

## Evaluation of Anti-Urolithiatic Activity of Sandoricum Koetjape

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**ABSTRACT:**Urolithiasis, commonly known as kidney stone disease, is characterized by the formation of renal calculi through a complex series of physicochemical events. This study examines the antiurolithiatic properties of Sandoricum koetjape bark extract, investigating its potential to dissolve calcium oxalate and phosphate, two primary constituents of renal stones. Experimental kidney stones were synthesized, and the efficacy of the aqueous extract of Santol bark was analyzed through quantitative dissolution methods. Results indicated a 50.66% dissolution rate for calcium oxalate and a 12% dissolution for calcium phosphate, suggesting that the Santol bark extract possesses considerable antiurolithiatic activity. This study reaffirms the need for further investigation into the clinical applications of the extract for managing urolithiasis. The research acknowledges the support and guidance provided by Dr. R.V. Celestin Baboo in the culmination of this pivotal study.

### KEYWORDS:

Sandoricum koetjape,  
Urolithiasis, Titrimetry, Colorimetry, Extracts

### I. INTRODUCTION

Urolithiasis is a Greek word that comes from two words "ouros" meaning urine and "lithos" meaning stone. They also refer to renal calculi or renal crystal as the process of kidney stone formation. Stones' development is a difficult process through the renal tubules because of a series of physicochemical events like great saturation, growth, nucleation, retention and aggregation. Salt with minerals and other substances found in urine is the primary constituent of kidney stones. They come over a period of days to months. Regardless of their size, some are as small as sand particles while others are like pearls or as big as a golf ball. Kidney stones in some instances appear in such places where the pelvis occupies the entirety of the excretory duct. Their outer surface could be regular (smooth), prickly

one or could have some indentations. Kidney stones are often brown, yellowish, or pinkish. The pain caused from pushing out kidney stones depends on their size. Tiny kidney stones can migrate silently with the urine. Big kidney stones are likely to cause some form of pain during their passage through the urinary tract; it may be very painful if the stone has sharp edges as it passes through the urinary passages.

This disease is harmful for all sexes; however, males have higher chances of getting infected and these days even younger females can suffer from this illness. According to the investigations, 80% of all renal stones consist of calcium oxalate or calcium phosphate. They include struvite that is magnesium ammonium phosphate-10%. Nine per cent uric acid; one percent cystine or ammonium acid urate in medication-related kidney stones. There is a belief that an increased presence of calcium, oxalate, and uric acid in the urine lead to increased formation of stones in the urinary system and decreased levels of urinary salts like citrates. The urine has two main factors that are known to inhibit the formation of stones hence when levels of these factors drops or there is lack of these factors leads to stone formation. People between 20 and 49 years of both sexes are prone to urolithiasis. Doctors often use surgical techniques and high-powered lasers to remove stones from the body. But high costs and frequent returns make this method unpopular with many people. In the absence of preventive measures, the recurrence rate for this problem ranges from one in ten within the first year; one in three in five years; half of all individuals after ten years.

### II. MATERIALS AND METHODS

#### Plant material:

Sandoricum koetjape tree bark was collected from an organic farm located in Beeranchira, Tirur, Malappuram district. The plant was identified and authenticated by botanist A.K

Pradeep, who is an Assistant Professor in the Department of Botany at Calicut University, Kerala.

#### Drugs and chemicals:

The Cystone herbal product was obtained from Himalaya Drug Company as used in this study. All reagents of solvent and chemical nature that were used during the investigation were of high quality and could be found at local markets.

#### Preparation of plant extract:

The dried bark of Sandoricum koetjape was ground to produce coarse powder. The decoction was used to extract water and lasted for 4 days. The extract was filtered and concentrated after 4 days to give a concentrated extract. The percentage yield of the extract was calculated using the weight of the herb and the weight of the extract obtained. After that, the extract was kept in an airtight container for further research.

#### Quantitative analysis:

We analysed the bark of Sandoricum koetjape using quantitative methods. The extract was subjected to quantitative analysis to determine two phytochemical constituents which are flavonoids and phenols.

#### Determination of antiurolithiatic activity:

To create experimental kidney stones (made up of calcium oxalate and calcium phosphate) by homogenous precipitation:

Equal volumes of calcium chloride dihydrate (AR) solution in oncotic distilled water medium and sodium oxalate (AR) substrate in 10 millilitres 2N H<sub>2</sub>SO<sub>4</sub> were combined in a beaker with extra distilled water. The produced substance was calcium oxalate. A beaker with just enough amount of distilled water contained equal quantities by mass of Calcium chloride dihydrate (AR) and Disodium hydrogen phosphate (AR) that had been pre-dissolved. To this in 10 ml of 2N H<sub>2</sub>SO<sub>4</sub> solution was added Disodium hydrogen phosphate, then finally made up to volume with distilled water. What remained after filtering off the solution formed was calcium phosphate. They were subsequently washed with Ammonia solution, rinsed thoroughly with distilled water, and dried at 60°C for 4 hours before weighing.

