

# ***In vitro Antiurolithiatic Activity of Methanolic Extract of Fruit Pulp of Two Varieties of *Lansium domesticum* Correa (Meliaceae)***

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## **Abstract**

*Lansium domesticum* Correa, native to Southeast Asian countries, is a tree bearing exotic fruit that belongs to the family Meliaceae. The tropical fruit tree consists of bunches of edible fruits borne on its branches. The aril of the fruits appeared as a white translucent part and is the edible portion for consumption. *Lansium domesticum* var. *domesticum* (LDD) and *Lansium domesticum* var. *pubescens* (LDP) are commonly known as Duku and Langsat, respectively. This is a seasonal fruit that contains vitamins, minerals and is used traditionally as medicine.

In addition to the aril of the fruits, their peel and seeds also have several medicinal properties and are used as raw medicines. The peel is used as an insect repellent and is visible as brownish in color. The present analysis clearly gives the vision about the antiurolithiatic potential of the plant. The methanolic extract from the fruit pulp of (mg/g) LDD ( $0.9997 \pm 1.1 \times 10^{-4}$ ) and LDP ( $0.9994 \pm 0.00$ ) has a higher potential to dissolve the calcium oxalate crystals than the standard drug, Cystone and potassium citrate. The methanolic fruit pulp extract of *Lansium domesticum* can be used as a natural drug for treating various urogenital diseases, especially kidney stones.

**Keywords:** *Lansium domesticum*, antiurolithiatic, kidney stones.

## **Introduction**

*Lansium domesticum* is a tropical fruit native to Southeast Asian countries including Malaysia, Thailand, Indonesia and the Philippines. The plant belongs to the family Meliaceae, the Mahogany family. About 560 species and 50 genera were included in the family, having wide-ranging distribution in tropical and subtropical regions<sup>2</sup>. The plant is an exotic fruit variety categorized as an underutilized fruit category in Kerala. *Lansium domesticum* is one of the most common species in both wild and cultivated forms in the Southeast Asian countries<sup>13</sup>. The two main varieties of *Lansium domesticum* are *Lansium domesticum* var. *domesticum* (LDD) and *Lansium domesticum* var. *pubescens* (LDP). These two varieties are known by the common names 'Duku' and 'Langsat'. Its taste is sweet-sour and acidic in nature depending upon its growth<sup>8</sup>. *Lansium domesticum* var. *domesticum* (Duku) fruits are round, comparatively

larger in size, thick-skinned, contains mild latex or latex-free.

*Lansium domesticum* var. *pubescens* (langsat) are oblong-ovoid, small in size, thin-skinned and contain latex. The fruit is consumed as a dessert and it is very popular in Indonesia. Traditionally, the fruit peel is a non-edible part and is considered toxic to domestic animals<sup>11</sup>. The peel appears in a natural brownish color in ripe fruits. At the early stage, the peel color appears green and turns brownish when it attains maturity. Both the fruits possess seeds inside the pulp of the fruits, but Duku fruits are comparatively seedless.

The number of seeds present in *Lansium domesticum* is 2-3 and the seeds are small in size for Langsat and comparatively large for Duku. It is greenish in color and bitter in taste. The fruit pulp is white, translucent in nature, consisting of 5-6 segments called aril and seeds are present inside this fleshy part. *Lansium domesticum* fruit pulp is the edible portion and the peel and seeds are not edible for consumption but have several medicinal properties. The leaves of *Lansium domesticum* are odd pinnately compound with an alternate arrangement of leaflets. The *Lansium domesticum* var. *pubescens* leaf is elliptical, long and the *Lansium domesticum* var. *domesticum* is ovate and larger in appearance.

The fruiting season of the plant varies in accordance with the habitat and climate. In Kerala, according to the present study, the flowering season of the plants started in late January–March and the fruiting season in late June–August. The flowers appeared as yellowish-white in color and are borne in clusters on tree branches. The tree attains an average height of up to 15 ft.

