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Evaluation of Anti-Urolithiatic Potential of *Pongamia pinnata* Leaves on Kidney Stone

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Abstract

The anti-urolithiatic potential of *Pongamia pinnata* leaf extract, obtained through ethanol extraction, and its correlation with the phytochemical composition is described in this study. Phytochemicals such as resins, flavonoids, carbohydrates, saponins, alkaloids, proteins, and gums are present in the plant extract. Nucleation and aggregation studies revealed the efficacy of the extract against calcium oxalate crystal formation. Additionally, the extract demonstrated antibacterial activity against *Propionibacterium acnes* and *Pseudomonas aeruginosa*, enhancing its potential for managing urolithiasis-related infections. Spectral analyses, including UV-Visible and FT-IR spectroscopy, confirmed the presence of phytochemicals having potential for their anti-inflammatory, antioxidant, and diuretic activities. These results suggest that *Pongamia pinnata* holds promise as a natural remedy for preventing and managing kidney stones, and a promising candidate for further investigation for its clinical applications.

Keywords: Pongamia pinnata, anti-urolithiatic, kidney stone, nucleation inhibition, aggregation inhibition, calcium oxalate, antibacterial activity, urolithiasis.

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Introduction

Urolithiasis, also known as kidney stones, is a widespread urological disorder that impacts a large percentage of people worldwide. The estimated prevalence of urolithiasis varies widely between 1% and 13%, depending on the region, which highlights the geographical variability in its occurrence. This variation can be attributed to factors such as genetic predisposition, environmental influences, and healthcare access, which can differ significantly across populations [1,2].

Recent research indicates that the prevalence of urolithiasis is increasing worldwide [3]. Several factors contribute to this upward trend. First, changes in social conditions, such as urbanization, sedentary lifestyles, and increased obesity rates, can influence the risk of stone formation. These lifestyle factors are often associated with dietary habits, such as increased consumption of salt, protein, and oxalate-rich foods, which are known to promote kidney stone formation.

Stone formation occurs when the concentration of substances that form stones in the urine exceeds the saturation point, leading to crystallization [4]. This is often due to excessive excretion of stone-forming compounds, insufficient inhibitory factors in the urine, or low urine output, resulting in highly concentrated urine.

Stone formation, or lithiasis, is classified based on the location of the calculi: nephrolithiasis refers to stones that form in the kidneys, while urolithiasis applies to those found in the bladder, ureter, or other areas of the urinary tract, as outlined in reference [5].

Urologists frequently face the challenge of recurrent stone formation, which remains resistant to both successful lithotripsy and surgical interventions. Adding to the complexity, the treatment of kidney stones is associated with substantial costs. Therefore, there is a pressing need to explore alternative approaches and valuable resources, including natural and waste products.

Natural products from plant sources have demonstrated as potential remedy for several diseases including anti-inflammatory, anti-microbial[6], anti-hyperglycemic [7] among others. Throughout history, traditional remedies have

played a significant role in addressing kidney stone disease. Notable examples include *Pedalium murex L.*[8], *Phyllanthus amarus* [9], *Cactus fruit* [10], *Cynodon dactylon* [11]*, and Ammi visnaga* [12] etc.

Pongamia pinnata has traditionally been used as an herbal remedy for a variety of health conditions, including ulcers, tumors, hemorrhoids, and skin disorders [13, 14]. The root of the plant is particularly beneficial for treating gonorrhea, improving oral health by cleaning gums and teeth, and healing ulcers. It is also used to address vaginal and skin infections [15].

In Ayurvedic practices, indigenous communities in southern India use *Pongamia pinnata* for wound healing. The stem bark of this plant is typically blended with *Aristida setacea* to form a poultice that is applied to wounds. Additionally, a combination of *Pongamia pinnata* oil and Eupatorium odorata leaves is also used to aid in healing, as noted in reference [16].

The objective of this study is to investigate the anti-urolithiatic effects of an alcoholic extract of *Pongamia pinnata* leaves, using in vitro assays to evaluate nucleation and aggregation, supported by microscopic examination for further insights.

Materials and Methods

Preparation of plant extract

A total of 2 kg of fresh *Pongamia pinnata* leaves were gathered from Nagapattinam district. These leaves were carefully dried in the shade over a period of ten days. Subsequently, the dried leaves were finely powdered and subjected to extraction using the Soxhlet technique, employing 50% ethanol in four separate 500 mL cycles. After the extraction process, the resulting solution was passed through Whatman filter paper for filtration. The filtrate was then concentrated under vacuum, resulting in the formation of a substance resembling green oil, which was subsequently stored in a refrigerated environment for future use.

For the experiments, various extract concentrations were prepared from the plant sample, including 25, 50, 100, 200, 400, and 1000 g/mL.

