In-vitro Anti-urolithiatic Evaluation of Methanolic Extract of *Salacia reticulata*

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Abstract

Urolithiasis can be defined as the process of forming stones in the kidney, bladder and urinary tract. It is found that 1 in 20 people develop kidney stones at some stage in their life time. Treatment includes relief of pain, hydration and antibiotics. Although many advanced allopathic medicines are available in the modern world, most people have been using the herbal medical treatments owing to its less side effects and low cost. *Salacia reticulata* is one of the herbs used to cure the urinary stones in the human body. This research was conducted to evaluate *In-vitro* anti-urolithiatic activity of *Salacia reticulata* stem extract on experimentally prepared calcium oxalate stones. Methanolic plant extract was prepared by using the soxhlet apparatus. Artificial stones of calcium oxalate were prepared by homogenous precipitation method. Semi-permeable membrane bags were prepared from chicken eggs. One sample of negative control, four samples of positive control with 10, 20, 30, 40 mg of standard drug cystone and four other samples with 10, 20, 30, 40 mg of methanolic extract of *Salacia reticulata* were prepared separately. The prepared samples were incubated for 7 hours at 37 ^{o}C in pH 7.4. Titrimetric method was followed in order to determine the anti-urolithiatic activity. The mean (±SD) dissolution percentage of extract of *Salacia reticulata* on experimentally prepared calcium oxalate for 10, 20, 30 and 40mg were 34.8, 38.6, 40.4 and 45.0% respectively. The dissolution percentage significantly increased with concentration. Dissolution percentages of cystone on artificial calcium oxalate stones for 10, 20, 30, 40 mg were 64.8, 70.6, 75.8 and 79.6% respectively. Both the methanolic extract of *S. reticulata* and the standard drug has a positive correlation with its concentration. The results of this study have confirmed the long history of the use of *Salacia reticulata* in traditional medicine for the treatment of Urolithiasis.

Keywords: *Salacia reticulata*, Anti-urolithiatic, Cystone, Methanolic extract.

Introduction

Kidney stone can be described as a formation of crystal within the kidneys, affecting 12% of the world population. Urolithiasis is one of the most common diseases of the urinary tract that has been afflicting human kind since ancient times[1]. Recurrent stone formation is probably the most important problem in the after-care patients who have undergone operations for renal and ureteric calculi.

The most common type of kidney stone is calcium oxalate formed at Randall's plaque on the renal papillary surfaces. 50% of these stones are pure calcium oxalate, 5% is calcium phosphate and 45% is a mixture of both. Magnesium phosphate represents 15%-20%, cystine represents 1% and uric acid represents 10% of the stones. The kidney stones are mostly calcium oxalate, 86%[2].

The causative factors for the stone formation are of huge range including epidermiological, biochemical and genetic[3]. Urolith formation is multifactorial which may relate to diet, urinary tract infection, altered urinary solutes and colloids, decreased urinary drainage and urinary stasis, prolonged immobilization, Randall's plaque and microliths

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etc.[4]. The mechanism of stone formation is a complex process. It results from several physicochemical events including supersaturation, nucleation, growth, aggregation and retention of kidney stones constituents within tubular cells[5].

Urolithiasis has a reoccurrence rate of 70%-80% in males and 47%-60% in females, usually more frequent in men than women and rare in children[6]. But the current population shows high kidney stone disease tendency in children as well.

Although exact data are not available about the kidney stones disease in Sri Lanka, nearly 3000 patients are subject to Extra-Corporeal Shock Wave Lithotripsy (ESWL) for a year, however only in Sri Lankan National hospitals[7]. This information shows that the gravity of kidney stone disease in Sri Lanka. Factors like quality of drinking water, climate and different dietary habits are the risk factors for the formation of kidney stones in Sri Lanka.

Various treatment methods have evolved over the years for the kidney stone disease, discrepancies exist regarding the efficacy of the treatment options. Surgical operation, lithotripsy and local calculus disruption using high power laser are widely used to remove the calculi.

Many remedies have been employed to treat renal stones and most of them were from plants and proved to be useful. In Ayurveda and Folklore medicine, many herbs are used in the treatment of urolithiasis [8]. The discoveries of *in-vitro, in-vivo* and clinical trials show that phytotherapeutic agents could be useful as either alternative or adjunct therapy in management of urolithiasis.

Medicinal plants are major parts of traditional systems in developing countries. Herbal medicine is defined as the branch of science where plant used formulations are used to ease the diseases. It is known as Botanical medicine or Phytomedicine. Many diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries[9]. Medicinal plants which form the backbone of traditional medicine have been the subject of very intense pharmacological studies in the last few decades.

Ayurveda, Siddha, Unani and Traditional systems are the main systems of indigenous medicines. Researchers are providing evidence through research, in validating the efficacy and safety indigenous drugs.

