

## Investigation Of *In-Vitro* Antiurolithiatic Activity Of *Passiflora Foetida* And *Stachytarpheta Indica* On Experimentally Prepared Calcium Oxalate Crystals.

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**Abstract:**-Urolithiasis or the formation of urinary stones is a major non communicable disease that affects the urinary system. Among the different types of renal stones  $\text{CaC}_2\text{O}_4$  are the overwhelming. Antiurolithiatic activity is the inhibition of formation of urinary stones. There is a high demand for herbal medicines to treat urolithiasis due to unavailability of a successful drug devoid of complications. *Passiflora foetida* (Udhalu) & *Stachytarpheta indica* (Balunakuta) plants are used in the treatment of kidney stones in traditional medicine. This study was aimed to investigate *in-vitro* antiurolithiatic activity of these plants on experimentally prepared  $\text{CaC}_2\text{O}_4$  crystals. The crude plant extracts were prepared by boiling the dried plant material in water followed by evaporation of the solvents via rotary evaporation. A concentration series of aqueous extract of the plants were prepared with negative and positive (cystone tablets) controls. Antiurolithiatic activity was evaluated using the UV/visible spectrophotometry by measuring optical density of each samples having different concentrations of the plant extracts at 620 nm. The mean absorbance of *P.foetida* extract at 0.125 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 1 mg/mL, 2 mg/mL, 4 mg/mL concentrations were 0.045, 0.062, 0.105, 0.216, 0.1393, 0.623 respectively. The mean absorbance of *S. indica* extract at 0.125 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 1 mg/mL, 2 mg/mL, 4 mg/mL concentrations were 0.180, 0.260, 0.201, 0.959, 0.862, 0.492 respectively. There is a concentration dependent increase in the mean absorbance for the samples. The results reveal that both extracts exhibit antiurolithiatic activity and the highest activity is exhibited by *S. indica*.

**Keywords:** Urolithiasis, calcium oxalate, *Passiflora foetida*, *Stachytarpheta indica*, antiurolithiatic activity

### Introduction:

Urolithiasis is one of the major diseases in the world. Urolithiasis means the formation of stones in the urinary system i.e. in the kidney, ureter, and urinary bladder or in the urethra. 'Urolithiasis' = ouron (urine) and lithos (stone). Globally, the prevalence and recurrence rates of urolithiasis disease are increasing. This affects about 12% of the world population at some stage in their lifetime. There are mainly five types of kidney stones, based on the mineral composition and the pathogenesis as

Calcium stones, Struvite or Magnesium Ammonium Phosphate stones, Uric acid stones or Urate, Cystine stones and drug-induced stones

There are two types of calcium stones namely calcium oxalate and calcium phosphate. Calcium stones are overwhelming renal stones containing about 80% of every urinary stones. Calcium oxalate is found in most of kidney stones and exists as  $\text{CaOx}$  monohydrate (COM, termed as mineral names: whewellite,  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ ), and  $\text{CaOx}$  dihydrate (COD, weddellite,  $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ ), or as a combination of both which accounts for greater than 60%. COM is the most thermodynamically stable form of stone and more frequently observed than COD in clinical stones. Stone development begins with the arrangement of crystals in supersaturated urine which at that point stick to the urothelium, subsequently making the nidus ensuing stone development. Crystals in urine stick together to arrange a small hard mass of

stone called crystal growth. This crystal growth occurs through aggregation of secondary nucleation of crystal on the matrix – coated surface. This crystal formation is a very slow process and it takes a long time to obstruct the renal tubules.

Antiuro lithiatic means preventing the formation of urine stones. To prevent the formation of urine stones, anti urolithiatic drugs have been discovered. Most of the synthetic anti urolithiatic drugs are having adverse effects. The traditional herbal medication is the basis of the modern complementary medical therapy. Nowadays, antiuro lithiatic drugs are not generally used by physicians. Instead they just treat for the pain until the stone passes out by itself. Standard drugs are not using due to its side effects and less effectiveness. Commonly using drugs are Allopurinol, Citrate, Cystone and thiazide diuretics

In this study, two plant species which are used as herbal remedies to treat urolithiatic patients in Sri Lanka have been investigated for antiuro lithiatic activity.

