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Research Article

EVALUATION OF *IN VITRO* ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF *PAVONIA ZEYLANICA* (L) CAV

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ABSTRACT

Objective: To evaluate *in vitro* antiurolithiatic activities of *Pavonia zeylanica* (L) Cav**.** Material and Methods: Whole plant of Ethanolic extract of *Pavonia zeylanica* was prepared and arranged in the three different concentrations (10mg/ml, 20mg/ml, and 30mg/ml). *In vitro* antiurolithiatic activity of Ethanolic extract of *Pavonia zeylanica* through turbidometric and titrimetric method was tested in terms of inhibition of calcium oxalate by nucleation and aggregation and dissolution of calcium oxalate, using the standard drug. Results: Standard drug has high percentage inhibition and dissolution ability and thus Ethanolic extract of *Pavonia zeylanica* also have a good percentage inhibition and dissolution ability was comparable to standard drug. Conclusion: The study concludes that the Ethanolic extract of *Pavonia zeylanica* have inhibitory effect on calcium oxalate for nucleation and aggregation assay. It also showed good dissolution of calcium oxalate crystals, so this extract has the good protection against Urolithiasis activity.

Keywords: Antiurolithiatic, Ethanolic, *Pavonia zeylanica* Nucleation, Aggregation, Dissolution

INTRODUCTION

The term 'Urolithiasis' comes from the Greek word, ouron means urine and *lithos* means "stone"[1] Urolithiasis is a condition in which the crystals of stone or formation of calcifications present in the urinary system, mainly in the kidneys or ureters and may also affect the bladder or urethra[2]. Kidney stones are related to an increased risk of chronic kidney diseases, end-stage kidney failure, cardiovascular diseases, diabetes, and hypertension. It has been suggested that urinary calculus could also be a systemic disorder linked to the metabolic syndrome[3].

Urolithiasis is the formation of uneven calculi, or the condition which called as urinary calculi is synonymous with the term uroliths, stones, or crystals. These uneven urinary calculi are produced by deposition of polycrystalline aggregates composed of various amounts of crystalloid and organic matrix. Calculi was in different in size and shape which found anywhere within the tract from kidney to the bladder [4].

Risk factors for crystallization is the level of urinary saturation with estimation to the stone forming constituents likecalcium, phosphorus, uric acid, oxalate, cystine, and low urine volume[5]. The mechanism of kidney stone formation involves urinary super saturation, crystal nucleation, crystal growth, and crystal aggregation[6].

Pavonia zeylanica (L) Cav shrub normally found in waste lands. Leaves are serrate, fruits are round in shape. This species is globally distributed in tropical Africa, southwest Arabia, Pakistan, India, Sri Lanka and Mauritius. Within India, it has been recorded in Rajasthan, Maharashtra, Karnataka and Tamil Nadu in waste land. The Synonyms for the *Pavonia zeylanica* is *Cancellaria zeylanica* (L.) *Mattei* in Tamil name chirtamutti and various other names called as Mammatti, sittamutti, sevagan, and Thengai poondu[7]. The plant is used in folk medicine, siddha and Ayurveda to treat inflammation, hemorrhage and dysentery activities have been reported. The Leaves and roots are regarded in Ayurveda as cooling demulcent, carminative, diaphoretic, and diuretic[8]. The aim of the present study was to evaluate *in vitro* antiurolithiatic activities of *Pavonia zeylanica* (L) Cav.

MATERIALS AND METHODS

Collection of plants

Dried whole plant of *Pavonia zeylanica* (L) Cav was collected from the local are around Anaikuttam, Sivakasi, Virudhunagar District, Tamilnadu (India), in the month of November 2021. It was authenticated by Dr. N. Senthilkumar Head and Associate Professor of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, Virudhunagar District. The plant specimen was certified as *Pavonia zeylanica* (L) Cav belonging to the family Malvaceae**.**

Preparation of Plant Extract

The whole plants of *Pavonia zeylanica* (L) Cav were cleaned and chopped into small pieces and dried under shade. The coarse powder was obtained by mixer grinding. The coarse powder material was subjected to continue hot extraction in Soxhlet apparatus at a temperature of (60 - 70ºC) by using ethanol (95% v/v) as solvent. After extraction is completed, the extract was dried, and it was stored at 4ºC in desiccator [9].

