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In-vitro evaluation of *Scoparia dulcis* Linn. for anti – urolithiatic activity

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ABSTRACT

Introduction: Urolithiasis is a complex process that occurs from series of several physicochemical event including super-saturation, nucleation, growth, aggregation and retention within the kidneys. Data from in-vitro, in- vivo and clinical trials reveal that phytotherapeutic agents could be useful as either alternative or an adjunct therapy in the management of Urolithiasis. *Scoparia dulcis* (L.) have been reported to possess antiurolithiatic property by various folk lore practitioners. **Methods**: The *in- vitro* antiurolithiatic study of the whole plant of *S. dulcis* (L). through titrimetric and turbidity method to check their potential in dissolving calcium oxalate crystals using Cystone as the standard compound **Result**: The aqueous extract showed relatively higher dissolution of 66.96% of stones than the alcoholic extract. The turbidity showed by the alcohol extract and the aqueous extract of test drug (*S. dulcis* (L).) was highly significant compared to the standard (Cystone).

Keywords: Urolithiasis, *in vitro*, *Scoparia dulcis* (L.) titrimetric, turbidity.

INTRODUCTION

Urolithiasis is the condition where urinary calculi are formed or located anywhere in the urinary system or the process of formation of stone in kidney, bladder or ureter. Calculi, is an aggregation of solute materials from urine such as calcium, oxalate, phosphate and uric acid which form stone. It is a serious, debilitating problem in all societies throughout the world, affecting approximately 12% of the population and men are three times more prone than women.It is more prevalent between the ages of 20 and 40 in both sexes [1]. Etiology is multifactorial and is strongly related to dietary lifestyle habits or practices.Increased rates of hypertension and obesity, also contribute to an increase in stone formation [2].

Surgical intervention and pain management are the main treatment procedures followed in this disease. The major part of the population is trying to find alternatives to modern medicines because of their side effects. Ayurveda, an indigenous Indian system of medicine, offers vast scope for the successful treatment of urinary tract problems including urolithiasis. Traditional system of medicine uses many herbs in different dosage forms with success stories without any side effects. But exact mode of action, evident facts are yet to be derived, to popularize such cost effective safe herbal drug practices. *Scoparia dulcis* Linn. commonly called as *Manithumbegida* in Kannada, *Kallurukki* in Malayalam is a popular herb used by folk lore practitioners in South India in the treatment of urinary calculi. The whole plant is used in the form of decoction to dissolve stones with different adjuvants. In the present study an effort has been made to evaluate anti-urolithiatic activity of *S. Dulcis* Linn. by titrimetric and turbidity method.

MATERIALS AND METHODS

1. Drug source

Whole plant of *S. dulcis* Linn.collected from the Udupi district of Karnataka and was authenticated. It was shade dried and powdered in a mixer grinder and stored in air tight jar for further study. Alcoholic and aqueous extract of test drug are prepared and those extracts are used for further study.

2. Preparationof calcium oxalate crystals [3]

By taking equimolar solution of Calcium chloride dihydrate (A.R) which was dissolved in distilled water and Sodium oxalate (A.R) was dissolved in 10 ml of 2N H_2SO4, sufficient quantity is allowed to react in a beaker. The resulting precipitate of calcium oxalate which was freed from traces of Sulphuric acid by washing with ammonia solution. Then again it was washed with distilled water and dried at

a temperature of 60 °C for 4 hours.

3. Preparation of the Semi permeable membrane from farm eggs [3]

The outer calcified shell was removed chemically by placing the eggs in 2 ml HCL for overnight, which caused complete decalcification. Further, washed with distilled water and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. It was then washed thoroughly with distilled water and placed it in ammonia solution, in the moistened condition for a while and then rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Titrimetry method [4]

Weighed exactly 1 mg of the calcium oxalate and 10mg of ethanolic extract, water extract and standard Cystone were packed in semi permeable membrane by suturing as shown in Model design (Fig 3). They were allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer. One group served as negative control (contained only 1 mg of calcium oxalate). Conical flask of all groups will be placed in an incubator pre heated to 37^0 C for 2 hours, for about 7-8 hours. Contents of semi permeable membrane from each group will be removed into a test tube. Added 2 ml of 1 N sulfuric acid and titrated with 0.9494 N KMnO4 till a light pink color end point obtained. 1ml of 0.9494 N KMnO4 equivalent to 0.1898 mg of Calcium oxalate. Percentage dissolution of calcium oxalate by various groups is shown in (Table 1).