The present study focused on antiurolithiatic activity of the fruit pulp of two varieties of *Lansium domesticum* Correa. The word "Urolithiasis" describes: "urolith" comes from the Greek word, "Urone" and "Lithos" which refer to "urine" and "stones" respectively. The word "anti" means "against" or "opposing". Altogether, the word antiurolithiatic referred to against "urine stones" specifically "kidney stones". The process of formation of stones in the kidney, bladder and urinary tract (Urethra) is known as "Urolithiasis". It is also known as "Nephrolithiasis". The word "nephro" also comes from the Greek word, which means "kidney". The substances that help to prevent or fight against kidney stones are called antiurolithiatic agents. The focus of the present investigation is to analyze the antiurolithiatic activity of the *Lansium domesticum* fruit pulp samples. As the fruits are

traditionally consumed for their medicinal properties: their potential as a natural antiurolithiatic agent, to inhibit the kidney stones or the potential to dissolve or break down the formation of stones in the kidney. The antiurolithiatic activity is reported in three species in the Meliaceae family, namely: *Azadirachta indica* (*Melia azadirachta*), *Melia dubia* and *Urena sinuate*. The present study is from the species *Lansium domesticum*.

## Material and Methods

**Collection of plant materials:** The plant materials used for the study include the ripe fruits (mature) of *Lansium domesticum* var. *domesticum* (LDD) and *Lansium domesticum* var. *pubescens* (LDP) collected during the months of June-August from home gardens near Kottayam and Ernakulam districts respectively in Kerala.

### Preparation of *Lansium domesticum* fruit pulp extract:

The collected fruit samples of *Lansium domesticum*- LDD and LDP were washed in running tap water and unwanted parts including peels and seeds, were removed using a sterilized blade. Then the fleshy parts were shade-dried for more than six weeks for the complete drying process. After the drying process, the dry fruit pulp samples were grinded, turned into fine powder and stored in labeled sample bottles for successive extraction.



Fig. 1: LDD (*Lansium domesticum* var. *domesticum*)

**Cold extraction:** 30 g of dried powder of the *Lansium domesticum* fruit pulp samples were extracted with 120 mL of methanol. These samples were placed in a gyratory shaker at 120rpm for 48 hours. The two extracts were filtered using Whatmann no.1 filter paper, dried to attain an even weight. The yield and quality of the extract were recorded. The final residues were stored in a refrigerator at 4 °C till further use. The samples were tested in the Phytochemistry Laboratory, PG and Research Department of Botany, University College, Palayam, Thiruvananthapuram, Kerala, India.

### Bioactivity Study-Antiurolithiatic assay

**Protocol:** Antiurolithiatic activity of the two fruit pulp samples of *Lansium domesticum* (LDD – *Lansium domesticum* var. *domesticum* and LDP – *Lansium domesticum* var. *pubescens*) was investigated with the help

of calcium oxalate dissolution assay by titrimetric method. For the analysis, the standard protocol by Atodariya et al<sup>1</sup> was employed. Dried mature fruit pulp samples of the *Lansium domesticum* fruit in powdered form were used for the assays. Cystone and potassium citrate were used as the standards.



Fig. 2: LDP (*Lansium domesticum* var. *pubescens*)

### Preparation of Experimental Calcium Oxalate (CaOx):

Prepare a solution of calcium chloride dihydrate in distilled water. Dissolve sodium oxalate in 10 mL of 2N sulphuric acid (H<sub>2</sub>SO<sub>4</sub>). Mix equal concentrations of the above solutions in distilled water to form a precipitate of calcium oxalate. Purify the precipitate using ammonia solution to neutralize residual sulphuric acid. Wash the precipitate thoroughly with distilled water. Dry the purified calcium oxalate at 60 °C for 4 hours.

### Preparation of Semi-Permeable Membranes from Farm Eggs:

Place eggs in 2M hydrochloric acid (HCl) overnight to decalcify (Fig. 3) and remove the shell (Fig. 4). Carefully remove the egg contents from the decalcified membranes (Fig. 5). Wash the membranes thoroughly with distilled water. Immerse the membranes briefly in ammonia solution to neutralize. Rinse the membranes again with distilled water and store them for use.

