

## EVALUATION OF IN- VITRO ANTI-UROLITHIATIC ACTIVITY OF *SCOPARIA DULCIS L*

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### *Abstract ;*

*The main objective of the study, the inhibitor effect of Scoparia dulcis L on the crystallization of calcium oxalate in synthetic urine. Our study of the calcium oxalate crystallization is based on the changes in turbidity. The effect of Scoparia dulcis aqueous extract on the crystallization of calcium oxalate was evaluated. The formation of calcium oxalate is caused by the addition of 0.01 M sodium oxalate solution to synthetic urine. The addition of different concentrations of inhibitors (200 µg, 400 µg, 600 µg, 800 µg, 1000 µg, 1200 µg, 1400 µg, 1600 µg, 1800 µg and 2000 µg) allowed us to provide information on the inhibition percentage. The aqueous extract of Scoparia dulcis leaves displayed a maximum nucleation inhibition of 66.928±0.021% observed at 2000 µg concentration. A strong dose-dependent inhibition of the aggregation was shown in the aggregation assay of Scoparia dulcis aqueous extract leaves with a percentage inhibition of 66.51±0.642 percent. In calcium oxalate urinary lithiasis, the aqueous extract of Scoparia dulcis strongly inhibits crystal formation.*

*Key words: Scoparia dulcis, Aqueous extract, calcium oxalate, urolithiasis.*

### **Introduction**

Approximately 75 percent of the world's population, especially in developing countries, relies on medicines for their health care needs around the world (Khan and Pradhans 2012). Urolithiasis is a chronic illness that for so many years has afflicted the human race. Urolithiasis is derived from the Greek terms ouron (urine) and lithiasi (stone). In the last of Egyptian mummies, urinary stones were discovered dating back to 7000 years and the symptoms of the condition were related by Hippocrates who proposed that drinking muddy river moiré object in urinate dejected sand. (Butt, 1956). The common symptoms are sudden pain, nausea, vomiting. The location of pain depends on the location of the stones. The formation of stones requires super saturated urine. Super saturation relies on ionic strength, pH, concentration of solutes, etc. CaOx crystallization starts with increased super-saturation of the urine, with the subsequent formation within the urinary tract of solid crystalline crystals. This is accompanied by nucleation, whereby stone-

forming salts coalesce into clusters in supersaturated urinary solution, which then increase in size by adding new constituents (Basavaraj et al., 2007). A treatment for the prevention of this disease would also be of considerable interest if its recurrence were to occur. Herbal medications are commonly used because they have less side effects and are more powerful and cheaper. *Dulcis* is a medicinal plant of rising importance worldwide. A variety of the medicinal properties hypothesized by S. Scientific analysis has validated *Dulcis*. These include antiviral activity (Hayashi et al, 1990), activity promoting antitumor activity (Nishing et al., 1998), hypoglycemic activity (Venkateswaran et al., 2002) and antioxidant activity (Pari et al., 2004). The plant is traditionally used for the treatment of kidney stones and urinary disorders (Hassan et al., 2008; Ailei et al., 2008).

## **Materials and Methods**

### **Collection of plant:**

#### ***1. Scoparia dulcis:***



The investigated plant *Scoparia dulcis* was collected from Kumaracoil, Thuckalay, Kanyakumari district during the month of August . The collected plant parts were separated from undesirable materials or plant parts. Then plant materials were washed with tap water and they were dried for 5 days at room temperature ( $28\pm 2^{\circ}\text{C}$ ). The plant parts were ground into a coarse powder with the help of a suitable grinding mixer. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

### **Preparation of extract**

The powdered plant material was successively extracted with the following solvents of chloroform, diethyl ether, acetone, ethanol, and water.

## **Experimental protocol**

The effect of extract on CaOx crystallization was determined by time course measurement of turbidity changes due to the crystallization in artificial urine on addition of 0.01M sodium oxalate solution. The precipitate of calcium oxalate at 37 °C and pH 6.8 has been studied by the measurement of absorbance at 620 nm using UV/Visible spectrophotometer.