The selected plant *Salacia reticulata* plays a considerable role in Sri Lankan folk medicines as treatment to Urolithiasis. In addition to the kidney stone disease, treatments of diabetes, rheumatism, gonorrhoea and other skin diseases are prepared by using *Salacia reticulata* which is known to provide safe and well- tolerated remedies for chronic illnesses which typically resulted from the combinations of secondary plant metabolites that are synthesized and deposited in specific parts of the plant. *Salacia reticulata* is an indigenous flowering plant grown in dry zone forests in Sri Lanka. In Ayurvedic medicine, it is known as *Kothalahimbutu* in Sinhala and *Karanthi, Ponkoranthi* in Tamil. It is a climbing, perennial, woody shrub which has a dichotomous branching pattern. The bark of this plant is a thin smooth one with grey colour outside and white colour internally. The leaves are opposite and elliptic oblong. Its flowers are bisexual and they are arranged as 2-8 clusters in leaf axils. The fruit is a drupe which is globose and tubular. The extract from the plant has been used for the treatment of urolithiasis, diabetes, rheumatism and gonorrhoea in Ayurveda [10].

This study was aimed to evaluate In-vitro anti-urolithiatic activity of methanolic extract of *Salacia reticulata* on experimentally prepared calcium oxalate stones and to compare it with the in-vitro anti- urolithiatic activity of the standard formulation.

Materials and methods

Extraction process

Plant *Salacia reticulata* was collected from Hettipola area in Kurunegala, Sri Lanka (Figure 1). The plant material was taxonomically identified and authenticated by the taxonomist of Department of Botany, University of Jaffna, Sri Lanka. Finely cut stem parts of the plant *Salacia reticulata* were shade

dried at room temperature and finely pulverized by using blender. Methanolic extract was obtained by using 200g of powdered stem and using 400ml of methanol (analytical grade, Merk specialties private Limited) in soxhlet apparatus until all the compounds were extracted in to the solvent. The extract was concentrated by using rotary evaporator at 40^0C.

Figure 1: Plant *Salacia reticulata*

Preparation of calcium oxalate crystals by homogeneous sprecipitation method

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

Calcium Chloride dihydrate (4.41g, analytical grade, Park Scientific Limited) dissolved in distilled water and Sodium Oxalate (4.02g, analytical grade, Research – Fine Chem Industries) dissolved in 2N Sulphuric acid were taken in two separate beakers and both solutions were mixed together with stirring until Calcium oxalate precipitate formed. Excess Sulphuric acid was removed by washing with Ammonia solution (analytical grade, Merk specialities private Limited) and distilled water respectively and allowed to dry at 60°C for 4 hours.

Preparation of semi-permeable membrane from chicken eggs

Semi permeable membrane was prepared from chicken egg by decalcifying outer shell and removing inner contents of egg. Apex of egg was punctured by a glass rod to remove the entire content. Empty egg shells were washed thoroughly with distilled water and placed in a beaker consisting 2M HCl (analytical grade, techno pharmache) for an overnight which caused complete decalcification. Then membranes were washed with distilled water and placed in ammonia solution for neutralization in the moistened condition for a while. Then they were rinsed with distilled water and Stored in a refrigerator at a pH of 7- 7.4[11]. Preparation of semi-permeable membrane and prepared membranes are shown in Figure 2.

Figure 2: Preparation of semi-permeable membrane and prepared membranes

Evaluation of anti-urolithiatic activity by the titrimetric method

The dissolution percentage of Calcium Oxalate was evaluated as described below by using titrimetric method with different three groups (Table 1).

Table 1: The dissolution percentage of Calcium Oxalate was evaluated as described below by using titrimetric method

Group	Concentration
Negative control	5 mg Calcium Oxalate
Positive control	5 mg Calcium Oxalate + 10, 20, 30, 40 mg of Cystone
Salacia reticulata.	5mg Calcium Oxalate + 10, 20, 30 and 40 mg of *Salacia reticulata*

Each sample was packed in separate semi permeable membranes and mixed well. Then semi permeable membranes were allowed to suspend in separate conical flasks which contained 100mL of the buffer solution. Conical Flasks containing the samples of all groups were kept in an incubator at 37°C for 7 hours. The contents of the semi permeable membranes from each group were removed into separate test tubes. A 5mL of 1 N Sulphuric acid was added to each test tube and the resultant mixture was titrated with 0.01 N KMnO4 until a light pink colour end point is obtained.

Calcium oxalate is reacted with the extract/standard and get dissolved based on the ability of test substances to dissolve it in a semipermeable membrane which is suspend in a buffer at pH 7.4. The undissolved Calcium oxalate remaining inside the semipermeable membrane is estimated in acidic condition by titrating against standarized KMnO4, purified from Merk specialities Private Limited. The percentage of dissolution of Calcium oxalate is calculated using the insoluble calcium oxalate.