IN VITRO ANTIUROLITHIASIS STUDIES

Turbidometric method

The *in vitro* anti-urolithiatic activity of the ethanolic extracts was test in terms of inhibition of calcium oxalate nucleation and aggregation in the presence of inhibitors (standard drug and plant extracts) and absence of inhibitors. A UV/Visible spectrophotometer was employed to measure the turbidity changes in each assay. Three different concentrations (10mg/ml, 20mg/ml, and 30mg/ml) of the ethanolic extracts were test in

each assay[10].

Nucleation assay

Solutions of calcium chloride and sodium oxalate were prepared at the concentrations of 5 mmol/L and 7.5 mmol/L respectively in a buffer solution containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950 µL of calcium chloride solution was mixed with 100 µL of ethanolic extract at different concentrations (10mg/ml, 20mg/ml and30mg/ml). Crystallization was formed by adding 950 µL of sodium oxalate solution and the temperature was maintained at 37°C. The optical density of the solution was detected at 620 nm. The rate of nucleation was estimated by comparing the inductiontime in the presence of the plant extract with that of control. Standard (Cystone) were used as the standard solution. The rate of nucleation was estimated by comparing the induction time in the presence of Standard with that of the control. Percentage inhibition of nucleation was calculated using this following formula below:

$$\% \text{ Inhibition of nucleation} = [(C-S)/C] \times 100$$

Where, C is the turbidity without extract and S is the turbidity with extract[11].

Aggregation assay

The ethanolic extract of *Pavonia zeylanica* influence on calcium oxalate (CaOx) crystal aggregation was resolved through the assay. Calcium oxalate crystals were formed by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both mixers were equilibrated to 60°C in a water bath for 1 h and then cooled to 37°C overnight. The CaOx crystals were harvested by centrifugation and then evaporated at 37°C.The Calcium oxalate crystals were used at a concentration of 0.8 mg/ml, buffered solutions with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. The experiments were conducted in the absence and presence of the ethanolic extract after stopping stirring. Standard drugs were used as drug standard solution. The rate of aggregation was estimated using the formula given below:

$$Ir = (1 - (\text{Turbidity sample}/\text{Turbidity control})) \times 100$$

Where, Ir is the percentage aggregation inhibition rate[12].

Titrimetric method

This method was used to evaluate the activity of the ethanolic extracts in dissolving the already formed stones in the kidneys[13].

Step 1: Preparation of calcium oxalate crystals

Solution of Calcium chloride dihydrate which was dissolved in distilled water and Sodium oxalate was dissolved in 10 ml of 2N H_2SO4, sufficient quantity was 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.

Step 2 Preparation of the semi permeable membrane from farm eggs

The semi permeable egg membranes lie in between the outer calcified shell and the inner contents like albumin & yolk. The preparation included the following steps: Apex of eggs was punctured by a glass rod in order to squeeze out the whole inner content. Empty eggs were washed thoroughly with water and placed in a beaker containing 2M HCl solution for an overnight, which caused complete decalcification. Then, the semi permeable egg membranes were washed thoroughly with distilled water, placed in ammonia solution for neutralization of acid traces in the moistened condition for a while and was rinsed with distilled water. Finally, the semi permeable egg membranes were stored in 2%ammonia until used[14].

Step 3 Estimation of Calcium oxalate by Titrimetry

Weighed exactly 1 mg of the calcium oxalate and three different concentrations (10mg/ml, 20mg/ml, 30mg/ml) of ethanolic extract and standard drug were packed in semi-permeable membrane by suturing. They were allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer solution. One group served as negative control (containing only 1 mg of calcium oxalate). Standard was used as a positive control. Conical flask of all groups will be placed in an incubator pre heated to 37^0 C for 6 hours. Contents of semi-permeable egg membrane from each group will be removed into a test tube. Added 2 ml of 1 N H_2SO_4 and titrated with 0.9494 N KMnO4 till a light pinkish colour endpoint obtained. 1ml of 0.9494 N KMnO4 equivalentto 0.1898 mg of Calcium[14].

Group I: 1ml of CaOx (1mg/ml) + 1ml of distilled water,
Group II: 1ml of CaOx (1mg/ml) + 1ml of Standard solution,
Group III: 1ml of CaOx (1mg/ml) + 1ml of ethanolic extract of *Pavonia zeylanica*.