Qualitative Phytochemical Analysis

Carboxylic Acids

To detect carboxylic acids, 2 mL of sodium bicarbonate solution was added to 1 mL of plant extract. The appearance of a color change indicates the presence of carboxylic acids.

Tannins

A red color was expected after boiling 2 mL of plant extract with 2 mL of 10% hydrochloric acid for 5-6 minutes, as a signal for the presence of tannins.

Steroids

In a test for steroids, 0.5 mL of plant extract was mixed with 5 mL of chloroform and an equal volume of concentrated sulfuric acid. Appearance of red coloration in the upper layer and a yellow-green color in the lower layer would confirm the presence of steroids.

Flavonoids

To detect flavonoids, 4 mL of 1% ammonia was added to 0.5 mL of plant extract, followed by 1 mL of concentrated sulfuric acid. The development of a yellow color confirms the presence of flavonoids.

Glycosides (Borntrager's Test)

In this test, 2 mL of extract was combined with 3 mL of chloroform and shaken vigorously. After phase separation, the addition of 10% ammonia solution resulted in the appearance of a pink color was not observed, indicating the absence of glycosides.

Proteins (Bradford Method)

The protein content was measured by mixing 500 µL of plant extract with 5 mL of Bradford reagent, followed by incubation in the dark for 10-15 minutes. The optical density was then measured at 575 nm to determine the presence of protein.

Phenols (Ferric Chloride Test)

For phenol detection, 50 mg of extract was dissolved in 5 mL of distilled water, and a few drops of 5% ferric chloride solution were added. The appearance of a dark green color indicates the presence of phenolic compounds.

Saponins
A test for saponins involved mixing 50 mg of plant extract with 20 mL of distilled water and shaking vigorously for 15 minutes. The formation of a 2 cm layer of foam suggests the presence of saponins.

Alkaloids (Mayer's Test)
To detect alkaloids, two drops of Mayer's reagent were added to a test tube containing a small amount of plant extract. The formation of a white, creamy precipitate indicates the presence of alkaloids.

Saponification Test
For saponification, 2 mL of plant extract was boiled with 2 mL of 10 N sodium hydroxide for 2 minutes. The appearance of soap or fat confirms a positive result for saponification.

Gums
To test for gums, 100 mg of plant extract was dissolved in 2 mL of distilled water, followed by the addition of 2 mL of absolute alcohol with continuous stirring. The formation of a white, cloudy precipitate indicates the presence of gums and mucilages.

Flavanoglycosides
A solution of 50 mg of plant extract in 5 mL of ethanol was treated with a few drops of magnesium sulphate and concentrated hydrochloric acid. The appearance of a pink color indicates the presence of flavanoglycosides.

Carbohydrates
Carbohydrates were detected by mixing 0.5 mL of extract with 0.5 mL of Benedict's reagent, then boiling the mixture for 2 min. A color change and the formation of a precipitate suggest the presence of carbohydrates.

Resins
To identify resins, 0.5 mL of plant extract was treated with 3 mL of copper sulphate solution. After shaking for 1-2 minutes, the appearance of a green color precipitate confirms the presence of resins.

Biuret Test
A biuret test was performed by adding 1 drop of 2% copper sulphate solution to 2 mL of plant extract, followed by 1 mL of 95% ethanol and 2-3 sodium hydroxide pellets. The development of a pink color confirms the presence of proteins.

Quantification of Alkaloids

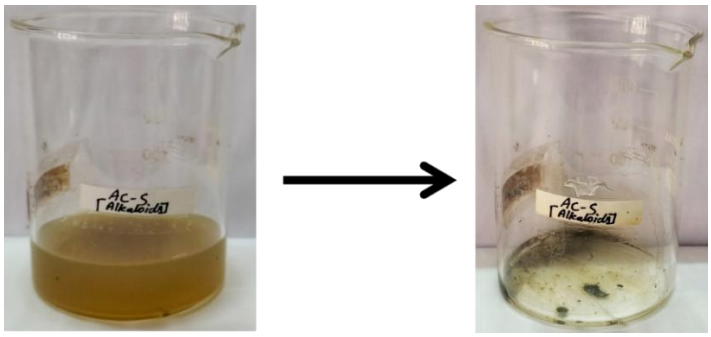

Figure 1. Quantification of alkaloid in the plant extract.

A solution of 200 mL of 10% acetic acid in ethanol was prepared and combined with 1.25 g of the plant extract. The solution was left undisturbed for about 4 h. The mixture was reduced one-quarter of its original volume by heating. Then the solution was filtered and added 15 drops of concentrated ammonium hydroxide and kept for 3 h. Following a 3-h incubation period, the precipitate was collected, rinsed with 20 mL of 0.1 M ammonium hydroxide, and then dried.