Turbidity method [5]

Growth of stone nucleus in vitro in the absence of any inhibitor was done. For this, a volume of 1.0 ml of 0.025M $CaCl_2$ and 2ml of Tris-buffer (pH 7.4) were added in a test tube. Then 1.0 ml of 0.025M Sodium oxalate was added. Formation of the turbidity results immediately after mixing of above chemicals and then the measurement of turbidity formed (in terms of absorption at 620 nm in UV/Vis spectrophotometer) was started immediately up to period of 10 min (600 seconds) after the mixing of the chemicals. This control experiment was done in six replications (Table 2). Absorptions were noted down and data obtained was used as the un-controlled growth of the stone nucleus for the comparison of growth in the presence of the standard drugs and plant extracts.

RESULTS AND DISCUSSION

Urolithiasis is a common painful disease, which afflict human population since ancient times [6]. Those composed of calcium oxalate are the most common uroliths accounting for more than 80% of the stones [7]. The mechanisms involved in the formation of calcific stones are not fully understood but it is generally agreed that urolithiasis is a multifaceted process involving events leading to crystal nucleation, aggregation and growth of insoluble particles [8]. Various therapies like diuretics are being used in attempt to prevent recurrence of hyper calciuria and hyper oxaluria induced calculi but scientific evidence for their efficacy is less convincing [9].

Medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheap source of drugs for majority of world's population. Several pharmacological investigations on the medicinal plants used in traditional antiurolithic therapy have revealed their therapeutic potential in the in vitro models [10, 11].

Estimation of calcium oxalate by titrimetric method

Titrimetric estimation measures undissolved calcium oxalate by using $KMnO_4$. 1 mg of the Calcium oxalate was weighed and 10mg of the Ethanolic extract, water extract, and standard Cystone, and control were packed separately in semi permeable membrane by suturing. They were allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer. One group served as negative control (contained only 1 mg of calcium oxalate). Placed the conical flask of all groups in an incubator, pre heated to 37^0C for 2 hours, for about 7-8 hours. The contents of semi permeable membrane were removed from each group into a test tube. To this 2 ml of 1 N Sulphuric acid was added and titrated with 0.9494 N $KMnO_4$ till a light pink colour end point obtained. 1ml of 0.9494 N $KMnO_4$equivalents to 0.1898 mg of Calcium oxalate.

The amount of calcium oxalate that was dissolved was subtracted from the total quantity of calcium oxalate used in the experiment. This shows the actual quantity of calcium oxalate the test drug can dissolve. In dissolution study the negative control shows zero dissolution. The standard group (Cystone) showed dissolution of 83.7 %. The aqueous extract and the alcohol extract of test drug(*S. Dulcis*(L))showed dissolution of 66.96 % and 50.22 % respectively. Except standard group the aqueous extract of test drug (*S. Dulcis*(L.)) showed maximum dissolution of 66.96 %. (Table 1, Figure1)

Table 1: Results of Dissolution studies of calcium oxalate by plant extract *Scoparia dulcis* Linn.

Group	Vol $KMnO_4$	wt of calcium estimated	wt of calcium reduced	% dissolution
Negative	0.12	0.0227	0	
Std(Cystone)	0.02	0.0037	0.019	83.7
Aqueous extract	0.04	0.0075	0.0152	66.96
Alcoholic extract	0.06	0.0113	0.0114	50.22

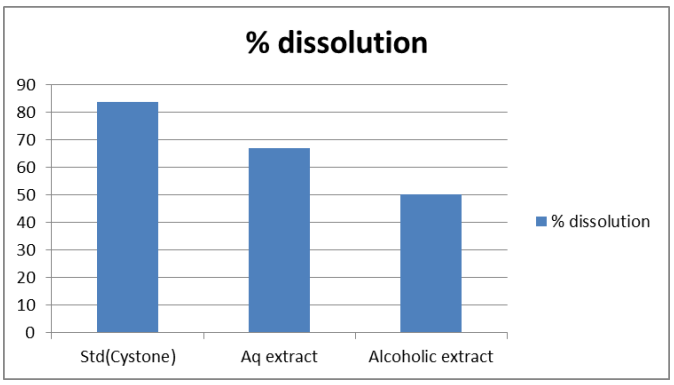

Figure 1: Percentage dissolution of calcium oxalate by *Scoparia dulcis* Linn. extract groups