Table 1 Result of Ethanolic Extract of *Pavonia zeylanica* (L) Cav on Nucleation Assay

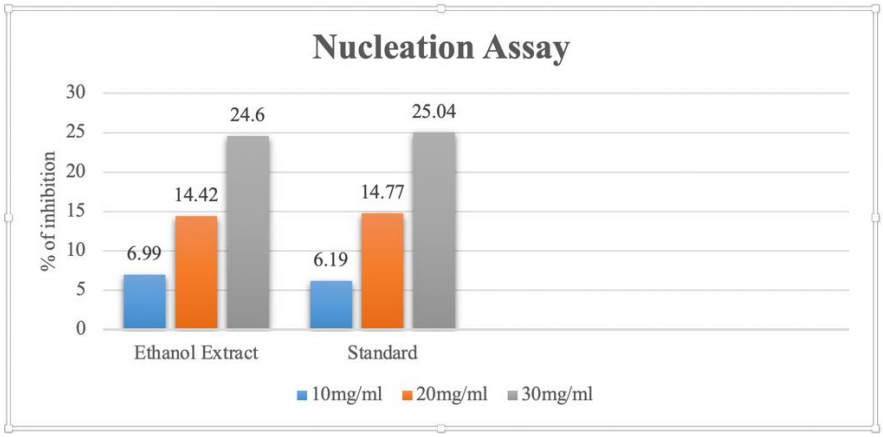
Sample Types	Concentration	% inhibition of Nucleation
Ethanol Extract	10mg/ml	6.99
	20mg/ml	14.42
	30mg/ml	24.60
Standard	10mg/ml	6.19
	20mg/ml	14.77
	30mg/ml	25.04

Table 2 Result of Ethanolic Extract of *Pavonia zeylanica* (L) Cav on Aggregation Assay

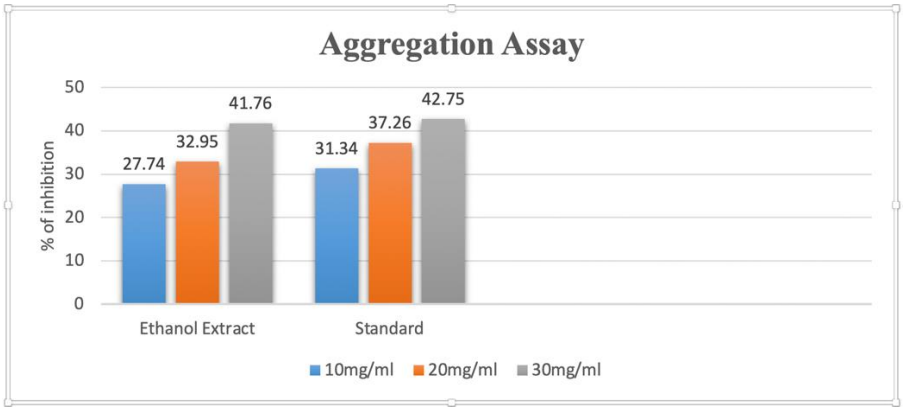
Sample Types	Concentration	% inhibition ofAggregation
Ethanol Extract	10mg/ml	27.74
	20mg/ml	32.95
	30mg/ml	41.76
Standard	10mg/ml	31.34
	20/mg/ml	37.26
	30mg/ml	42.75

Table 3 Result of Ethanolic Extract of *Pavonia zeylanica* (L) Cav on Dissolution study