The weight of the resulting residue was used to quantitatively assess the alkaloid content in the plant extract. The following equation is used to calculated the %alkaloid present in the plant extract.

$$\%\boldsymbol{Alkaloid} = (\boldsymbol{Weight\ of\ alkaloid/Weight\ of\ sample})\ \boldsymbol{x}\ \mathbf{100}$$

Quantification of Total Phenol

Total phenolic composition was determined using the Folin-Ciocalteu method. 1 mL of sample and standard gallic acid were mixed with 5 mL distilled water and 0.5 mL of Folin-Ciocalteu's reagent, followed by a 5-minute incubation. Then, 1.5 mL of 20% sodium carbonate was added, and the volume was adjusted to 10 mL with distilled water. After 2 hours of incubation at room temperature, the absorbance was measured at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per 100 g of dry mass, based on a calibration curve using gallic acid as the standard.

Estimation of glycoside

1 g of each plant extract was mixed with 200 mL distilled water and autolyzed for 2 hours. Distillation was performed in a 250 mL flask containing 20 mL of sodium hydroxide and tannic acid as an antifoaming agent. The distillate was treated with 100 mL of cyanogenic glycoside, 8 mL of 6 M NH_4OH, and 2 mL of 5% potassium iodide solution, then mixed thoroughly. Titration was conducted using 0.02 M $AgNO_3$ with a microburette, and the endpoint was determined by observing turbidity. The cyanogenic glycoside content in the plant extract was calculated based on the titration results.

$$Cyanogenic\ glycoside\ \left(\frac{mg}{100g}\right) = \frac{Titre\ value\ (mL)\ x\ 1.08\ x\ Volume}{Distillate\ volume\ (mL)\ x\ sample\ weight\ (g)} x\ 100$$

Quantification of saponin

5 g of plant extract were mixed with 100 mL of 20% aqueous ethanol and heated at 55°C for 4 hours while stirring in a water bath. After filtration, the residue underwent a second extraction with an additional 100 mL of 20% aqueous ethanol, followed by heating at 55°C for 4 hours. The combined extracts were concentrated to 40 mL at 90°C. To the concentrated solution, 20 mL of diethyl ether was added, and the mixture was agitated in a separating funnel. The aqueous layer was retained, and the ether layer discarded, with this process repeated twice. Next, 60 mL of n-butanol was added, followed by two extractions with 10 mL of 5% NaCl, and the NaCl layer was discarded. The remaining solution was heated for 30 minutes in a water bath and then dried in a crucible. The final quantity obtained reflects the saponin content.

$$\%\boldsymbol{Saponin} = (\boldsymbol{Weight\ of\ saponin/Weight\ of\ sample})\ \boldsymbol{x}\ \mathbf{100}$$

Spectral Studies

The plant extract was subjected to UV-Visible spectrophotometer and FT-IR spectrometric analysis.

Antibacterial Studies

Antibacterial Assay Procedure

The Nutrient Agar medium was prepared by dissolving 2.8 g of commercially available Nutrient Agar (HiMedia) in 100 mL of distilled water, followed by autoclaving at 121°C for 15 minutes at 15 lbs of pressure. The molten medium was poured into 100 mm petri plates, with 25-30 mL dispensed per plate. Nutrient broth was prepared by dissolving 2.8 g of Nutrient Agar in 100 mL of distilled water, boiling to dissolve completely, and sterilizing by autoclaving at 121°C for 15 minutes.

For the antibacterial assay, 20 mL of nutrient agar was poured into each petri dish, and the plates were seeded with a 24-hour bacterial culture of *Pseudomonas aeruginosa* (424) and *Propionibacterium acnes* (1951), adjusted to an optical density (OD) of 0.5 according to the McFarland standard. Wells were cut into the agar, and plant extract was added at concentrations of 500, 250, 100, and 50 µg/mL. The plates were incubated at 37°C for 24 hours, and antibacterial activity was evaluated by measuring the inhibition zone diameter around the wells. Gentamicin was used as a positive control. Data analysis was performed using GraphPad Prism 6.0 software (USA).

Nucleation assay

A buffer solution containing 0.05 mol/L Tris, 0.15 mol/L NaCl, and pH 6.5 was used to prepare 5 mmol/L calcium chloride and 7.5 mmol/L sodium oxalate solutions. The experiment commenced by mixing 950 µL of calcium chloride

solution with 100 µL of herb extract at concentrations ranging from 100 µg/mL to 1000 µg/mL. Crystallization was triggered by adding 950 µL of sodium oxalate solution. The reaction was conducted at 37°C, and the optical density was measured at 620 nm. Nucleation rates were evaluated by comparing the induction times in the presence of the extracts to those of the control group.