The main objectives of this study are to investigate in-vitro antiuro lithiatic activity of the plants *Passiflora foetida* and *Stachytarpheta indica* on experimentally prepared calcium oxalate crystals.

#### Methodology

The study was carried out to evaluate in-vitro antiuro lithiatic activity of selected medicinal plants; *Passiflora foetida* and *Stachytarpheta indica* on experimentally designed calcium oxalate crystals as a laboratory based experimental study in natural urine medium. The study was carried out in the laboratories of the Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University Ratmalana.

The *Passiflora foetida* and *Stachtarpheta indica* plants including its flowers and fruits were collected at daytime from the local areas of Ratnapura (6.70560N, 80.38470E) and Galle (6.05350N, 80.22100E) districts.

The specimens were identified by the National herbarium, Royal botanical garden, Peradeniya, Sri Lanka. The collected plants were washed with running water and air dried for seven days to remove the moisture and grinded as a coarse powder. Then they were labelled and stored in air tight bottles.

Phytochemical screening was carried out for both plants to detect the presence of particular compounds using standard procedures

The crude plant extracts were prepared by using standard methods with minor modifications. From the stored plant powder, 50 g were taken from each, soaked in 750 mL of distilled water and boiled for 2 hours separately. The two solution mixtures were filtered through a filter paper. The filtrates were subjected to rotary evaporation to remove the water and solid crystals of the crude extract were obtained. The crystals were kept in separate air tight bottles and stored under 2-4°C.

A concentration series of plant extract was prepared using the doubling method. First, 40mg of plant extract was taken and dissolved in 10.0 mL of distilled water (10 mL). This process was repeated to make a successive dilution series as 4 mg/mL, 2 mg/mL, 1 mg/mL, 5 mg/mL, 0.25 mg/mL, 0.125 mg/mL.

A volume of 1.0 mL of the plant extracts having different concentrations were added to six test tubes each containing 2 mL of healthy urine. Into those test tubes, 50  $\mu$ L of  $\text{Na}_2\text{C}_2\text{O}_4$  solution having a concentration of 0.0005 mg/dm<sup>-3</sup> and 50  $\mu$ L of  $\text{CaCl}_2$  solution having a concentration of 0.003 mg/dm<sup>-3</sup> were added. For this mixture, Tris buffer with the concentration of 0.05 mol/L was added drop wise until the pH is adjusted to 6.5 and 10  $\mu$ L of dil. Sulphuric was added to facilitate the formation of crystals.

For the positive control, two Himalaya Cystone tablets were crushed and 40 mg was taken and dissolved in 10.0 mL of distilled water. A volume of 1.0 mL of this solution was added to test tubes containing 2.0 mL of healthy urine along with the 0.05 mol/L of Tris buffer solution to

maintain the pH 6.5. For this mixture, 50  $\mu\text{L}$  of  $\text{Na}_2\text{C}_2\text{O}_4$  with a concentration of  $0.0005 \text{ mg/dm}^{-3}$  and 50  $\mu\text{L}$  of  $\text{CaCl}_2$  with a concentration of  $0.003 \text{ mg/dm}^{-3}$  were added. Into the final mixture, 10  $\mu\text{L}$  of dil. Sulphuric acid was added to facilitate the formation of crystals.

For the negative control, 1.0 mL of distilled water was added instead of plant extract and the rest of the chemicals and conditions were not changed.

The samples were incubated at  $37^\circ\text{C}$  for 2 hours. And OD of the formed crystals were measured using a UV-Visible spectrophotometer at 620 nm wavelength.

### Results and discussion

A graph was plotted for the absorbance values obtained for different concentrations of the aqueous extracts of *P.foetida*, *S. indica* and positive control as a comparison among each other (Figure 1).