### **Preparation of artificial urine:**

The artificial urine (AU) was prepared according to the method Burns and Finalayson (1980) with slight modification with the following composition: sodium chloride 105.5 mM, sodium phosphate 32.3 mM, sodium citrate 3.21 mM, magnesium sulfate 3.85 mM, sodium sulfate 16.95 mM, potassium chloride 63.7 mM, calcium chloride 4.5 mM, sodium oxalate 0.32 mM, ammonium chloride 0.0028 mM. The AU was prepared fresh each time and the pH was adjusted to 6.0.

### **Study without inhibitor:**

A volume of 1.0 ml of AU was transferred into the cell and 0.5 ml of distilled water added to it and blank reading was taken. Then 0.5 ml of 0.01 M sodium oxalate was added, to the previous volume, and the measurement is immediately started for a period of ten minutes.

### **Study with inhibitor:**

The aqueous extract of *Scoparia dulcis* was dissolved in distilled water, filtered through membrane filter and concentration of 200, 400, 600, 800 and 1000 µg/ml was obtained. A mixture of 1 ml of AU and 0.5 ml of plant extract solution is versed in the cell. A blank reading was taken and then 0.5 ml of 0.01 M sodium oxalate solution was added and immediately the absorbance was measured for a period of ten minutes at 620 nm. The percentage of inhibition of calcium oxalate crystal formation was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of Control}}$$

Where; Ab Test: Absorbance in the presence of inhibitor (Extract), Ab Control: Absorbance of without inhibitor (control).

### **Nucleation Assay:**

The method used was similar to that described by Hennequin *et al.*, 1993, with slight modifications. Solutions of calcium chloride and sodium oxalate were prepared at the final concentration of 3 mM and 0.5 mM, respectively, in a buffer containing Tris 0.05 M and NaCl

0.15 M at pH 6.5. Both solutions were filtered through a 0.22 µm filter; 33 mL of calcium chloride solution was mixed with of the aqueous extract of *Scopariadulcis* of different concentrations. Crystallization was started by adding 33 mL of sodium oxalate solution. The final solution was magnetically stirred at 800 rpm. The temperature was maintained at 37°C. The absorbance of the solution was monitored at 620 nm. The percentage inhibition produced by the herb extract was calculated as:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test}) \times 100}{\text{Absorbance of control}}$$

Where; Ab Test: Absorbance in the presence of inhibitor (Extract), Ab Control: Absorbance of graph without inhibition (Control).

## RESULTS AND DISCUSSION

Table I showed the percentage inhibition of the crystallization of calcium oxalate (CaOx) by different extracts of *Scoparia dulcis* L with inhibitors. It is inhibited the crystallization in a concentration dependent pattern. The % inhibition was calculated using the above mentioned formula

Table:I Percentage inhibition of crystallization of calcium oxalate

Conc. of extract	Aqueous (%)		
	SDR	SDL	SDW
2 mts	4.46± 0.188	15.46± 0.126	11.506±0.226
4 mts	10.096±0.079	25.388±0.108	20.27± 0.131
6 mts	15.668±0.406	33.352±0.134	30.552±0.407
8 mts	21.42± 0.252	47.668±0.235	38.738±0.058
10 mts	27.992±0.421	65.548±0.179	42.21± 0.130

Table 1 described the percentage inhibition of crystallization of calcium oxalate by *Scoapria dulcis* with inhibitors. The maximum percentage inhibition of calcium oxalate was maximum in Leaf in *Scoparia dulcis* of 65.548±0.179 in 10 min.

Table 2 explained the percentage inhibition of calcium oxalate crystallization by different extracts of *Scoparia dulcis* by nucleation assay. The concentration of the plant extract used for the present study were 200 µg,400µg,600 µg,800 µg,1000 µg,1200 µg,1400 µg,1600 µg,1800 µg and 2000 µg. Of the three parts tested ,leaves at 2000 µg showed maximum inhibition.

