RESEARCH ARTICLE

INVESTIGATION OF POTENTIAL *IN VITRO* ANTI-UROLITHIASIS ACTIVITY FROM *MUSA* PSEUDO STEM LATEX IN COMBINATION WITH *CUMINUM CYMINUM* EXTRACT

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Abstract:

Urolithiasis, characterized by the formation of kidney stones, is a prevalent urinary tract disorder that poses significant health concerns worldwide. Conventional treatments are often associated with side effects, highlighting the need for alternative therapeutic approaches derived from natural sources. The present study investigates the potential in vitro anti-urolithiasis activity of *Musa pseudo stem latex* in combination with *Cuminum cyminum* extract. Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, phenolics, and alkaloids in both extracts, which are known for their antioxidant and anti-inflammatory properties. The anti-urolithiasis potential was evaluated using in vitro assays including nucleation, aggregation, and crystal growth inhibition tests, simulating conditions of calcium oxalate stone formation. The combination of *Musa pseudo stem latex* and *Cuminum cyminum* extract exhibited significant inhibition in all tested phases compared to the individual extracts, suggesting a synergistic effect. The results indicate that this combination could serve as a promising natural remedy for preventing or managing urolithiasis. Further in vivo studies and clinical trials are recommended to validate its therapeutic efficacy and safety profile.

Keyword: Urolithiasis, *Musa pseudo* Stem Latex, *Cuminum cyminum*, Phytochemicals, Calcium Oxalate Inhibition.

Introduction:

Urinary stones are polycrystalline aggregates composed of various organic components and crystalline matrices. Lipids have been shown to be an important component in the stone matrix [11] where the stone matrix contributes only 2–3% of the dry weight [1] from urolith. The stone matrix contains various macromolecules such as protein (64%), non-amino sugar (9.6%), hexosamine as glucosa- mine (5%), water-binding (10%) and resting as ash [3]. The formation of stones because of

lifestyle changes and nutritional factors is the most painful urology disease and becomes prevalent in the current population [16]. The characterization of stone formation or lithiasis is based on the formation of calculi, either nephrolithiasis or urolithiasis. Nephrolithiasis is a stone formation in the kidney while the formation of calculi in the bladder, ureter or any part along the urinary tract other than the kidney is known as urolithiasis [5]. Urologists often feel concerned with the problem of recurrent stone formation which neither successful lithotripsy nor surgery can prevent it. Moreover, the treatment of kidney stones is costly. This necessitates the need for finding alternate methods and valuable sources from natural or waste products. Traditional remedies have been used for a long time in treating kidney stone disease. Most of the remedies used in traditional medicine systems are taken from plants although they do not have strong clinical evidences to support their causes. However, many remedies produced from plants have a positive effect on patients, especially some composite plants and herbal drugs such as *Herniaria hirsuta* where the extract can reduce the crystal size [2], *Bergenia ligulata* [18], *Piper nigrum* [12], *Dolichos biflorus* [7], *Plantago major* [19] etc. Previously, most of the studies were carried out in vivo using animal or human [14]. Synthetic drugs are a common substance widely used over the last decade and due to the unintended side effects of its use, it has been approved as safe and effective. However, it has been found that synthetic drugs have proven side effects as a result of long-term usage. Drugs such as Tamsulosin and nifedipine are opted for patients with kidney stones. Tamsulosin is an alpha-blocker that has the ability to relax the ureter, making urination easier, and for stones to pass through. Nifedipine is a calcium channel blocker. The expulsion rate differs across different drugs but was significantly high in tamsulosin (97.1%), followed by nifedipine (77.1%) and phloroglucinol (64.3%) in a study done by Dellabella *et al.* [6]. Tamsulosin and nifedipine are drugs that have been validated with minor side effects on patients, and these include effects such as low blood pressure, headache, dizziness, and nausea [4]. Though generally acknowledged with minor side effects, Porpiglia *et al.* [17] reported adverse effects in a patient with transient hypotension and palpitations in the nifedipine treatment group and another patient with severe asthenia in the tamsulosin treatment group. Additionally, Islam *et al.* [8] reported only one patient who experienced serious effects associated with hypotension and palpitations in the nifedipine treatment group. To come up with a solution for the effects caused by drugs when treating kidney stones, a safe alternative was introduced in this study from natural derivatives. Bananas have been known to have medicinal properties, especially in traditional medicine, and hence in this study, four different types of Musa variations were chosen to determine the potential extract on anti-urolithiasis in overcoming the kidney stone disease. Banana cultivation generates a huge amount of biomass post-harvesting the fruits and these wastes include the pseudo-stems. Different parts of Musa pseudo-stems have been used traditionally for treating inflammation, high blood pressure, diabetics, diarrhoea, peptic ulcer, rheumatism, high blood pressure, burns, and wounds, as well as pseudo-stem in treating nephritis, uremia, and urolithiasis [10, 13, 15]. The banana cultivar Monthan corm extract has been reported to have antilithiatic potentials [9]. Despite the stated advantages, studies on banana pseudo-stems have not yet been explored extensively. Therefore, the aims of this study are to investigate the juice from Musa species Pseudo Stem Latex in Combination with Cuminum cyminum Extract and

