Original Article

Evaluation Of In- Vitro Anti-Urolithiatic Activity Of Achyranthes Aspera

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Abstract

Species of plant in the Amaranthaceae family. It is distributed throughout the tropical world commonly called as Putkhanda and Prickly chaff flower (English), Kadaladi, Vankadaladi, Valiyakadaladi (Malayalam), Nagurivi, Shiru, Kadaladi (Tamilnadu) is an important medicinal plant used in folk medicine for the treatment of urolithiasis. It is an essential source of bioactive compounds viz., flavonoids, alkaloids and saponins. Present study is to evaluate the anti-urolithiatic activity of ethanolic extract of seeds of Achyranthes aspera by in- vitro methods Collection and authentication of the plant Achyranthes aspera. Extraction of seeds of Achyranthes aspera by maceration method. Evaluation of the in-vitro method.by inhibition of calcium oxalate crystallization

Key words: Urolithatic; Achyranthes aspera; UV Spectrophotometry; Cystone; In-vitro.

INTRODUCTION

Medicinal plants which form the backbone of traditional medicine in have been the subject of very intense pharmacological studies in the last few decades. Traditional Medicine concept they are the main approach of Siddha, Unani, Ayurvedha, aboriginal drugs. Researchers are identified evidence and research, in validating efficacy and safety of utilizing earliest awareness for health and healing. Recent trends great economic impact of Therapeutic value plants are of in the Indian subcontinent. the Ancient and medieval period provided valuable important drugs of the recent day the documentation of traditional knowledge especially on the medicinal uses of plants in. Urolithiasis is one the urinary tract of the common diseases of which has been afflicting human kind since antiquity. The industrialized countries population Urinary stones affect 10-30% of the repeatedly affected two different people Compared men to women but higher number of men affected but very slightly in child. Urolithiasis is one of the reason with calculus formation at any time in the urinary collecting system, but calculus often increases in the renal.(1) probably the most important problem in the time of stone formation is the after care patients who have undergone surgery for kidney and ureteric calculi. Multifactorial process urolithiasis formation is a mostly affected to unnutrition diet, unhygine condition affected urinary tract infection, changes of urinary solutes and colloids, lowered urinary drainage and urinary stasis, prolonged immobilization, Randall's plaque and microliths etc.,(2) infect the urinary tract when the urea-splitting organisms, the presence of urease enzyme what happen bacteria disintegrate the urea excreted urine in, which subsequently trigger the formation of ammonia in the urine alkaline.

In when the time of strong form calculi, in the state of large amount alkaline, contain precipitated crystals of calcium oxalate, magnesium phosphate and calcium carbonate from urine leads. Bacterial infection may make create stone formation by crystal adherence. Historically documented as far back as Egyptian mummies form of Urinary tract stone disease. (3-6)

MATERIALS AND METHODS

The seeds were collected from Chennai Dt, TN (INDIA) in the month of May 2019. It is authenticated by Dr. K N Sunil Kumar, Research officer and Head Of the Department, Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai.

Turbidity Method And Titrimetry Method Used By *In-Vitro* Anti-Urolithiatic Activity

In vitro Anti-Urolithiatic activity of *Achyranthes aspera* seed extract were tested in terms of inhibition of calcium oxalate formation by the extracts in the presence (standard drugs and extract) and absence of inhibitors. The precipitation of calcium oxalate at 37ºC and pH 6.8 was calculated by the measurement of turbidity by UV/Vis spectrophotometer at

620nm. It has been employed to determine the turbidity caused due to formation of calcium oxalate. To estimate calcium oxalate inhibition of plant extract by absorptions were noted and in microscopic study the comparison of un-controlled growth of the stone nucleus for the comparison of growth in the presence of the standard drugs and seed extract were also observed [7-12]

STUDY WITHOUT INHIBITOR

Volume of 1.0 ml of 0.025M calcium chloride dihydrate and 2ml of Tris-buffer (pH7.4) were added in a test tube. Then 1.0ml of 0.025M sodium oxalate was added. After mixing of above solution immediately due to the formation of turbidity and then up to the period of ten minutes to calculate the turbidity of solution by UV/Vis spectrophotometer at 620nm. This control experiment has been done in three replications.