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

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

Growth Assay

The inhibitory effect on CaOx crystal growth was assessed using the seeded, solution-depletion assay described by Nakakawa et al. [18]. An aqueous solution of 10 mM Tris-HCl and 90 mM NaCl (pH 7.2) was prepared. A stone slurry (1.5 mg/mL) was prepared in a 50 mM sodium acetate buffer (pH 5.7). A solution containing 1 mM CaCl2 and 1 mM sodium oxalate was seeded with sodium oxalate crystals. The interaction of CaCl2 and Na2C2O4 with the crystal seeds leads to CaOx deposition, reducing free oxalate detectable at 214 nm. When an aqueous extract is added, inhibition of CaOx crystal growth results in decreased depletion of free oxalate ions. The rate of oxalate reduction was measured before and after a 30-second incubation with or without the extract. The relative inhibitory activity was calculated using the formula: % Relative Inhibitory Activity = [(C - S)/C] × 100, where C is the rate of oxalate reduction without the extract and S is the rate in the presence of the extract.

Microscopical Examination

Samples obtained from the nucleation and aggregation assays were observed under a microscope (MicroVis, India) and digital photos are taken using a 50 MP camera to visualize the formation and inhibition of calcium oxalate crystals.

Results and Discussion

Phytochemical Composition

Table 1. Phytochemical composition of *Pongamia pinnata.*

Phytochemical compound	Result
Resins	+
Carboxylic acid	-
Tanins	-
Steroids	-
Flavonoid	+
Carbohydrates	+
Glycosides	-
Saponification	+
Protein	+
Phenol	+
Biuret	+
Soponin	+
Gum	+
Flavanoglycosides	-
Alkaloids	+

In the present research, the leaves of *Pongamia pinnata* was dried and extracted the phytochemicals using 50% ethanol. The qualitative phytochemical composition of the extract is given in Table 1. The ethanolic extract of Pongamia pinnata was evaluated for anti-urolithiatic potential and the correlation between the phytochemical composition and the possible interaction of the plant extract with the kidney stone are given below.

Resins are known for their anti-inflammatory and antimicrobial properties. The presence of resins in the plant extract indicates that it can assist in reducing inflammation in the urinary tract, potentially aiding in the prevention of

stone formation or alleviating discomfort associated with urolithiasis. The absence of carboxylic acids (Table 1) suggests that the plant does not rely on these compounds, which are often involved in the solubilization of stones or in enhancing the body's ability to dissolve them.

Tannins, which possess astringent properties, are not present in the plant. This absence could imply that the plant does not primarily act through astringency or by reducing mucosal swelling in the urinary tract. The lack of steroids suggests that the plant does not contain compounds typically associated with anti-inflammatory or hormonal regulatory effects, which can influence kidney function and stone formation. Flavonoids are known for their antioxidant, anti-inflammatory, and diuretic properties. The presence of flavonoids in the plant extract can contribute to the prevention of urolithiasis by reducing oxidative stress and promoting increased urine flow, thus helping in the expulsion of crystals or preventing their formation.

Carbohydrates are often involved in providing energy and supporting metabolic processes.
Presence of carbohydrates aids the overall health benefits of the plant extract. The absence of glycosides suggests that the plant does not rely on compounds that may have cardiotonic, or diuretic which are sometimes used for treating conditions like urolithiasis.

Saponins possess anti-inflammatory, antimicrobial, and diuretic properties. Availability of the saponins in the plant extract can help break down kidney stones and facilitate their removal through urine, which is beneficial in anti-urolithiatic treatments. Proteins are essential for the overall health of the body and can play a role in regulating various metabolic processes. In the context of urolithiasis, presence of proteins may contribute to kidney function.

Gums are recognized for their soothing and anti-inflammatory effects, which may help alleviate irritation in the urinary tract and ease symptoms related to stone formation or movement. The absence of flavanoglycosides indicates that the plant does not rely on these compounds, typically associated with health benefits like antioxidant activity. Alkaloids, known for their diverse pharmacological actions, including anti-inflammatory, analgesic, and diuretic properties, may contribute to anti-urolithiatic activity by reducing pain, inflammation, and promoting stone excretion or preventing their formation.

In this study, alkaloids, total phenols, cyanogenic glycosides, and saponins were quantified. Alkaloids, nitrogen-containing compounds found in many plants, are recognized for their broad biological effects. The high alkaloid content (47.2%) in the ethanolic extract suggests a significant role in the plant's therapeutic potential. Phenolic compounds, known for their antioxidant properties, help neutralize free radicals, reducing oxidative stress and preventing cellular damage. Additionally, phenols exhibit anti-inflammatory, antibacterial, and antifungal properties.

Cyanogenic glycosides are compounds that can release cyanide when metabolized. While these compounds can be toxic at high concentrations, in smaller quantities, they are often found in plants used in traditional medicine. The presence of cyanogenic glycosides (0.65 mg/100g) in the plant extract suggests the potential for toxicity if consumed in large amounts. However, many plants contain these glycosides in controlled quantities and may have detoxifying mechanisms.