determine their anti-urolithiasis activity via in vitro nucleation, aggregation assay and Titrimetric Method.

Materials and Methods:

Plant Material

Collection and Authentication

The plant *Musa paradisiaca Linn,* synonym Musa which is known in Tamil as Vazhai were collected from Veppampalayam village of Erode District, Tamil Nadu. The plant was authenticated by Botanical Survey of India, Coimbatore.

The collected plant was authenticated by botanical survey of India, Coimbatore for its taxonomical confirmation. (Reference No:BSI/SRC/5/23/2022/Tech/114,115,116)

Musa × *paradisiaca* is the accepted name for the hybrid between *Musa acuminata* and *Musa balbisiana*. Most cultivated bananas and plantains are triploid cultivars either of this hybrid or of *M. acuminata* alone. Linnaeus originally used the name *M.paradisiaca* only for plantains or cooking bananas, but the modern usage includes hybrid cultivars used both for cooking and as dessert bananas. Linnaeus's name for dessert bananas, *Musa sapientum*, is thus a synonym of *Musa* × *paradisiaca*. [20]

Cumin (*Cuminum cyminum*) is a flowering plant in the family Apiaceae, native to the Irano-Turanian Region. Its seeds – each one contained within a fruit, which is dried – are used in the cuisines of many cultures in both whole and ground form. Although cumin is used in medicine, there is no high-quality evidence that it is safe or effective as a therapeutic agent. [21]

Method of Collecting Latex

- The inner core of banana pseudo stem (*musa* sp.) was identified from the local banana plantation.
- The outer surface of the pseudo-stem was cleaned with deionized water.
- By using the sharp toothed knife, the pseudo-stem was drilled and made into deep hole.
- Cumin seeds were added into the hole, and after 24 hours, approximately 150 mL of latex containing cumin phytochemical constituents was collected.
- It is a traditional method for collecting the latex by local peoples.

1. Phytochemicals Test

The prepared extracts were subjected to qualitative chemical tests to detect the presence of different classes of phytoconstituents as briefed below:

1.1. Test for Alkaloids

The detection of alkaloids in qualitative chemical tests are due to the character of alkaloids being susceptible to precipitation as salts of organic acids or with the compound of heavy metals like Hg, Au, Pt, etc. A total of 50 mg of samples was dissolved in 5 ml of distilled water. The solution was added with 2 M hydrochloric acid until an acid reaction occurred and then filtered. The filtrate was tested for the presence of alkaloids as detailed below:

- **Dragendorff's Test:** Approximately 2 ml of the filtrate was added with 1 ml of Dragendorff's reagent along the side of the test tube. The formation of orange or an orange to reddish-brown precipitate indicates the test as being positive.