#### Creating a semi-permeable membrane out of eggs:

Eggs' semi-permeable membrane is located amid the outer calcified shell and the inner contents like albumin & yolk. This ended in total

decalcification after the eggs were put into 2M HCl for a whole night for the purpose of eggshell chemical removal. The eggs were then rinsed using distilled water and a small hole was carefully put on top to scoop out the contents before the egg membrane would be effectively cleaned by thorough washing using distilled water; then it was soaked in ammonia solution with moisture on it thereafter rinsing using distilled water followed by its preservation inside refrigerator under PH range 7 – 7.4.

#### Estimating Calcium oxalate through Titrimetry:

Exact amounts of calcium oxalate, such as 1mg, and an extract/compound/standard, such as 10 or 20mg, were weighed and placed together in a semi-permeable membrane by suturing it. The whole thing was then suspended within a conical flask that contained 0.1 M TRIS buffer and 100 ml of liquid. A single group participated under no treatment (carrying only 1mg of calcium oxalate). All the groups conical flasks were put into an incubator that had been preheated to 37°C for two hours then left for about 7-8 hours; the semi-permeable membrane contents were transferred into test tubes from each group. Then 2 ml of 1 N sulfuric acid were added and titration was done until a light pink colour end points with 0.9494N KMnO<sub>4</sub> was achieved. One millilitre of 0.9494 N KMnO<sub>4</sub> = 0.1898 mg Calcium.

$$\text{The quantity of calcium oxalate} = \frac{(S \times 0.18981)}{w}$$

where, S = amount of sample being titrated (ml)  
w = the weight of sample being used

#### Estimation of Calcium phosphate by colorimetry:

To prepare molybdic-sulphuric acid reagent: Mix 5% w/v of sodium molybdate solution (28 g Na<sub>2</sub>MoO<sub>4</sub>), 13 mL conc. H<sub>2</sub>SO<sub>4</sub> in 80 mL distilled water and make volume up to 100 ml distilled water.

#### Preparation of molybdic-sulphuric acid reagent:

Molybdic-sulphuric acid reagent was prepared by 5% w/v of sodium molybdate solution, 13ml of conc. H<sub>2</sub>SO<sub>4</sub> in 80ml of distilled water. Finally, the volume was adjusted to 100ml with distilled water.

Preparation of reducing solution:

Mix 1 gram of p-phenylene diamine with 100 millimeters of a 3% w/v solution of sodium meta-bisulfite. Following this, remove the contents from a semi-permeable membrane and transfer them to a test tube. Add IN sulfuric acid (2 milliliters), Molybdic-sulfuric acid reagent (2.5 milliliters) and reducing solution (1 milliliter) into this test tube. Thus, the volume should be adjusted to 10 milliliters of distilled water to make 10 milliliters. I prepared standard dilutions with varying concentrations of 200, 400, 600, 800 and 1000 µg/ml of calcium phosphate. To each

standard dilution I added 2.5 milliliters of Molybdic-sulfuric acid reagent, 1 milliliter of reducing solution, and after that 10 milliliters of distilled water were added to make up their volume. I measured the optical density of both standard dilutions and study groups at 620 nm in a colorimeter. Further, I identified the undissolved calcium phosphate from the standard calibration curve by extrapolation.

The initial quantity was subtracted from the total amount of calcium oxalate/phosphate that was insoluble to ascertain the quantity that the test substance(s) could dissolve.

$$\% \text{ Dissolution} = \frac{\text{Amount of sample taken} - \text{Amount undissolved}}{\text{Amount of sample taken}} \times 100$$

Amount of sample taken



Fig 1: Decalcification of egg membrane

Fig 2: Packing of membrane



Fig 3: Titration with 0.9494 N KMnO4

**III. RESULT AND DISCUSSION:**

**Anti-urolithiatic Activity:**

Standard calibration of calcium phosphate:

Concentration (µg/mg) v/s absorbance (nm)

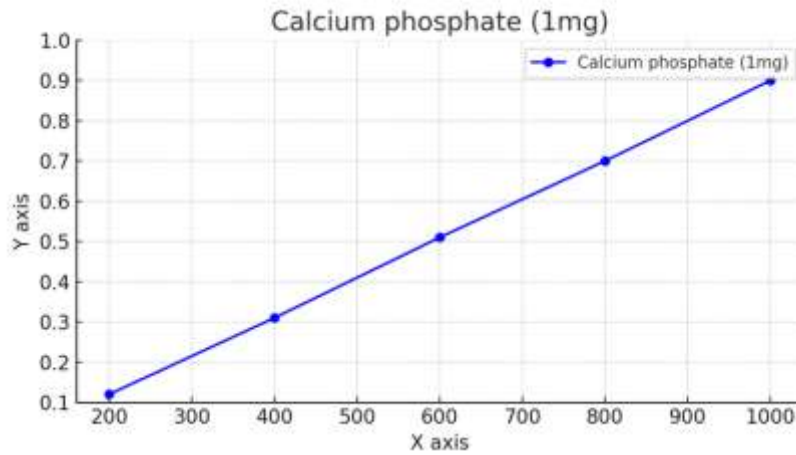


Fig 4: Standard calibration of calcium phosphate (1mg/ml)

