallic acid solutions with 0.5ml of Folin Ciocalteu reagent and 0.4 ml of sodium carbonate solution. After 30 min the absorbance was measured at 765 nm. 1 ml of extract was mixed separately with the same reagents, as performed for a standard curve. After 1 hr, the absorbance was measured to determine the total phenolic contents in all the extracts separately using the following formula.

$$\mathbf{C} = \frac{C1XV}{m} \quad \ldots (2)$$

C = Total amount of phenolic content in mg/g, in GAE (Gallic acid equivalent),

C1 = concentration of Gallic acid established from the calibration curve in mg/ml,

V = volume of extract in ml, and

m = the weight of the plant extract [10]

2.3.2 Determination of alkaloids

1g of the sample was separately weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 hrs. The whole content was filtered and reduced to one-quarter of the original volume. Concentrated ammonium hydroxide (NH4OH) was added drop-wise to the extract until the precipitation was complete. The whole solution

was allowed to settle, and the precipitate was collected by filtering, then it was washed with dilute ammonium hydroxide. The total residue is the alkaloid, which was weighed after drying and estimated using the Gravimetric method.

2.3.3. Determination of Flavonoids

1g of the sample is extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered, and the filtrate was later transferred into a pre-weighed crucible and evaporated into dryness over a water bath. Final residue was considered as total flavanoids content and calculated using formula 3[12]

2.4 NUCLEATION ASSAY

It is a classical model for the study of oxalate crystallization because of its simplicity and satisfactory reproducibility. This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of any chemical species used [13].

Procedure

Solution of calcium chloride and sodium oxalate were prepared at the final concentrations of 5mmol/L and 7.5mmol/L respectively in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950µL of calcium chloride solution mixed with 100µL of herb extracts at different concentrations (100µg/ml–1000µg/ml). Crystallization was started by adding 950 µL of sodium oxalate solution. The temperature was maintained at 37 °C. The OD of the solution was monitored at 620 nm.The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control [14].

The growth of crystals was expected due to the following reaction:

$$CaCl_2 + Na_2C_2O_4 \rightarrow CaC_2O_4 + 2NaCl$$

Percentage inhibited is calculated by using the formula:

Percentage Inhibition = (1-OD of Sample/OD of Control) × 100

AGGREGATION ASSAY

Principle

In this process crystals in solution stick together and form a larger particle. Aggregation of particles in solution is determined by a balance of forces, some with aggregating effects and some with disaggregating effects. A small inter particles distance increased attractive force and favours particle aggregation. In addition, Tamm-Hors fall glycoprotein and other molecule may act as glue and increase viscous binding. Furthermore, aggregate may be stabilized by solid bridges formed by crystalline material connecting two particles. The main force that inhibits aggregation is the repulsive electrostatic surface charge, known a Zeta very goodial.

The principle of an aggregation assay for anti-urolithiasis involves studying the ability of a substance to inhibit the formation or growth of urinary stones (urolithiasis) by assessing its impact on the aggregation of crystals (*Sarmistha, et al., 2013*)

Procedure for Aggregation Assay

(Sarmistha, et al., 2013)

Step 1: The COM crystals were prepared by mixing both the solutions of calcium chloride and sodium oxalate at 50mmol/L.

Step 2: Both solutions were then equilibrated in a bath for 1 h at 60 C.]

Step 3: The solutions were then cooled to 37 C and then evaporated in a china dish.

Step 4: The COM crystals were then dissolved with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5 to a final concentration of 1mg/mL

Step 5: The absorbance at 620 nm was recorded

Step 6: The rate of aggregation was estimated by comparing the slope of turbidity in the presence of the extract with that obtained in the control.

Step 7: Percentage inhibition of nucleation was calculated using the following formula [15-18].

In a comparative analysis of phenolic content among various plant extracts, the following results were obtained:

- Trigonella foenum-graecum (fenugreek): 4.825±0.121
- Coriandrum sativum (coriander): 7.114±0.008
- Tribulus terrestris: 2.869±0.031
- Sterculia foetida: 5.112±0.012

These findings indicate that Coriandrum sativum possesses the highest phenolic content among the studied plants, suggesting its potential as a rich source of natural antioxidants.