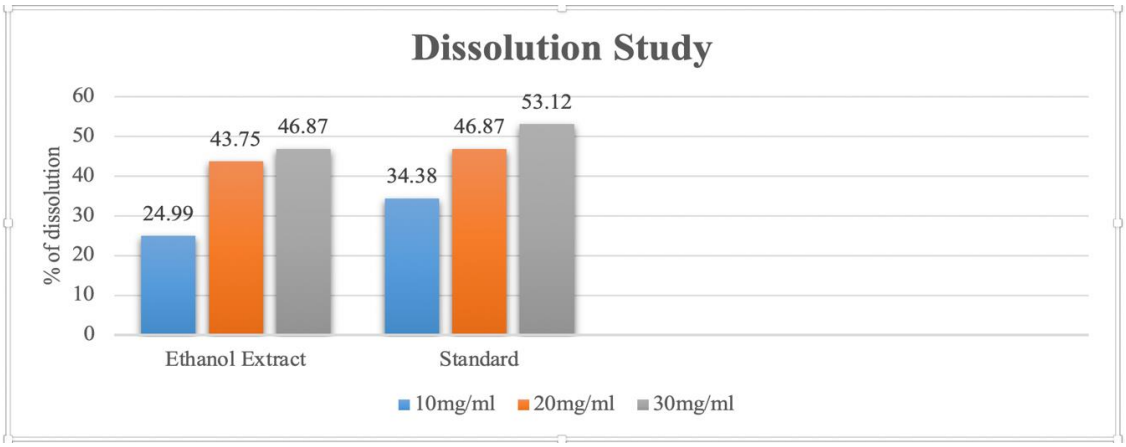
Group	Concentration	Volume of Standard KMnO4 (ml)	Weight ofcalcium estimated	Weight of calciumreduced	% Dissolution
Negative	---	3.2	0.6073	---	---
Ethanol Extract	10mg/ml	2.4	0.4555	0.1518	24.99
	20mg/ml	1.8	0.3416	0.2657	43.75
	30mg/ml	1.7	0.3226	0.2847	46.87
Standard	10mg/ml	2.1	0.3985	0.2088	34.38
	20mg/ml	1.7	0.3226	0.2847	46.87
	30mg/ml	1.5	0.2847	0.3226	53.12



Graph 1: Effect of Ethanolic Extract of *Pavonia zeylanica* (L) Cav on Nucleation Assay



Graph 2: Effect of Ethanolic Extract of *Pavonia zeylanica* (L) Cav on Aggregation Assay



Graph 3: Effect of Ethanolic Extract of *Pavonia zeylanica* (L) Cav on Dissolution study

RESULTS AND DISCUSSION

INVITRO ANTIUROLITHIASIS STUDIES
Turbidimetry method

Nucleation Assay
Addition of Sodium oxalate solution in the reaction mixture of Calcium chloride resulted in the formation of numerous Calcium oxalate crystals. The three different concentrations (10mg/ml 20mg/ml, 30mg/ml) of Ethanol extract and Standard in the reaction mixture produced a percent reduction of calcium oxalate in nucleation. The highest percentage inhibition was obtained from Standard drug at a concentration of 30mg/ ml (25.04%). Percent reduction in size of Calcium oxalate crystals produced by ethanolic extract of *Pavonia zeylanica* (L) Cav was comparable to that produced by Standard. The results were shown in the Table 1 and Graph 1.

Aggregation Assay
In the aggregation assay, three different concentrations (10mg/ml, 20mg/ml, 30mg/ml) of Ethanol extract and Standard produced a percent reduction of calcium oxalate. The highest percentage aggregation inhibition was obtained from Standard at a concentration of 30mg/ ml (42.75%). The percentage inhibition of Ethanolic extract of *Pavonia zeylanica* (L) Cav at the concentration of 30mg/ml (41.76%) slightly close to Standard. So, the percent reduction in aggregation produced by Ethanolic extract of *Pavonia zeylanica (l) cav* was comparable to that of Standard. The results were shown in the Table 2 and Graph 2.

Titrimetry method
In dissolution study, the Negative control shows zero dissolution. The three different concentrations (10mg/ml, 20mg/ml, 30mg/ml) of Ethanol extract and Standard of test drugs in the semipermeable membrane produced a dissolution of calcium oxalate. The results finding revealed that Standard drug has high dissolution ability, and thus Ethanolic extract of *Pavonia zeylanica* also have a good dissolution ability comparable to Standard. The results were shown in the Table 3 and Graph 3.

CONCLUSION

From this study we conclude that the ethanolic extract of *Pavonia zeylanica* (L) Cav through turbidometric and titrimetric method showed leading inhibition of all phases of calcium oxalate crystallization which include nucleation and aggregation. It also showed good dissolution ability of calcium oxalate crystal. Further conclusion of present study of ethanolic extract of the plant *Pavonia zeylanica* (**L**) **Cav** produce better protection against for urolithiasis activity.

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ABBREVIATIONS
EEPZ: Ethanolic Extract of *Pavonia zeylanica*
CaOx: Calcium Oxalate

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