1.2. Test for Flavonoids

- **Shinoda's test:** A piece of magnesium followed by concentrated hydrochloric acid was added to a stock solution, with the acid being added by drops, and heated. The appearance of magenta coloration shows the presence of flavonoids.
- **Alkaline Reagent Test:** Approximately 5 drops of 5% sodium hydroxide was added into 1 ml of stock solution which resulted in an increase in the intensity of a yellow hue which later on becomes colorless upon the addition of a few drops of 2 M hydrochloric acid. This also indicates the presence of flavonoids.
- **Zn/HCl or Mg/HCl Reduction:** A few fragments of a magnesium ribbon and concentrated hydrochloric acid was added to a stock solution, with the acid being added by drops. The presence of flavanol glycosides was inferred by the development of a pink to crimson color.

1.3. Test for Tannins

- **Ferric Chloride Test:** A few drops of neutral 5% ferric chloride solution were added into a stock solution. A dark green color indicates the presence of phenolic hydroxyl group compounds.
- **Gelatin Test:** Approximately 2 ml of 1% gelatin solution containing 10% sodium chloride was added into a stock solution. A white precipitate indicates the presence of phenolic compounds.

1.4. Test for Saponins

- **Foam test:** A small amount of stock solution was shaken with a small quantity of water. A persisting foam suspension (for about 10 min) indicates the presence of saponins.

1.5. Test for Steroids

Approximately 1 mg of pseudo-stem extract was dissolved with 10 mL of chloroform and concentrated sulphuric acid (10 mL) in test tube. The upper layer in the test tube was turned into red and sulphuric acid layer will show yellow with green fluorescence which indicates the presence of steroids.

1.6. Test for Tri-terpenoids

- **Salkowski test:** A total of 5 ml of each stock solution was mixed in 2 mL of chloroform and then followed by adding 3 mL of concentrated H2 S0 4 carefully to form a layer. A reddish-brown coloration formed in the interface shows positive results for the presence of terpenoids.

1.7. Test for Carbohydrates

- **Molisch's test:** A total of 1 ml of stock solution was added with a few drops of 1% alpha-naphthol and 2–3 ml concentrated sulfuric acid along the side of the test tube. A reddish-violet or purple ring was formed at the junction of two liquids which thus confirms the test.
- **Barfoed's test:** 2 ml of reagent was added to 2 ml of the stock solution, mixed and then placed in a boiling water bath for 1 min. A red precipitate formed indicates the presence of monosaccharides.

1.8. Test for Proteins

- **Biuret test**: To 2 ml of the test solution, 5 drops of 1% copper sulphate solution and 2 ml of 10% Sodium hydroxide (NaOH) were added. After mixing thoroughly, the purple or violet hue formed confirmed the presence of proteins. [22, 23]