In a comparative analysis of alkaloid content among various plant extracts, Trigonella foenum-graecum (fenugreek) exhibited an alkaloid concentration of 0.389±0.110, Coriandrum sativum (coriander) had 0.268±0.004, and Tribulus terrestris showed 0.389±0.110. Notably, Sterculia foetida contained a higher alkaloid content of 0.489±0.004.

3.0 RESULT AND DISCUSSION

3.1 Determination of total phenols

Table 2. Total phenols content

S. No	Concentration of the Gallic acid			Absorbance
1.	5			0.104
2.	10			0.257
3.	20			0.516
4.	40			1.019
5.	80			1.896
S.NO	**Total Phenolics in 1gm sample (µg)**			
	A	**B**	**C**	**D**
1	4.825±0.121	7.114±0.008	2.869±0.031	5.112±0.012

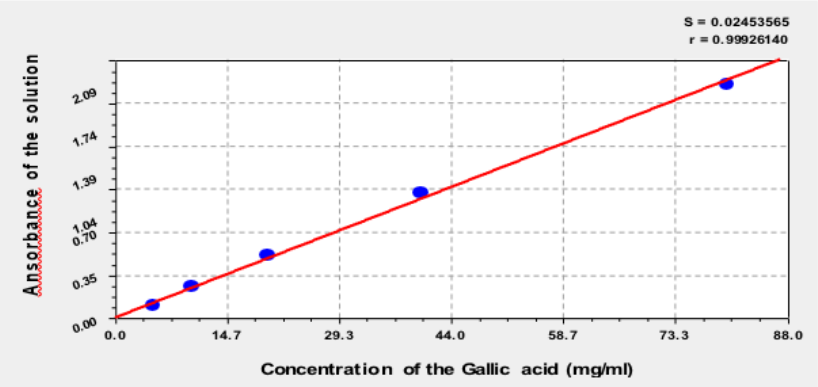

Figure 11. Total phenols Content

3.2 Determination of alkaloids

Table 3. Total alkaloids content

S.NO	Total alkaloids in 1gm sample (µg)			
	A	B	C	D
1	0.389±0.110	0.268±0.004	0.401±0.020	0.489±0.004

3.3 Determination of flavonoids

Table 4. Total flavonoids content

S. No.	Total Flavonoids in 1gm sample(µg)			
	A	B	C	D
1	1.062±0.010	0.919±0.080	0.071±0.071	1.043±0.010

In a comparative analysis of total flavonoids content among various plant extracts, the following results were obtained:

- Trigonella foenum-graecum (fenugreek): 1.062±0.010
- Coriandrum sativum (coriander): 0.919±0.080
- Tribulus terrestris: 0.071±0.071
- Sterculia foetida: 1.043±0.010

These findings indicate that Trigonella foenum-graecum and Sterculia foetida possess the highest total flavonoids content among the studied plants, suggesting their potential as rich sources of natural antioxidants.

3.4 NUCLEATION ASSAY

Table 5. Nucleation Assay for all sample

S.No	Sample	Absorbance (MEAN± SEM)	% Percentage of Inhibition of Nucleation of CaOXCrystals
1	Control	0.4514±0.04843	-
2	Standard-1 (800 µg/ml)	0.3048±0.006807	32.59%
3	Test- 1 (800µg/ml	0.3260±0.01675	27.71%
4	Test- 2 (800 µg/ml)	0.2654±0.01973	41.11%
5	Test- 3 (800 µg/ml)	0.2476±0.01169	57.79%
6	Test- 4 (800 µg/ml)	0.2466±0.009896	55.89%

In a comparative study, evaluating the inhibition of calcium oxalate (CaOx) crystal nucleation,

Tribulus terrestris extract a significant inhibitory effect of 55.89%. This efficacy was comparable to Sterculia foetida (57.89%) and superior to the standard drug Cystone (32.59%), as well as other plant extracts such as Trigonella foenum-graecum (27.71%) and Coriandrum sativum (41.11%). These findings suggest that Tribulus terrestris may serve as a potent natural agent in preventing CaOx crystal formation.