**Experimental Setup:** Weigh 1 mg of experimental calcium oxalate and 10 mg of the *Lansium domesticum* fruit extract. Place the mixture inside the semi-permeable membrane and seal it. Prepare the following experimental groups:

**Group 1:** Blank (1 mg calcium oxalate only).

**Group 2:** Positive control (1 mg calcium oxalate + 10 mg Cystone).

**Group 3:** Positive control (1 mg calcium oxalate + 10 mg Potassium Citrate).

**Group 4:** Experimental (1 mg calcium oxalate + 10 mg methanolic extract of LDD).

**Group 5:** Experimental (1 mg calcium oxalate + 10 mg methanolic extract of LDP).

Suspend each membrane in a separate conical flask containing 100 mL of 0.1 M Tris buffer (Fig. 6).



Fig. 3: Decalcification process of eggshell in 2M HCl



Fig. 4: Decalcified eggs



Fig. 5: Egg membrane removed after decalcification of eggs

**Incubation:** Place the flasks in an incubator preheated to 37 °C. Incubate for 2 hours to allow calcium oxalate dissolution.

**Dissolution Analysis:** Carefully remove the contents of the membranes into individual 250 ml beakers. Add 2 mL of 1N sulphuric acid ( $H_2SO_4$ ) to each beaker. Perform titration using 0.9494 N potassium permanganate ( $KMnO_4$ ) until a light pink endpoint is observed. Fig. 7 represents the semipermeable membrane after titration with potassium

permanganate (0.9494 N - Normality of potassium permanganate).



Fig. 6: Egg membrane along with the contents suspended in Tris buffer

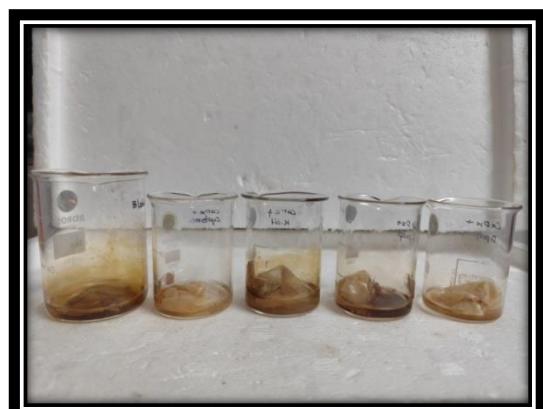


Fig. 7: Semipermeable membrane after being titrated with potassium permanganate (0.9494 N)

#### Calculation of Calcium Oxalate Dissolution:

1. Measure the amount of undissolved calcium oxalate in each group.
2. Subtract the undissolved amount from the initial 1 mg of calcium oxalate to determine the quantity dissolved.
3. Compare dissolution rates across groups to evaluate the efficacy of *Lansium domesticum* fruit pulp extracts (LDD and LDP).

**Statistical analysis:** Data represent mean values of four replicates repeated thrice. Mean values followed by the same letter in the superscript in a row do not differ significantly based on ANOVA at  $p \leq 0.01$ .

#### Results and Discussion

The dried fruit pulp of two varieties of *Lansium domesticum* (LDD and LDP) plant materials was extracted using methanol as a solvent. The present investigation aimed to study the antiulcerogenic potential of the fruit pulp samples. The analysis was done using the standard protocol.

**Antiulcerogenic activity- A bioactivity study:** The antiulcerogenic activity of fruit pulp samples of LDD and

LDP (*Lansium domesticum* var. *domesticum* and *Lansium domesticum* var. *pubescens* respectively) was carried out using the most polar solvent, methanol. For plant extraction, methanol is one of the organic solvents used frequently<sup>15</sup>. For the present study, the titrimetric method is selected to check the *Lansium domesticum* antiurolithiatic activity. There are two methods: the titrimetric and the turbidity method. The titrimetric method is mainly aimed at dissolving the already formed stones in the kidney or in the tract while the other method, the turbidity assay, is used to inhibit the pre-arranged crystals<sup>6</sup>. Here, the titrimetric method is selected for the present examination of the fruit pulp samples.

