



Research Article

Pharmacological Evaluation of Antiurolithiatic Phytochemicals of Methanolic Extracts of *Tetrapleura tetraptera* and *Syzygium cumini*: An In Vitro Study

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Abstract

Background: Urolithiasis is a widespread urinary problem, and its main feature is the production of hard crystalline deposits (renal stones) in the urinary tract. The most frequent type of stones formed by calcium oxalate is often connected with oxidative stress, supersaturation, crystal nucleation, and aggregation. Recurrence is common with conventional therapies, which results in the herbal alternatives gaining more attention. *Syzygium cumini* and *Tetrapleura tetraptera* have been used for their diuretic, anti-inflammatory, antioxidant, and lithotriptic properties. **Objectives:** The study aimed to perform a combination study for the prevention of kidney stones. This included the authentication of herbs, preparation of extraction, phytochemical testing, and evaluation via in-vitro methods to fill the gaps in current models. **Rationale:** Reoccurrence of urolithiasis with conventional treatments encourages a shift toward herbal therapies. While *S. cumini* and *Tetrapleura tetraptera* show potential, further in-vitro investigations are needed to confirm their efficacy against calcium oxalate stones (2-3). **Methodology:** Authenticated plant materials were extracted through methanolic maceration and screened phytochemically. In-vitro assays for antiurolithiasis included nucleation, aggregation, and growth inhibition, using spectrophotometric and gel diffusion techniques. The FTIR method identified the structure of crystals-extract interactions. **Results:** The two extracts, when compared with controls, effectively inhibited calcium oxalate crystal nucleation and aggregation. Both individual extracts and their combination demonstrated concentration-dependent inhibition of calcium oxalate nucleation and aggregation, with the combination showing substantial inhibitory activity at the tested concentrations. **Conclusion:** The mixture of *Syzygium cumini* and *Tetrapleura tetraptera* exhibited enhanced antiurolithiatic properties compared to individual extracts, supporting their usage as herbal remedies to prevent formation of calcium oxalate stone.

Keywords: Urolithiasis, *Syzygium cumini*, *Tetrapleura tetraptera*, Calcium oxalate, In-vitro study.

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Introduction

Urolithiasis is a common urinary tract disorder characterized by the formation of crystalline deposits within the urinary system. Stone formation involves several physicochemical processes, including urinary supersaturation, nucleation, crystal growth, aggregation, and retention within the urinary tract. Calcium oxalate is one of the most common constituents of urinary calculi. Several factors, including inadequate fluid intake, dietary habits, genetic predisposition, metabolic abnormalities, and urinary tract infections, may contribute to stone formation and recurrence (5-6). The most common stones are calcium oxalate, which are

diagnosed most frequently and comprise almost all cases globally. The main risk factors for urolithiasis are acute or chronic dehydration, improper dietary habits, genetic predisposition, metabolic disorders, and urinary infections. Chronic kidney disease (CKD), endothelial dysfunction, cardiovascular disorders, recurrent urinary infections, and metabolic comorbidities are some of the complications associated with urolithiasis. The growing phenomenon of stone recurrence despite surgical and medical treatments has stirred up the interest in the use of traditional herbal medicines. *Syzygium cumini* (L.) Skeels and *Tetrapleura tetraptera* (Schumacher & Thonn.) Taub. have been investigated for their various pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and diuretic activities. Phytochemical constituents such as flavonoids, tannins, saponins, and phenolic compounds have been proposed to contribute to the biological activities of medicinal plants (1-4). Herein, we report on the assessment of the in-vitro antiurolithiatic activities of hydroalcoholic extracts of *S. cumini* and *T. tetraptera* alone and

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in combination, through the use of validated crystallization assays (7).

Materials and Methods

Plant Collection and Authentication

Leaves of *S. cumini* and bark/pods of *T. tetraptera* were collected from local regions of Gujarat. Dried parts were processed into herbarium sheets and authenticated at the Department of Botany, Parul University.