3.5 AGGREGATION ASSAY

Table 6. Aggregation Assay for all sample

S.No.	Sample	Absorbance (MEAN± SEM)	% Percentage of Inhibition of Aggregation of CaOXCrystals
1	Control	0.3288±0.01218	-
2	Standard-1 (800 µg/ml) (Cystone)	0.1032±0.01746	68.59%
3	Test- 1 (800µg/ml	0.1224±0.007782	62.80%
4	Test- 2 (800 µg/ml)	0.09060±0.007096	72.56%
5	Test- 3 (800 µg/ml)	0.08880±0.01282	73.17%
6	Test- 4 (800 µg/ml)	0.08860±0.01371	72.6%

In a comparative aggregation assay evaluating the inhibition of calcium oxalate (CaOx) crystal formation, the following inhibition percentages were obtained.

- Standard drug: 68%
- Trigonella foenum-graecum (fenugreek): 62.8%
- Coriandrum sativum (coriander): 62%
- Tribulus terrestris: 72%
- Sterculia foetida: 72%

These results indicate that Tribulus terrestris and Sterculia foetida exhibit the highest inhibitory effects on CaOx crystal aggregation, surpassing both the standard drug and the other plant extracts tested.

4.0 SUMMARY AND CONCLUSION

4.1 SUMMARY

Plants play an essential role in primary health care and treatment of diseases and disorders in traditional medicine. Phytochemicals present in plant are classified as primary and secondary metabolites. Primary metabolites are necessary for plant life and include carbohydrates, amino acids, proteins, lipids, purines and pyrimidines of nucleic acids. On the contrary secondary metabolites are the remaining plant chemicals produced by the cells through metabolic pathways derived from the primary metabolic pathways. Secondary metabolites in plants are classified into three main groups based on their biosynthetic pathway; (a) nitrogen-containing compounds such as alkaloids, glucosinolates, and cyanogenic glycosides, (b) phenolic compounds such as phenylpropanoids and flavonoids and (c) terpenes Kidney disorders and urinary infections are common in people over the world and a large number of research works has been done to overcome these challenges.

Medicinal plants offer an attractive source for improving kidney function and treating the symptoms of renal disorders. Here in, we have systematically summarized the secondary metabolites of the medical plants introduced in traditional Persian medicine books. Based on

these data we conduct the study to estimate the total content of alkaloids, phenolic compound and flavanoids level in four plant such as *Trigonella foenum-graecum.,, Coriandrum sativum L., Tribulus terrestris., Sterculia foetida L.* All the plant exhibits presence of alkaloid, flavanoids and phenolic compound based on these data we conduct Anti- urolithiatic activity in *Sterculia foetida L* by using in-vitro method. From that experiment, the observation revealed that increasing the absorbance of control when compared to standard and test. When the absorbance of sample decreases, the percentage of inhibition increases.

The highest percentages of inhibition occur in standard at (800µg/ml- 32.59%, 68.59%) and test drug at **(**800µg/ml-55.89%, 73.17%) in nucleation and aggregation assay. Urolithiasis is a condition, in which stone are formed by following process such as super saturation, nucleation, aggregation, crystal adhesion and crystal growth. In this process super saturation, nucleation and aggregation is initial step for crystal growth. Inhibiting the nucleation and aggregation process helps to inhibit the further process. In this study.

we artificially synthesis crystal lattice and check whether the drug shows effect or not, study shows there is an significantly decrease in absorbance and increase in percentage of inhibition, o the plant shows anti-urolithiatic activity by inhibiting the nucleation process.

5. CONCLUSION

The present study compares the in vitro anti-urolithiasis potential of four herbal extracts, Trigonella foenum-graecum, Coriandrum sativum L., Tribulus terrestris, and Sterculia foetida L., using nucleation and aggregation assays. These plants contain phytoconstituents such as phenols, alkaloids, and flavonoids, which are responsible for anti-urolithiatic activity. The study provides primary evidence that these plants possess significant anti-urolithiatic properties. Among them, Tribulus terrestris and Sterculia foetida emerged as the most potent in inhibiting urolithiasis, exhibiting significant anti-urolithiatic activity in both aggregation and nucleation assays. The results show a significant decrease in absorbance and an increase in the percentage of inhibition, indicating that *Tribulus terrestris and* Sterculia foetida has better anti-urolithiatic potential compared to the other plants. However these findings provide a scientific basis for the traditional use of these plants in managing kidney stones although the further in vivo studies and clinical trials are necessary to confirm their therapeutic potential and to understand the mechanisms behind their anti-urolithiatic action.

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