Cystone and potassium citrate are two standard drugs used for comparison with the methanolic fruit pulp samples. The results are tabulated in table 1. Fig. 8 represents the antiurolithiatic activity of the standards and the samples of *Lansium domesticum* Correa. The two varieties of *Lansium domesticum* -LDD and LDP exhibited significant levels of antiurolithiatic activity when compared to the standard drugs Cystone and potassium citrate. LDD fruit pulp of *Lansium*

*domesticum* constitutes about  $0.9997 \pm 1.1 \times 10^{-4}$  mg /g and LDP,  $0.9994 \pm 0.00$  mg/g. Both the fruit samples of *Lansium domesticum* have high milligram (mg) of calcium oxalate dissolution when compared with the standards. So, the methanolic extract of *Lansium domesticum* fruit pulp can be used as a natural drug for treating urogenital infections and disorders, as it represents a plant-based component with minimal side effects.

Here are some illustrations of the results:

- **Calcium oxalate (Blank):** The lowest absorbance indicates the formation of calcium oxalate crystals without any inhibitors.
- **Potassium citrate (Positive control):** The highest absorbance indicates the strongest dissolution or inhibition of calcium oxalate crystals. This confirms potassium citrate as an effective anti-urolithiatic agent and sets the standard for maximum activity.
- **Cystone (Positive control):** The absorbance is higher than the blank but lower than potassium citrate. This indicates moderate anti-urolithiatic activity, consistent with its known medicinal properties.

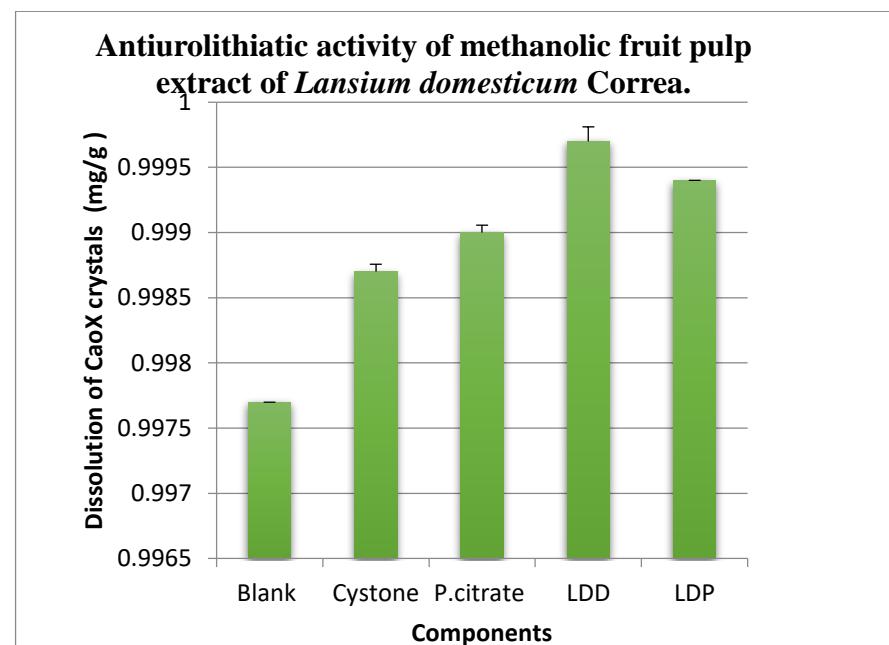


Fig. 8: Graphical representation of antiurolithiatic activity of *Lansium domesticum* Correa

Table 1  
Antiurolithiatic activity of methanolic fruit pulp extract of *Lansium domesticum* Correa.