Certificate No: - PIAS/PU/2025/03 & PIAS/PU/2025/08

The plant parts were selected based on their documented traditional use and reported phytochemical composition relevant to the pharmacological investigation. Leaves of *Syzygium cumini* and bark/pods of *Tetrapleura tetraptera* were therefore selected for the present study(8).

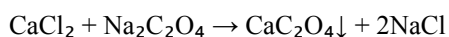
Extraction Procedure

Dried plant materials were powdered separately. Approximately 100 g of each powdered plant material was macerated separately with 500 mL of methanol for 48 h with intermittent stirring. The extracts were subsequently filtered, and the filtrates were concentrated at 40 °C using a water bath. The resulting crude extracts were stored at 4–8 °C until further analysis(9).

Qualitative Phytochemical Screening

The extracts were subjected to qualitative phytochemical screening using standard pharmacognostic procedures. Alkaloids were evaluated using Mayer's, Wagner's, Dragendorff's, and Hager's tests. Carbohydrates were assessed using Molisch's, Benedict's, and Fehling's tests. The extracts were also screened for flavonoids, saponins, tannins, phenolic compounds, glycosides, proteins/amino acids, and diterpenes using the respective standard qualitative chemical tests. Positive reactions were identified based on the characteristic colour change, precipitate formation, ring formation, or frothing reaction specified for each test(10).

Preparation of Calcium Oxalate Crystals: Calcium oxalate crystals were prepared by dissolving 1.47 g of calcium chloride in 100 mL of distilled water and 1.34 g of sodium oxalate in 100 mL of 2 M sulfuric acid. The two solutions were mixed under magnetic stirring to facilitate precipitation of calcium oxalate. Ammonia solution was subsequently added to remove the acidic medium. The precipitated crystals were washed with distilled water, filtered, and dried before further use.



Crystal characteristics: Hardness: 5.58, Size: 6.42 mm, Type: Calcium oxalate, Dissolution: Non-dissolving.

In-vitro antiurolithiatic studies

In vitro model:

Different *in vitro* assays conducted to assess the anti-urolithiatic activity of herbs. Nucleation, Aggregation, and Calcium Oxalate mineralization suppressing assays. Crystallization or suppressing assays were conducted with or without herbs to determine the potential inhibitory activity of herbs(11).

Experiment kidneys evaluations:

Calcium oxalate crystals were prepared by dissolving 1.47 g of calcium chloride in 100 mL of distilled water and 1.34 g of sodium oxalate in 100 mL of 2 M sulfuric acid. The two solutions were mixed under magnetic stirring to facilitate precipitation of

calcium oxalate. Ammonia solution was subsequently added to remove the acidic medium. The precipitated crystals were washed with distilled water, filtered, and dried before further use.

The prepared crystals were examined for their physical characteristics, including crystal type, size, hardness, and dissolution behavior. Crystal size was determined by direct measurement of the prepared crystals, and the observed value was recorded. Hardness was assessed using the laboratory procedure followed for physical characterization of the prepared crystals, and the corresponding value was recorded. The crystals were further evaluated for their dissolution behavior in the test medium. The prepared crystals were identified as calcium oxalate based on the experimental procedure used for their preparation.

Table 1: Characteristics of Prepared Calcium Oxalate Crystals

Sr. No	Evaluation Parameter	Result
1	Hardness	5.58
2	Size	6.42
3	Type	Calcium oxalate
4	Dissolution Time	Do not dissolve

Nucleation Assay (Turbidity method)

Calcium oxalate crystallization was induced using calcium chloride and sodium oxalate. Briefly, 1 mL of 0.025 M calcium chloride dihydrate was mixed with 2 mL of Tris buffer (0.05 mol/L, pH 7.4), followed by the addition of 1 mL of 0.025 M sodium oxalate solution.

For the negative control, 1 mL of calcium chloride solution was mixed with 1 mL of distilled water, followed by the addition of 1 mL of sodium oxalate solution to initiate crystallization. For the test groups, 1 mL of calcium chloride solution was mixed with 1 mL of the respective plant extract at the required concentration. After stable absorbance was obtained, 1 mL of anhydrous sodium oxalate solution (7.5 mM) was added to initiate crystallization. For the positive control, 1 mL of calcium chloride solution was mixed with 1 mL of 6 mM citric acid, followed by the addition of 1 mL of sodium oxalate solution. Absorbance was measured at 620 nm for 10 min at 10-s intervals using a UV-visible spectrophotometer.