The presence of saponins (2.2%) is particularly relevant to the plant’s anti-urolithiatic potential. Saponins can have diuretic effects, which promote increased urine output, thereby helping to flush out urinary crystals and prevent the formation of kidney stones. Additionally, saponins possess anti-inflammatory and antioxidant properties, which can reduce the formation of stones by decreasing oxidative stress and inflammation in the kidneys and urinary tract. These properties can help in dissolving the stones or preventing their formation by inhibiting crystal aggregation.

Spectral analyses and correlation with phytochemical analysis

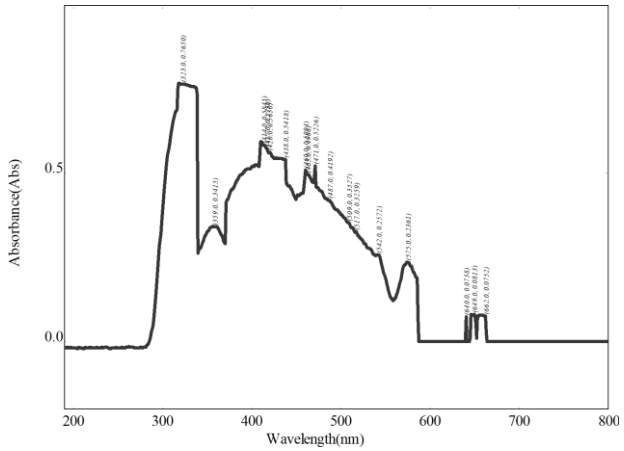

Figure 2. UV-Visible spectrum of plant extract.

The UV-visible spectrum of a plant extract provides valuable information about the presence of specific phytochemical compounds that can absorb light in the ultraviolet and visible regions. The absorbance maxima in the spectrum were predominantly observed at 323 nm, 414 nm, 438 nm, 471 nm, 350 nm, 575 nm, and 640 nm.

The absorption band at 323 & 350 nm is typically associated with flavonoids and phenolic compounds. Flavonoids, in particular, are known to absorb in the UV range (typically around 300-350 nm), with a peak near 323 nm indicating the presence of flavonoid derivatives like flavones or flavonols. Absorbance in the region of 400-420, 438 and 475 nm is commonly seen with anthocyanins, which are flavonoid pigments responsible for the coloration in many plants. This peak could also indicate the presence of polyphenolic compounds. Absorption at 438 nm is typical of flavonoids, especially flavonols and their glycosides. It could also be linked to the presence of carotenoids or other plant pigments. Chlorophyll typically absorbs in the 640-660 nm range.

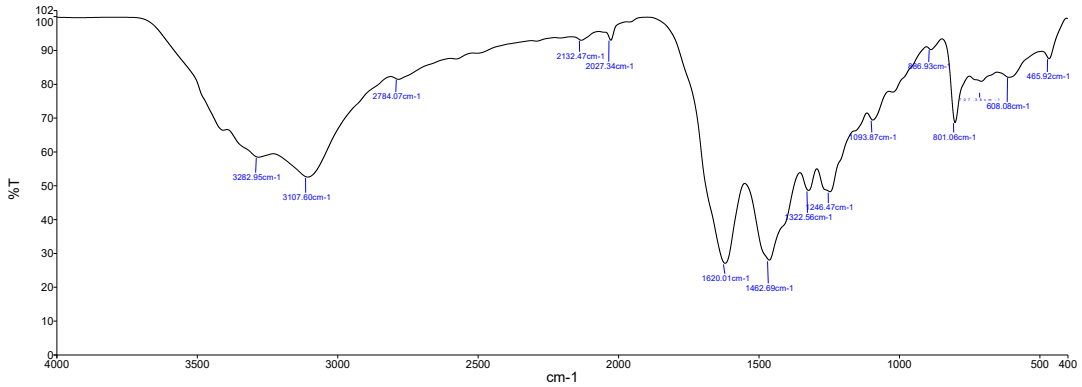

Figure 3. FT-IR spectrum of plant extract of *Pongamia pinnata*.

The Fourier-transform infrared (FT-IR) spectrum provides detailed information about the functional groups and molecular structures present in a plant extract. The broad band at 3282 cm^{-1} is typically associated with O-H stretching vibrations (Figure 3). It corresponds to the presence of phenolic groups, which are common in flavonoids and other polyphenolic compounds. The broadness of this peak suggests the presence of intermolecular hydrogen bonding, a characteristic feature of phenolic compounds.

The band at 3107 cm^{-1} corresponds to C-H stretching vibrations of aromatic compounds, indicating the presence of aromatic rings typically found in flavonoids and alkaloids. The weak band at 2027 cm^{-1} is associated with C≡N (nitrile) or C=O (carbonyl) stretching, commonly observed in cyanogenic glycosides or other nitrogen-containing compounds, aligning with the presence of cyanogenic glycosides (0.65 mg/100g) in the plant extract. A peak at 1620 cm^{-1} suggests C=C stretching in aromatic compounds or C=O stretching in carboxylic acids. The former is characteristic of flavonoids or alkaloids, while the latter may indicate phenolic acids.