S.N.	Components		Initial mass of calcium oxalate (mg)	Calcium oxalate dissolved (mg/g)
1	Blank (Calcium oxalate crystals)		1	0.9977
2	Standard Drug	Cystone	1	$0.9987^b \pm 5.8 \times 10^{-5}$
		Potassium citrate	1	$0.9990^a \pm 5.8 \times 10^{-5}$
3	<i>Lansium domesticum</i> fruit pulp samples	LDD	1	$0.9997^a \pm 1.1 \times 10^{-4}$
		LDP	1	$0.9994^b \pm 0.00$

[LDD-*Lansium domesticum* var. *domesticum*; LDP-*Lansium domesticum* var. *pubescens*; Data were shown as mean $\pm$ SD. Mean values followed by the same letter in the superscript in a row do not differ significantly based on ANOVA at  $p \leq 0.01$ ]

- **Sample 1-LDD** (*Lansium domesticum* var. *domesticum*): Showed significant anti-urolithiatic activity, with absorbance as high as that of potassium citrate. This suggests that LDD pulp has promising potential for inhibiting or dissolving calcium oxalate crystals.
- **Sample 2- LDP** (*Lansium domesticum* var. *pubescens*): Demonstrates notable anti-urolithiatic activity, comparable to LDD. This indicates that the LDP sample has the potential to be used as an alternative natural remedy for preventing or treating kidney stones.

The kidney stones, otherwise called urinary stones, are the third most frequent and very severe pain disease after causing infection in the prostate as well as in the urinary tract<sup>9</sup>. According to several literature reports, cystone is said to be a mineral plant compound and plays a very crucial role in the movement of stones through the urinary tract, thereby helping to remove the stone from the human body<sup>4,5,7,10</sup>. Hydroxyproline is a non-essential amino acid i.e. our body can produce this amino acid by its own. It constitutes approximately 13% of mammalian collagen and plays a very dominant role in its function and stability of collagen.

The gathering, settling and overabundance of calcium hydroxyproline and oxalic acid in urine are blocked by cystone and also disintegrate mucin. Following this, cystone which stops the mixing of crystal particles in our body, will assist in the expulsion of tiny stones through urine<sup>4,5,10</sup>. Actually, Cystone is an Ayurveda herbal formulation containing minerals and herbal extract. Cystone is widely used clinically for several urogenital infections and also for numerous renal disorders<sup>4,12</sup>. Potassium citrate, the standard drug, is used as a therapeutic agent acting as a metabolic alkalinizer and also as calculi dissolution agent. This is mainly used for the treatment of kidney stones and the condition is known as renal tubular acidosis.

Potassium citrate is used extensively because this can reduce the calcium stone formation in urine that results in the formation of kidney stones. The presence of citrate will act as a lipoprotective element. When potassium citrate reaches our body, it is converted into potassium ion (K + ion) which plays a crucial role in nerve impulse transmission and contraction of muscles. Citrate ions play a major role in Kreb cycle for energy production. In addition to this, citrate will bind to calcium and will stop it from forming insoluble stones, leading to the kidney stones and making the urine more alkaline to prevent the stone formation in the kidney. Plants are always sources of medicine or drugs for the treatment of different diseases or disorders and are said to be less toxic, safe and cost-effective<sup>3</sup>.

The earlier study about the plant *Lansium domesticum*<sup>14</sup> reported the presence of several phytoconstituents especially, phenols, terpenoids and saponins<sup>14</sup>. According to Velu et al<sup>16</sup>, the presence of high content of terpenoids in aqueous extract of *Ananas nanus* has been suggested as a reason for its good antiurolithiatic property which will prevent the formation of calcium oxalate crystals<sup>16</sup>.

## Conclusion

The fruit pulp of two varieties of *Lansium domesticum* (LDD and LDP) can be a natural medicine for various conditions of urolithiasis. Compared to both the standards, potassium citrate showed more effectiveness than Cystone. Both LDD and LDP methanolic fruit pulp samples of *Lansium domesticum* exhibited significant anti-urolithiatic activity, compared to potassium citrate, suggesting their promising role as natural alternatives for urolithiasis. In this, LDD methanolic fruit pulp extract had high potential to inhibit or dissolve calcium oxalate crystals, thereby reducing the formation of kidney stones.

The presence of bioactive compounds including saponins, phenols and terpenoids, in fruit pulp samples of *Lansium domesticum* may have the ability to inhibit calcium oxalate crystallization. The present investigation about the plant concluded that this extract can be used as a natural drug for reducing urinary stones and in the treatment of several urogenital infections.

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