The percentage inhibition of nucleation was calculated using:

$$\% \text{ inhibition} = [1 - (P_{ai}/P_{si})] \times 100$$

where P_{ai} represents the turbidimetric slope in the presence of the plant extract and P_{si} represents the turbidimetric slope in the absence of the plant extract.

Aggregation Assay

Calcium oxalate (CaOX) crystals were produced *in vitro* through a process that took the following steps: a water bath was used to mix and heat calcium chloride (50 mM) and sodium oxalate (50 mM) solutions together for one hour at 60°C. The solution obtained was cooled at room temperature for one night and then filtered twice using Wattman paper the next day. The solid that was left over was a combination of weighed and dried residue, and the oven was set to 37°C for drying. A wash of CaOX at the concentration of 1 mg/mL was made from the dry residue in a buffer of Tris-HCl 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. From a 100 mg/mL stock solution, the concentrations A1(1mg/mL), A2 (2 mg/mL), A3 (5 mg/mL), and A4(7.5mg/mL) were prepared for each extract. At the same time, two controls, one positive (citric acid as an inhibitor) and one negative (no

inhibitor), were also prepared. Each concentration of 1 mL was added to 1 mL of pre-prepared CaOX solution. Absorbance was evaluated at 620 nm with and without extracts every 30 min for a total of 3 hours. The following formula was used to determine the percentage of inhibition of aggregation:

$$PA \% = [1 - (P_i/P_c)] \times 100$$

PA -represents the percentage of aggregation inhibition,

P_i -is the turbidimetric slope in the presence of extract,

P_c -is the turbidimetric slope in the absence of extract

Growth Inhibition Method

An aqueous solution of 0.5M Ammonium dihydrogen phosphate was mixed with sodium metasilicate solution of specific gravity 1.05 in the necessary amount by using magnetic stirrer so that the pH value 7.0, pH of the reaction was determined by using a pH probe meter. The gel solution of 10 mL was dispensed into the test tubes of 140 mm length and 25 mm diameter. After the gel was formed, 5 mL of supernatant solutions of 0.5 and 1% concentration of test drug in 1.0 M magnesium acetate were gently poured on the gels in test tubes to observe the growth inhibition of Struvite crystals.

Detection method: 5 ml of 1.0 M magnesium acetate without test drug was added as supernatant to the control tubes which constitute the crystal control group. All the operations were performed in an aseptic medium in the laminar flow hood to prevent microbial contamination. All test tubes and other glassware were autoclaved at 120 °C for 15 min. After abstraction of the supernatant solution, the test tubes were sealed with airtight stoppers. The experiment was carried out at room temperature.

Crystal growth was evaluated for five consecutive days, after which the samples were subjected to FTIR analysis.

FTIR Analysis

FTIR analysis was performed to characterize the functional groups present in the investigated samples following the crystal growth experiment. The FTIR spectrum of *T. tetrapetra* showed characteristic peaks at 3310, 2923, 2115, 1716, and 1648 cm⁻¹, while *S. cumini* showed characteristic peaks at 3240, 3017, 2924, 1520, and 1442 cm⁻¹. The combination extract showed characteristic peaks at 3297, 3456, 2987, 1756, and 1578 cm⁻¹. These spectral features were assigned to characteristic molecular vibrations based on their corresponding absorption regions(12).

Results and Discussion

Phytochemical Analysis: The extracts were selected because they were highly enriched in flavonoids, tannins, saponins, alkaloids, and phenols, which are known to be potent impurities influencing the crystallization process.