Turbidity method

Turbidometric method measures the turbidity in terms of calcium oxalate formation in synthetic urine using spectrophotometer at 620nm and crystallization inhibition measured by turbidity reduction. Stone nucleus was grown *in- vitro*in the absence of any inhibitor. For

this, a volume of 1.0 ml of 0.025M $CaCl_2$ and 2ml of Tris-buffer (pH 7.4) were added in a test tube. Then 1.0 ml of 0.025M Sodium oxalate was added. Formation of the turbidity results immediately after mixing of above chemicals and then the measurement of turbidity formed (in terms of absorption at 620 nm in UV/Vis spectrophotometer) was started immediately up to period of 10 min (600 seconds) after the mixing of the chemicals. This control experiment was done in six replications at 60 sec., 120 sec, 240 sec, 360 sec, 480 sec, and 600sec. Absorptions were noted down and data obtained were used as the un-controlled growth of the stone nucleus. For the comparison of growth in the presence of the standard drug and plant extracts, it was taken at the concentration of 1mg/ ml each and were added to the above chemicals and the turbidity formed were measured.

In this turbidity test, the turbidity showed by alcohol extract at 0 sec, 60sec, 120 sec, 240sec, 360sec, 480sec, and 600 sec were 0.041, 0.047, 0.058, 0.093, 0.097, 0.098, 0.098. The turbidity showed by the aqueous extract were 0.018, 0.025, 0.092, 0.121, 0.124, 0.131, 0.133 at 0sec, 60sec, 120 sec, 240sec, 360sec, 480sec, and 600sec respectively. Turbidity with the standard (Cystone) were 0.09, 0.158, 0.186, 0.218, 0.22, 0.232, 0.238 at 0 sec, 60sec, 120sec, 240sec, 360sec, 480sec, 600sec. For control group the turbidity was 0.111, 0.152, 0.250, 0.315, 0.317, 0.319, and 0.321 at 0sec, 60sec, 120 sec, 240sec, 360sec, 480sec, and 600sec respectively. The turbidity showed by the alcohol extract and the aqueous extract of test drug(*S. dulcis*)was highly significant compared to the standard (Cystone).(Table 2, Figure 2)

Table 2: Results of turbidity method by plant extract *Scoparia dulcis* Linn.

Time (sec)	Control	Turbidity (Alc. ext)	Turbidity (cystone)	Turbidity (Aq.ext)
0	0.111	0.041	0.09	0.018
60	0.152	0.047	0.158	0.025
120	0.250	0.058	0.186	0.092
240	0.315	0.093	0.218	0.121
360	0.317	0.097	0.22	0.124
480	0.319	0.098	0.232	0.131
600	0.321	0.098	0.238	0.133

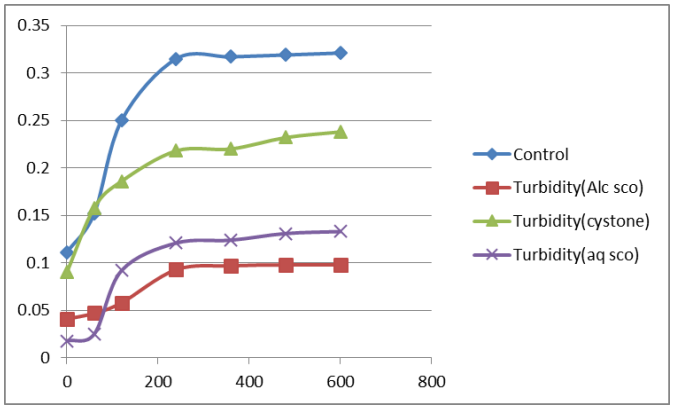

Figure 2: Effect of *Scoparia dulcis* Linn. extract groups in turbidity method

Egg shell membrane (Semipermeable)

The calcium oxalate stone with Control, Std (Cystone), Aqueous extract, alcoholic extract

Figure 3: Estimation of calcium oxalate (Stones) by titrimetry

CONCLUSION

The *in- vitro* antiurolithiatic study of the whole plant of *Scoparia dulcis* Linn.through titrimetric and turbidity method has showed extremely significant action on urinary calculi. Titrimetric estimation measures undissolved calcium oxalate by using $KMnO_4$. The aqueous extract and the alcohol extract of test drug (*S. Dulcis(L.)*) showed dissolution of 66.96 % and 50.22 % respectively which was significant compared to standard group(Cystone 83.7%). Turbidometric method measures the turbidity in terms of calcium oxalate formation in synthetic urine using spectrophotometer at 620nm and crystallization inhibition measured by turbidity reduction. The turbidity showed by the alcohol extract and the aqueous extract of test drug (*S. dulcis*) was highly significant compared to the standard drug.

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