FT-IR analysis provides strong evidence for the presence of bioactive compounds, including alkaloids, phenolic compounds, saponins, and flavonoid glycosides, all linked to anti-urolithiatic activity. The anti-inflammatory, antioxidant, and diuretic properties of these compounds, supported by both the phytochemical composition and FT-IR data, suggest the plant's potential as a natural remedy for preventing or treating kidney stones. The FT-IR results corroborate the known biological activities of these compounds, reinforcing the scientific basis for the plant's use in urolithiasis treatment.

Antibacterial Studies

The antibacterial activity of the plant extract was evaluated against *Propionibacterium acnes* and *Pseudomonas aeruginosa* at concentrations of 500 µg/mL, 250 µg/mL, 100 µg/mL, and 50 µg/mL, along with a positive control. The results, expressed as zones of inhibition (mm), demonstrated significant antibacterial effects against P. acnes. At the highest concentration (500 µg/mL), a 10.5 mm zone of inhibition was observed, which is comparable to the 12 mm inhibition reported in a previous study for *P. acnes* at the same concentration. The data revealed a dose-dependent antibacterial response, with larger inhibition zones at higher concentrations and smaller ones at lower concentrations. Specifically, at 250 µg/mL, the inhibition zone was 9.25 mm; at 100 µg/mL, it reduced to 7.25 mm; and at 50 µg/mL, it further decreased to 5.25 mm. These results indicate that the antibacterial efficacy is concentration-dependent, with higher concentrations required for optimal effectiveness.

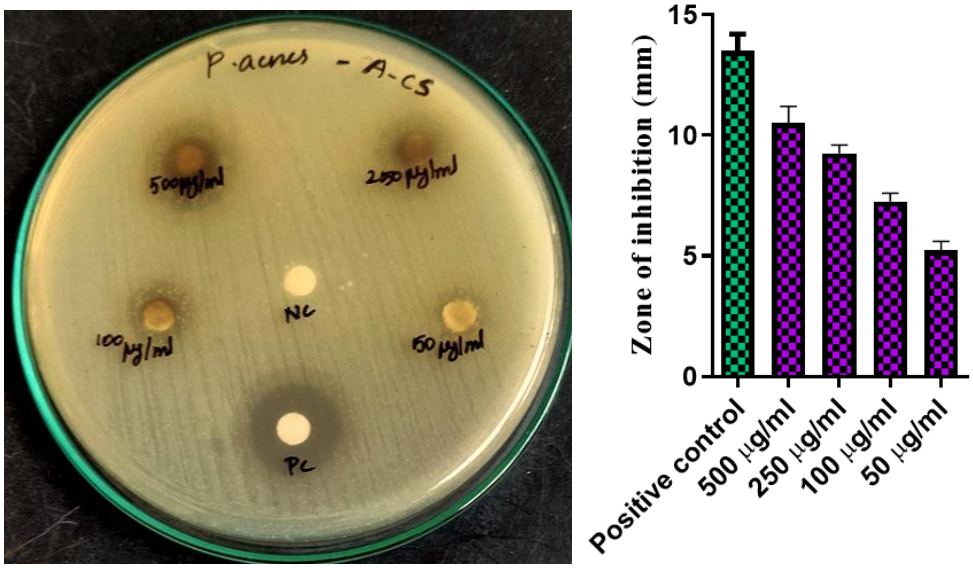

Figure 4. Antibacterial efficacy of plant extract against *Propionibacterium acnes.*

Table 2. Antibacterial activity of the ethanolic extract of *Pongamia pinnata* leaves.

Organism	Zone of inhibition (mm)*				
	500 µg/mL	250 µg/mL	100 µg/mL	50 µg/mL	PC
Propionibacterium acnes	10.5±0.70	9.25±0.35	7.25±0.25	5.25±0.35	13.5±0.71
Pseudomonas aeruginosa	15.5±0.71	14.25±0.30	10.25±0.35	9.25±0.35	17.5±0.71

*p< 0.05.