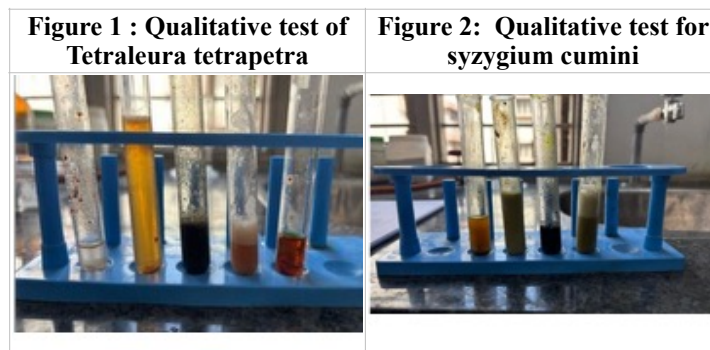


Table 2: Nucleation Assay

Standard Drug				Syzygium Cumini			
Conc.	Abs.	Slope	%inhibition	Conc.	Abs.	Slope	%inhibition
Blank	0.2279	0.2279	-	Blank	0.2279	0.2279	-
5mg/ml	0.5129	0.0570	74.79%	5mg/ml	0.4992	0.0853	69.95%
10mg/ml	0.8927	0.0329	85.57%	10mg/ml	0.5129	0.0570	74.79%
30mg/ml	1.2231	0.0257	88.73%	30mg/ml	0.9998	0.0412	86.95%
<i>Tetraleura tetrapetra</i>				Combination of Syzygium Cumini & Tetraleura tetrapetra			
Conc.	Abs.	Slope	%inhibition	Conc.	Abs.	Slope	%inhibition
Blank	0.2279	0.2279	-	Blank	-	-	-
5mg/ml	0.4982	0.2840	63.15%	5mg/ml	0.5129	0.0570	70.87%
10mg/ml	0.8927	0.0664	70.87%	10mg/ml	0.6127	0.0317	75.10%
30mg/ml	0.9989	0.0338	85.17%	30mg/ml	1.1127	0.0217	87.01%

Table 3: Aggregation Assay

Standard Drug				Syzygium Cumini			
Conc.	Abs.	Slope	%inhibition	Conc.	Abs.	Slope	%inhibition
Blank	0.3728	0.3728	-	Blank	0.3728	0.3728	-
5mg/ml	0.6787	0.0927	75.14%	5mg/ml	0.4992	0.0827	65.2%
10mg/ml	0.9782	0.0812	78.21%	10mg/ml	0.8727	0.0672	72.07%
30mg/ml	2.1213	0.0582	84.39%	30mg/ml	1.5127	0.0312	80.07%
<i>Tetraleura tetrapetra</i>				Combination of Syzygium Cumini & Tetraleura tetrapetra			
Conc.	Abs.	Slope	%inhibition	Conc.	Abs.	Slope	%inhibition
Blank	0.3728	0.3728	-	Blank	0.3728	0.3728	-
5mg/ml	0.4276	0.0992	63.67%	5mg/ml	0.5787	0.0917	70.01%
10mg/ml	0.7278	0.0672	70.17%	10mg/ml	0.8992	0.0801	73.17%
30mg/ml	1.3217	0.0327	73.27%	30mg/ml	1.8927	0.0412	75.87%

Nucleation inhibition: up to 87%

Aggregation inhibition: up to 85%

Combination of the herbs in some assays was found to be better than Cystone alone. The hydroalcoholic extracts of *S. cumini* and *T. tetraptera* exhibited in-vitro potent antiurolithiatic activity(13). The flavonoids and phenolic compounds probably bind calcium ions, leading to precipitation reduction. Saponins could be preventing crystal build-up through lowering surface tension, whereas tannins and alkaloids might be modifying nucleation kinetics(14). The combination extract demonstrated substantial inhibitory activity against calcium oxalate nucleation and aggregation; however, the present study did not include a formal synergy analysis, and therefore a synergistic interaction cannot be conclusively established(15).

Conclusion

The combination of *Syzygium cumini* and *Tetrapleura tetraptera* hydroalcoholic extracts significantly inhibited calcium oxalate crystal formation, growth, and aggregation in vitro. This supports their potential use as a management herbal therapy for urolithiasis. Further preclinical and clinical studies are recommended.

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Conflict of Interest

The authors declare no conflict of interest.

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