Each 1 mL of 0.01N KMnO4 is equivalent to 0.64 mg of Calcium oxalate.

Amount of Calcium Oxalate in dissolution sac in the beginning = 5mg

Estimation of Calcium Oxalate in dissolution sac after incubation = (VKMnO4 × 0.64)

Reduced Calcium Oxalate amount

= 5mg - (VKMnO4 × 0.64)

Dissolution Percentage

= (Reduced Calcium Oxalate)/5mg ×100

Total quantity of dissolved Calcium Oxalate weight was obtained by subtracting the remaining insoluble Calcium Oxalate from the total quantity used in the beginning of the experiment[12].

Results and Discussion

Yield percentage of methanolic extract of Salacia reticulata

Yield percentage of methanolic extract of Salacia reticulata is shown in Table 2.

The mean (±SD) dissolution percentages of methanolic extract of Salacia reticulata on experimentally prepared Calcium Oxalate for 10, 20, 30 and 40mg concentration were 34.8%, 38.6%, 40.4% and 45.0% respectively.

Table 2: Yield percentage of methanolic extract of Salacia reticulata

Initial weight	Crude weight	Yield percentage	Colour of the crude product
100g	15.50	15.5%	Dark brown

The mean (±SD) dissolution percentages of standard drug Cystone, Himalaya company, India on experimentally prepared Calcium Oxalate for 10, 20, 30 and 40mg concentration were 64.8%, 70.6%, 75.8.%, 79.6% respectively (Figure 3 and Table 3).

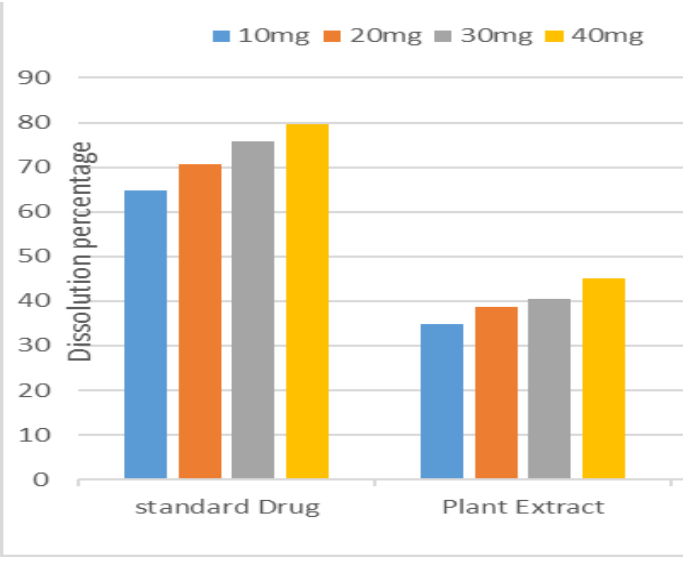

Figure 2: Dissolution percentages of Calcium Oxalate by the standard and the selected plant extract

Table 3: Dissolution percentage of Calcium oxalate by plant extract and the standard

Groups	Concentrations	Volume of $KMnO_4$*(ml)	Weight of Calcium oxalate estimated** (mg)	Weight of Calcium oxalate reduced*** (mg)	Dissolution percentage (%)
Negative control	5mg	05.20	3.33	1.67	33.4
Standard	10mg	02.75	1.76	3.24	64.8
	20mg	02.30	1.47	3.53	70.6
	30mg	1.89	1.21	3.79	75.8
	40mg	1.60	1.02	3.98	79.6
Salacia reticulata	10mg	05.10	3.26	1.74	34.8
	20mg	04.80	3.07	1.93	38.6
	30mg	04.65	2.98	2.02	40.4
	40mg	04.30	2.75	2.25	45.0

*After the incubation remaining calcium oxalate in the sac was titrated with $KMnO_4$.

**Weight of calcium oxalate estimated by titration with $KMnO_4$

***Weight of calcium oxalate reduced due to the dissolution by plant extracts and the standard.

The study could be proceeded by using stones removed from the patients who affected by the kidney stones with aqueous extract.

Conclusion

Titration method was used in the study to evaluate the anti- urolithiatic activity of *Salacia reticulata* against the experimentally prepared calcium oxalate stones. The mean dissolution percentages of methanolic extract of *Salacia reticulata* on experimentally prepared calcium oxalate at 10, 20, 30 and 40 mg were 34.8%, 38.6%' 40.4% and 45.0% respectively. The dissolution percentage was significantly increased with concentration. There was a significant correlation between dissolution percentage and concentration. Dissolution percentages for the standard drug against calcium oxalate stones in the given concentration were 64.8%, 70.6%, 75.8% and 79.6% respectively. Both the methanolic extract of the plant and the standard drug have a positive correlation with the concentration. The results of this study have confirmed the long history of the use of *Salacia reticulata* in traditional medicine for the treatment of Urolithiasis.

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