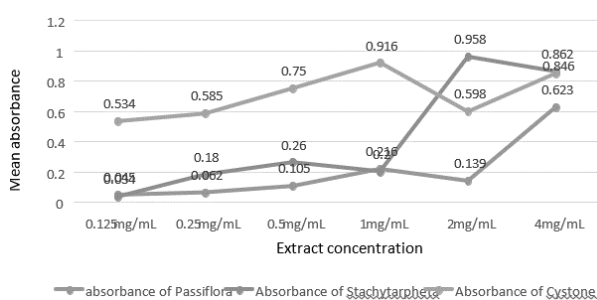


Figure 1 - Graph of comparison of mean absorbance of *P.foetida*, *S.indica* and positive control

As shown in the graph, the mean absorbances of the two plant extracts and the positive control have shown an increasing pattern with the increase of concentrations from the lower concentration towards the higher concentrations with slight fluctuations (figure 1)

The graph depicts that there is a concentration dependent increase in the mean absorbance for both the *P foetida* and *S indica* extracts except for slight fluctuations at the concentrations 2mg/mL and 1 mg/mL respectively.

According to the graphical representation, aqueous extract of *S.indica* has shown higher

absorbance values with compared to *P.foetida* aqueous extract. The highest absorbance value for *S.indica* has shown at its 2mg/mL concentration which is 0.958 while the highest absorbance value for *P.foetida* is 0.623 which has been shown at its 4mg/mL concentration. The highest absorbance value of *S.indica* is higher than that of the positive control cystone which is 0.916 shown at its 1mg/mL concentration.

Crystal formation and presence of those free particles in the urine do not necessarily lead to stone formation, but crystal retention is essential for the formation of stones so that interference with crystal growth and aggregation can be applied as a therapeutic strategy for the prevention of recurrent stone formation. Therefore, the main focus of the study was to investigate the inhibitory activity of the two plants *Passiflora foetida* and *Stachytarpheta indica* on crystal aggregation.

Optical density of a solution is affected by many characteristics such as its particle number, particle size and shape. Particle number and particle size are the main factors which affect the OD. The particles in a suspension will scatter light. (Thus preventing it from reaching the light detector) Therefore, the turbidity changes and OD readings can be taken as evidences for the changes in the number of particles of a solution.

If there is an effect of the plant extracts to inhibit the formation of calcium oxalate stones, aggregation of crystal particles in the test samples should be prevented. This principle has been used to determine the effect of the plant extracts on the inhibition of kidney stone formation by the spectrophotometric method. According to the theory, if the particles are aggregated, the turbidity of the solution should be reduced.

In other words, in the spectrophotometric analysis, the increase in the absorbance at 620 nm with increasing concentrations of the extracts depicts an increase in the number of

crystals in the samples preventing the crystal growth and aggregation. Inhibition of crystal

growth and aggregation can inhibit the stone formation. Because the dispersed crystals can be eliminated easily through urinary tract.

In this study, the antiurolithiatic property was investigated *in-vitro* with the mean absorbance results obtained by the spectrophotometer. They show a concentration dependent increase in the mean absorbance values with slight fluctuations which can be taken as a proof to demonstrate the inhibition of particle aggregation with the increase in the concentration of the plant extracts in natural urine. In *P.foetida*, the extract in the concentration 4 mg/mL has shown a mean absorbance value of 0.623 which is higher than that of the negative control which is 0.492 and plant *S.indica* has shown its highest mean absorbance value of 0.861 at 4 mg/mL concentration.

### Conclusion

The present study reveals that aqueous extracts of *Passiflora foetida* and *Stachytarpheta indica* exhibit antiurolithiatic activity on experimentally prepared calcium oxalate stones in natural urine medium. They may be beneficial in the treatment of urolithiasis due to calcium oxalate kidney stones. Aqueous extract of *Stachytarpheta indica* exhibits the highest antiurolithiatic activity following the standard drug cystone (Himalaya drug company, India) and aqueous extract of *Passiflora foetida*.

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