2. Evaluation for Anti-Urolithiatic Activity

2.1. Nucleation Assay (Turbidity Method)

In this method the in vitro anti-urolithiatic activity of the extract was tested in term oh inhibition of calcium oxalate formation by the method of prachi khare *et al.* With modification. The inhibition of calcium oxalate formation in the presence of extract was compared with the inhibition of calcium oxalate formation in the presence of the standard (cystone). The precipitate of calcium oxalate at 37c and pH 6.5 was studied by the measurement of turbidity at 620nm using UV/VIS Spectrophotometer. The turbidity caused due to formation of calcium oxalate by the reaction of calcium chloride with sodium oxalate. In the control, turbidity due to the formation of calcium oxalate was determined in the absence of any inhibitor. For this a volume of 0.95ml of 50mM CaCl2 (in Tris buffer pH 6.5) and 1 ml of water were added in a test tube. Then 0.95 ml of 75 mM sodium oxalate (in Tris buffer pH 6.5) was added. Formation of the turbidity results immediately after mixing of above chemicals. The measurement of turbidity was done by measuring the absorption by UV/Visible spectrophotometer at 620 nm after shaking the mixture for 1 min. Then the measurement of absorbance was carried out after 1min interval upto a period of 5 mins. Absorption was noted down. The study was continued to know the effect of plants latex against stone nucleus formation (formation of calcium oxalate) in vitro. In this experiment the effect of the latex on inhibition was carried out in three concentrations of the extract. For this, in one test tube a volume of 0.95 ml of 50mM CaCl2 (in Tris buffer pH 6.5) and 1ml of 100µg/ml latex in water were added, and then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. In another test tube volume of 0.95 ml of 50mM CaCl2 (in Tris buffer pH 6.5) and 1ml of 250µg/ml latex water were added, and then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. In another test tube a volume of 0.95 ml of 50mM CaCl2 (in Tris buffer pH 6.5) and 1ml of 500µg/ml latex water were added, and then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. The measurement of turbidity was done by measuring the absorption by UV/Vis spectrophotometer at 620 nm after shaking the mixture for 1 min. Then the measurement of the absorbance was carried out after 1 min interval up to a period of 5 min. Absorptions was noted down. After that the effect of the standard (Cystone) on the inhibition of the formation of calcium oxalate was studied. The standard drug is a poly herbal formulation. For this in a test tube a volume of 0.95 ml of 50mM CaCl2 (in Tris buffer pH 6.5) and 1ml of 100µg/ml of standard in water were added, and then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. In another test tube 0.95 ml of 50mM CaCl2 (in Tris buffer pH 6.5) and 1ml of 250µg/ml of standard in water were added, and then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. In a third test tube a volume of 0.95 ml of 50mM CaCl2 (in Tris buffer pH 6.5) and 1ml of 500µg/ml of standard in water were added, and then 0.95 ml of 75mM sodium oxalate (in Tris buffer pH 6.5) was added. The measurement of turbidity was done by measuring the absorption by UV/Vis spectrophotometer at 620nm after shaking the mixture for 1 min. Then the measurement of the absorbance was carried out after 1 min intervals up to a period of 5 min. Absorptions were noted down. (24,25)

Inhibition in stone nucleus formation was calculated by the graphical method using the following mathematical formula:

% of Inhibition = {1-[Si / Sc]} x 100

Where; Si: slope of graph in the presence of inhibitor (drugs/latexs);

Sc: slope of without Inhibitor (Control)

2.2 Aggregation Assay

2.2.1 Preparation of the Semi – Permeable Membrane from Eggs

Apex of eggs was punctured by a glass rod in order to squeeze out the entire content with distilled water. Empty eggs were thoroughly washed and put in a beaker consisting of 4ml of concentrated HCl in 200ml distilled water. It was held overnight for the full decalcification of semi-permeable membranes. It on the next day, semi-permeable membranes were carefully removed from egg shells; washed thoroughly with distilled water and put in ammonia solution for acid traces neutralization, and then rinsed with distilled water were placed in a incubator at 37oC for 30 min. 10 mg of calcium oxalate in 10ml of distilled water as negative control. 5ml of latex was taken. 500mg tablet of cystone was placed in absolute ethanol for removing colour coating and 400mg was obtained cystone tablet was crushed into powder form and dispersed into 100ml of filtered and distilled water. Filtrate of cystone was used as positive control for in vitro anti-urolithiasis activity. **(**24,25,26)

2.2.2. Spectrophotometric Calcium Oxalate calculation using Dissolution Method

Synthesis of Calcium Oxalate by Homogenous Precipitation:

1.47gm calcium chloride dehydrate was dissolved in100ml distilled water and 1.34gm sodium oxalate was dissolved in 100 ml of 2 N H2SO4.The both were mixed equally in a beaker to precipitate out calcium oxalate with stirring. Resultant calcium oxalate was freed from traces of sulphuric acid by ammonia solution; washed with distilled water and dried at a temperature 60°c for 2 hours.