Extract	Volume of 0.9N KMnO4(ml)	Wt of Calcium estimated(ml)	Wt of Calcium reduced(ml)	% Dissolution
Standard	28ml	0.5314	0.4686	46.86%
Aqueous	26ml	0.4934	0.5066	50.66%

Table 1: Dissolution of Calcium oxalate of Sandoricum koetjape

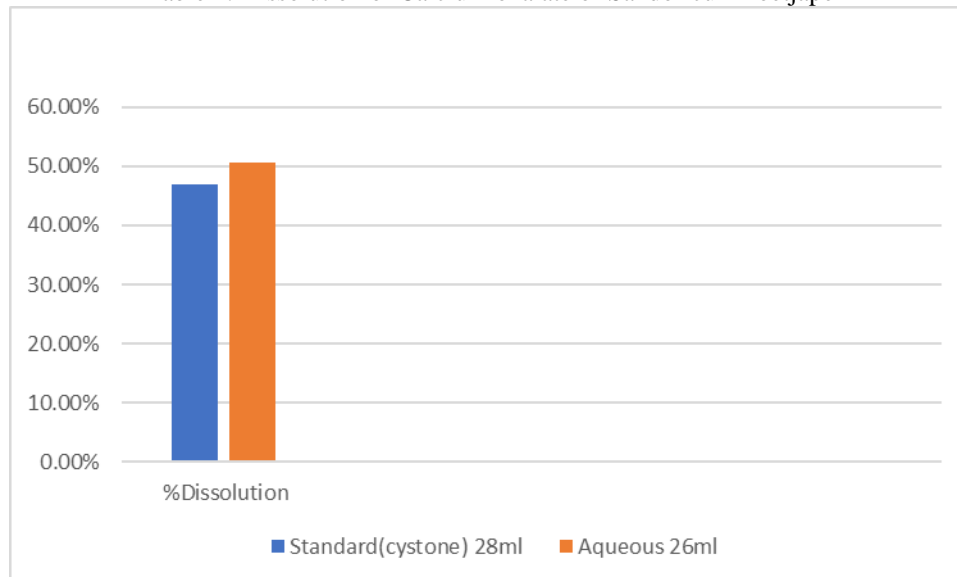


Fig 5: Graphical representation of Dissolution of Calcium oxalate of Sandoricum koetjape

Extract	Absorbance	Wt of Calcium estimated(ml)	Wt of Calcium reduced(ml)	% Dissolution
Standard	0.818	0.86	0.14	14%
Aqueous	0.872	0.88	0.12	12%

Table 2: Dissolution of Calcium Phosphate of Sandoricum koetjape

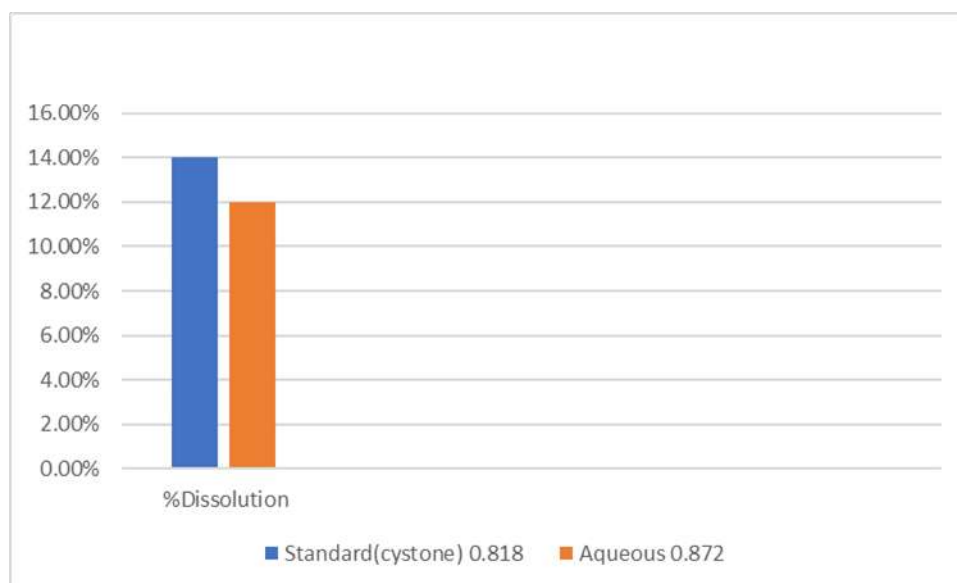


Fig 6: Graphical representation of Dissolution of Calcium phosphate of Sandoricum koetjape

#### IV. CONCLUSION:

This study sought to ascertain whether the bark extract obtained from *Sandoricum koetjape* had any antiurolithiatic capabilities. Our results displayed strong indications that this extract might be a workable therapy in the fight against urolithiasis. It implies that further studies on this matter should be conducted to enable us to understand its significance including possible remedies for urolithiasis. It would be advisable to examine the practical uses of this extract in clinical contexts more closely through the continuation of research.

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