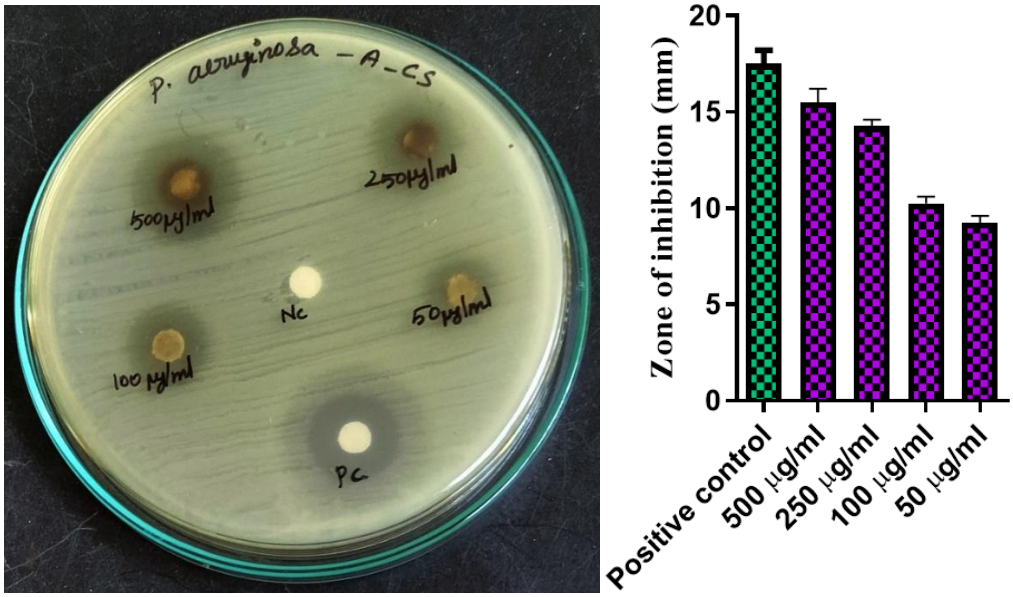

Figure 5. Antibacterial efficacy of plant extract against *Pseudomonas aeruginosa.*

The plant extract showed significant antibacterial activity against *Pseudomonas aeruginosa* compared to P. acnes. At 500 µg/mL, the inhibition zone is 15.5 mm, which is very close to the 17.5 mm observed with the positive control. This suggests that the plant extract has potent antibacterial effects against *P. aeruginosa*, and the inhibition is nearly as effective as the standard antibiotic. At 250 µg/mL, the zone of inhibition is 14.25 mm, at 100 µg/mL it is 10.25 mm, and at 50 µg/mL, it is 9.25 mm. While the activity is still noticeable at lower concentrations, the antibacterial effect is more pronounced at higher concentrations, confirming the concentration-dependent nature of the extract. The inhibition zone at 500 µg/mL (15.5 mm) is slightly less than the positive control (17.5 mm), but still highly significant, indicating that the plant extract is highly effective against P. aeruginosa, especially at higher concentrations. This

finding is particularly valuable because P. aeruginosa is often resistant to many conventional antibiotics, and this plant extract could serve as an alternative therapeutic agent.

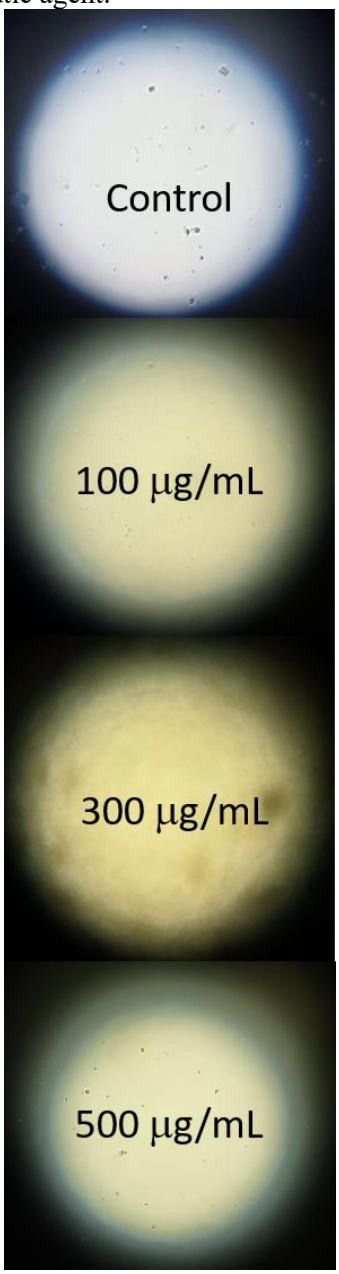

Figure 6. Nucleation inhibition of kidney stone by plant extract.

As discussed above, the plant contains alkaloids, flavonoids, phenolic compounds, and saponins, all of which are known to exhibit antibacterial properties. The observed antibacterial effect on *P. acnes* and *P. aeruginosa* is likely due to these bioactive compounds. The presence of alkaloids (47.2%) and flavonoids could be primarily responsible for the inhibition of bacateria, as both groups are known to disrupt bacterial cell functions. The slightly lower inhibition compared to the positive control might indicate that higher concentrations or further refinement of the extract could enhance its effectiveness.

Nucleation Inhibition Assay

The process of crystal formation commences with nucleation, which represents a pivotal initial phase. Subsequently, crystals progress and amalgamate into aggregates. Consequently, an in vitro crystallization study was carried out to assess the plant extract's capacity to impede calcium oxalate formation. The outcomes of the nucleation assay substantiated the presence of components within the extract that deter nucleation and aggregation (Figure 6).