It was found that the aqueous extracts of the different parts of *Scoparia dulcis* in leaf,root,whole plant of the concentrations increases the percentage inhibition of calcium oxalate crystallization is also increases. Of the Aqueous extracts leaves of SDL of *Scoparia dulcis* of aqueous exhibited maximum inhibition (66.928±0.021%).

Maximum inhibition of nucleation 66.928±0.021 % observed at concentration of 2000 µg.

Table II:Nucleation Assay in Aqueous extract of *Scoparia dulcis*

Conc. of extract	Aqueous (%)		
	SDR	SDL	SDW
200 µg	3.10± 0.027	14.53± 0.075	12.24± 0.040
400 µg	3.172± 0.016	16.368± 0.021	15.768± 0.027
600 µg	5.436± 0.026	17.138± 0.030	16.422± 0.019
800 µg	5.676± 0.040	19.344± 0.035	26.82± 0.073
1000 µg	6.028± 0.035	21.142± 0.873	37.838± 0.043
1200 µg	7.238± 0.036	28.54± 0.041	38.45± 0.030
1400 µg	7.94± 0.033	40.036± 0.029	38.616± 0.020
1600 µg	8.632± 0.038	52.046± 0.040	39.842± 0.034
1800 µg	9.63± 0.029	54.52± 0.015	40.56± 0.038
2000 µg	10.07± 0.046	66.928± 0.021	42.254± 0.035

Table 3 explained the aggregation assay of different parts leaf,root,whole plant of *Scoparia dulcis*. In the present work,the concentration of the plant work extract used for the present study were 200 µg,400µg,600 µg ,800 µg and 1000 µg.It was found that the extracts tested as the concentrations increases the aggregation of calcium oxalate crystallization is also increases. The aqueous extracts of *S.dulcis* leaves of exhibited maximum inhibition (66.51±0.642%)

Table III Aggregation Assay in Aqueous extract of *Scoparia dulcis*

Conc of the extract	Aqueous %		
	SDR	SDL	SDW
200 µg	12.31±0.251	22.44±0.191	18.48±0.077
400 µg	15.158±0.079	30.396±0.069	24.41±0.097
600 µg	20.402±0.142	43.30±0.090	34.45±0.077
800 µg	26.53± 0.112	54.65±0.139	44.54±0.052
1000 µg	29.92±0.548	66.51±0.642	50.57±0.225

In the aggregation assay of *Scoparia dulcis* leaf of aqueous extract showed a significant dose-dependent inhibition of the aggregation with percentage inhibition 66.51±0.642%. The percentage inhibition of turbidity (aggregation) in the presence of extracts was lower than in the low concentration. The inhibited aggregation associated with concentration. This inhibition was greatest with aqueous extract of leaf when compared with all other extracts of whole plant & root. The herbal extracts contain various phytochemicals that can inhibit the growth of CaOx crystals. Due to the presence of phytochemicals, the binding of the CaOx to epithelial cell surfaces reduces and easily excreted through urine (Wesson *et al.*, 1998). The phytochemicals present in the extracts inhibit CaOx crystals through aggregation, agglomeration and inhibition. So kidney stones can pass through the urinary tract (Kok and Khan, 1994). Kidney stone formation occurs due to several physicochemical events like supersaturation, nucleation, growth, aggregation and retention with in renal tubules (Khan, 1997). Different systems are aimed at investigation of all or atleast some of these partial events. (Achilles 1997). Nucleation is the formation of a solid crystal phase in a solution. It is an essential step in renal stone formation. (Finlayson 1978; Khan *et al.*, 1990) So our preliminary studies showed that *Scoparia dulcis* useful as antiurolithiatic, *in vivo* studies are required to provide further support for its use.

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