Method:

The inhibition of in-vitro calcium oxalate crystal formation by various solvent latex's was investigated by synthetic calcium oxalate crystals. In this method through adding 0.02 KMnO4solution, calcium oxalate formation is caused and the inhibition percentage of the calcium oxalate crystals from at different concentrations of various solvent latex of Musa paradisica investigated by the UV-620nm

Group I: Control

Group II: 1ml of Calcium Oxalate (1mg/ml) + 1ml of Cystone Solution

Group III: 1ml of Calcium Oxalate (1mg/ml) + 1ml Latex(100mg/ml)

Group IV: 1 ml of Calcium Oxalate (1mg/ml) + 1ml Latex (200mg/ml)

Group V: 1 ml of Calcium Oxalate (1mg/ ml) + 1ml Latex (300mg/ml)

In egg semi permeable membrane tied with thread at one end and was suspended with in a conical flask containing 150 ml 0.1 m tris buffer. Each tied by a stick placed on the mouth of conical flask and covered with aluminum foil at another end of thread. All groups have been kept in an incubator, pre-heated to 37°c for 4hours, kept for 3 days. The entire material of each group was separated from the semi-permeable membrane sutured and transferred individually to the test tube. 4ml of 1N H2SO4 and 60-80ml of 0.02m KMnO4 were added and kept aside for 2 hours color change from dark pink to colorless was observed after 2 hours. Change in intensity of color was assessed spectrophoto-metrically against 620nm.

Concentration of un-dissolved calcium oxalate was calculated using the measured absorbance readings from the normal calibration curve of calcium oxalate.

2.2.3 Calcium oxalate measured by Titrimetric

The dissolution percentage of calcium oxalate was evaluated by taking exactly 10mg of calcium oxalate and 100mg, 200mg, 300mg & 400mg of the latex, packed it together in semi permeable membrane of egg. This was allowed to suspend in a conical flask containing 100 ml of 0.1M Tris buffer. First group served as blank containing only 10mg of calcium oxalate. The second group served as positive control containing 10 mg of calcium oxalate and along with the 200mg,300mg and 400mg of standard drug, i.e. cystone. The 3rd and 4th groups along with 10mg of calcium oxalate containing, latex. The conical flasks of all groups were kept in an incubator preheated to 37°C for 2 hrs.

Remove the contents of semi permeable membranes from each group into separate test tubes, add 2ml of 1N sulphuric acid to each test tube and titrated with 0.9494 N KMnO4 till a light pink color end point obtained. The amount of remaining un-dissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved calcium oxalate by various solvent latex.

Each ml of 0.9494 N KMnO4 equivalents to 0.1898mg of Calcium oxalate **(**28-31)

Result:

The Phyto-chemical evaluations were performed by using the above combined latex of *Musa paradisica* and cumin and found the presence of Carbohydrates, Proteins, Tri-terpenoids Steroids, Tannins, Saponins, Flavonoids, Alkaloids. The list of Phyto-constituents presents in the combined latex of *Musa paradisica* and cumin shown in table 1.

Table 1: List of Phyto-Constituents Present in the Combined Latex of *Musa paradisica* and Cumin

Sr. No.	Phytoconstituents	Remark
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Saponins	+
5	Steroids	+
6.	Tri-terpenoids	+
7	Carbohydrates	+
8	Proteins	+

Anti-urolithiatic activity of the combined latex of *Musa paradisica* and cumin was evaluated by Spectrophotometric Calcium Oxalate calculation using Dissolution Method and by Titrimetric method. In Nucleation Assay the % inhibition of different concentration of combined latex of *Musa paradisica* and cumin (100mg/ml, 200mg/ml, 300mg/ml & 400mg/ml) were compared with the standard cystone in the concentration of 400 mg/ml. The result was shown in figure 1.