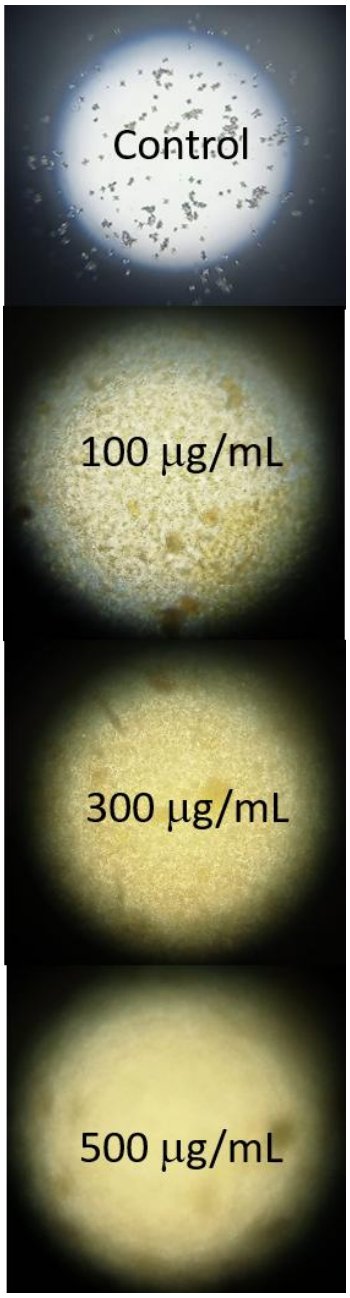

Figure 7. Aggregation inhibition of kidney stone by plant extract.

Effectively regulating the crystallization process, known as nucleation, offers a promising strategy for preventing and treating urolithiasis. This can be accomplished by harnessing the properties of plant extracts, extensively employed in traditional and Ayurvedic medicine for kidney stone treatment. Plant extracts encompass a distinctive blend of diverse bioactive compounds, which can be extracted using a variety of extraction methods.

In our study, the plant extract exhibited inhibition of nucleation, as evidenced by a decrease in optical density after 1 hour of incubation in comparison to the negative control (untreated). Over time, optical density at 620 nm escalates due to crystal growth. Additionally, visual examination under the microscope, in conjunction with a comparative analysis against a negative control, serves as a means to demonstrate the hindrance of crystal formation.

In an aggregation assay, plant extracts demonstrated a capacity for size reduction. Aggregation is the second most significant factor in the formation of kidney stones; it is the mechanism by which the size, composition, and structure of urinary stones are increased. The crystals that adhered to one another are difficult to separate and remain in position. This process has a significant impact on lithiasis.

Conclusion

In the present study the dried leaves of *Pongamia pinnata* was extracted with aqueous ethanol and found to possess flavonoids, alkaloids, saponins, resins, carbohydrates, proteins, and gum. These compounds likely contribute

to the extract's ability to block crystallization by either inhibiting the binding of crystal nuclei or interfering with crystal aggregation. The alkaloids and flavonoids, in particular, have been previously reported to exert inhibitory effects on crystallization, making them key contributors to the extract's anti-lithiasis potential.

The study comprehensively explored the potential of the plant extract in preventing calcium oxalate (CaOX) crystallization, which plays a critical role in the formation of kidney stones, a condition known as urolithiasis. The results of the in vitro crystallization study have highlighted the plant's effectiveness in impeding both the nucleation and aggregation stages of crystal formation.

The plant extract significantly hindered the initial phase of crystal formation (nucleation), as demonstrated by a decrease in optical density after 1 hour of incubation compared to the untreated control. This observation, coupled with microscopic examination, confirms that the extract contains bioactive compounds capable of interfering with the nucleation process. The extract's ability to prevent the formation of new crystals is an essential factor in the management and prevention of kidney stones.

The aggregation assay further substantiated the plant's potential by showing a reduction in crystal size. As aggregation is crucial in the growth and expansion of kidney stones, the extract's ability to prevent this process could significantly reduce the risk of stone formation and enhance the dissolution of existing stones.

The plant extract's antibacterial properties against *Propionibacterium acnes* and *Pseudomonas aeruginosa* further enhance its therapeutic value, suggesting a broader range of actions that could be beneficial for treating or preventing kidney stone-related infections, which often accompany urinary tract stones. The antibacterial effects of the plant also point to its potential role in managing infections related to urolithiasis, a common complication in patients with kidney stones.

In conclusion, the plant extract exhibits significant anti-urolithiatic potential, effectively inhibiting both nucleation and aggregation of calcium oxalate crystals. This indicates that the plant could be a valuable therapeutic agent for preventing and managing kidney stones, especially when integrated into traditional or modern treatment strategies for urolithiasis. The phytochemical composition of the extract further supports its multifaceted biological activities, making it a suitable candidate for further research and development as a natural remedy for kidney stone formation and related disorders.

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