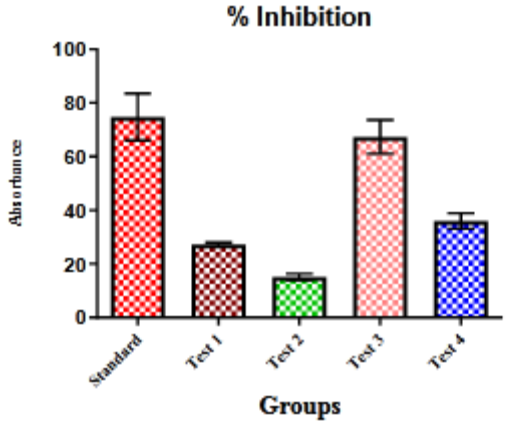

Figure 1: % Inhibition of Different Concentration of Combined Latex of *Musa paradisica* and Cumin Compared with Standard Cystone

In Aggregation Assay the % inhibition of different concentration of combined latex of *Musa paradisica* and cumin (100mg/ml, 200mg/ml, 300mg/ml and 400mg/ml) were compared with the standard cystone in the concentration of 400 mg/ml. The result was shown in table 2. The percentage of dissolution of calcium oxalate stone by Titrimetric method was performed by using $KMNO_4$.

Table 2: % Dissolution of Calcium Oxalate by Titrimetric Method

Sr. No.	Compounds	% of inhibition
1	Cystone + Calcium Oxalate	83.02 %
2	100 mg of combined latex of *Musa paradisica* and cumin + 10 mg of calcium oxalate	42.78 %
3	200 mg of combined latex of *Musa paradisica* and cumin + 10 mg of calcium oxalate	52.53 %
4	300 mg of combined latex of *Musa paradisica* cumin + 10 mg of calcium oxalate	65.41 %
5	400 mg of combined latex of *Musa paradisica* and cumin + 10 mg of calcium oxalate	71.39 %

The result shows that the combined latex of *Musa paradisica* and cumin (300mg/ml & 400mg/ml) significantly (65.41% & 71.39%) dissolved the formed calcium oxalate stones when compared with the standard cystone (83.02%)

Discussion:

The inner core of the banana pseudo stem was cleaned and bored using a sterile knife. Cumin seeds were inserted into the cavity and left for 24 hours, allowing the Phyto active latex to infuse and collect. The resulting latex extract—containing constituents from both.

The combined latex tested positive for the Phyto-constituents reported Carbohydrates, Proteins, Tri-terpenoids Steroids, Tannins, Saponins, Flavonoids, Alkaloids-phytochemicals known for anti-urolithiatic activity.

Urolithiasis begins with urinary supersaturation, leading to nucleation, growth, aggregation, and retention of calcium oxalate crystals. Inhibiting nucleation is vital to prevent stone formation. The

combined latex of Musa and cumin exhibited strong inhibition of crystallization, likely due to the presence of saponins (which disrupt mucoprotein aggregation) and other bioactive.

- **Nucleation Assay:** The combined latex (300 mg/ml) showed 71% inhibition of calcium oxalate crystal formation, comparable to the standard drug cystone (78%).
- **Aggregation Assay:** At the same concentration, the extract showed 59% inhibition of crystal aggregation, vs. 69% for cystone.
- **Titrimetric Assay:** Dose-dependent dissolution of calcium oxalate was observed, with significant activity at 100–400 mg/ml.

These effects are attributed to the synergistic action of saponins, flavonoids, alkaloids, and tannins, which interfere with both the formation and aggregation of calcium oxalate crystals

Conclusion:

The findings of the present study also highlight the ability of Musa latex combined with cumin seed extract to prevent the nucleation and aggregation of calcium oxalate crystals as proved in in vitro studies. These data suggest that the presence of antiurolithic effects in the Musa latex with cumin seed extract is possibly due to calcium oxalate crystal inhibition, and further pre-clinical and clinical studies are needed to evaluate and establish the use of Musa pseudo-stem combined with cumin seed extracts as antiurolithiatic activity.

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Declaration of Competing Interest:

The authors declare that they have no competing interests.

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