STUDY WITH INHIBITOR

In this experiment, Add 1ml of 0.025M calcium chloride dihydrate, take 2ml Tri-buffer and 1ml (10 mg/ml solution) of ethanolic plant extract were poured in a two varies of test tubes. More than two test tubes were poured 1ml of 0.025M calcium chloride dihydrate, 2ml Tri-buffer and poly herbal formulation, Cystone. Then 1ml of 0.025M sodium oxalate was added to each test tube and then waits for 10 minutes to measure the change in turbidity of the solution by UV/Vis spectrophotometer at 620nm. Three times each procedure was done. Inhibition in stone nucleus formation was calculated by graphical method using the following formula:

Percentage inhibition = {1-[Si /Sc]} x 100

Where;

Si - slope of graph in the presence of inhibitors (drugs/plant extracts)

Sc - slope of without inhibitors (control)

RESULTS AND DISCUSSION

Phtyochemical analysis

The phytochemical analysis of the ethanolic extract of the seeds of *Achyranthes aspera* was performed by using standard protocol.

Invitro Anti-Urolithiatic Activity By Turbidity Method And Titrimetry Method

India, as in many less developed areas, phytotherapy is a common method of primary health care. *Achyranthesaspera*is widely distributed in southern parts of India. Some species of the genus *Achyranthes* have been used in the traditional system of medicine for the treatment of various diseases.

In kidney, stone formation, calcium oxalate and calcium phosphates, or other chemicals in the urine form crystals on the inner surfaces of kidneys. This stage is called is initial mineral phase formation. Over a period of time crystals may combine to form a small, or mass called as stones and stage is referred as crystal growth.[13-22]

Preliminary phyto-chemical analysis of ethanolic extract of seeds of *Achyranthes aspera* revealed the presence of various components flavanoids glycosides, quinines, coumarins, saponins and steroids. Active phyto chemical such as flavanoids and saponins are known to responsible for the diuretic activity.[29]

The percentage inhibition of calcium oxalate crystallization of ethanolic extract was calculated by using formula. Our present study clearly indicates the Ethanolic extract of *Achyranthes aspera* seeds showed calcium palate crystallization inhibition (83%) for the turbidity method. While Cystone a prescribed medicine for renal calculi showed highest inhibition (87%) in terms of formation of calcium oxalate precipitation.[30,28,27]

The % inhibition of turbidity (aggregation) in the presence of herb extract was lower than in the control, showing that crystals were less aggregated. The inhibited aggregation associated with the extract increased with concentration. This inhibition was greatest with ethanolic extract of seeds of *Achyranthes aspera*.

The ethanolic extract of seeds of *Achyranthes aspera* showed percentage dissolution of calcium oxalate(62%).While Cystone a prescribed medicine for renal calculi showed highest percentage dissolution (71%) of calcium oxalate (Fig.5 and values depicted in the table 3) by the Titrimetry method.

CONCLUSION

From the recent study we conclude that the primary phytochemical analysis of Achyranthes aspera specified the presence of Flavonoids, quinones, glycosides, coumarins and steroids.

In-vitro Calcium oxalate crystallization prohibition studies were evaluated. From this study conclude that the Ethanolic extract of seeds of Achyranthes aspera. Prohibition the calcium oxalate crystallization in 83% respectively. In-vitro turbidity methods were evaluated. From this study conclude that the ethanolic extract of Achyranthes aspera prohibit the

calcium oxalate nucleation in 62% respectively. The ethanolic extract produces more potent Anti Urolithiatic activity. In the present study provided basic evidence that the plant, Achyranthes aspera possess potent Anti Urolithiatic activity.

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Table.1: In-vitro Anti- Urolithiac Activity for Ethanolic extract of Achyranthes aspera

S.No.	Concentration (µg/ml)	OD at 620nm	% Inhibition
1	200	1.32	46
2	400	1.29	53
3	600	1.26	61
4	800	1.19	78
5	1000	1.17	83

Table.2: In-vitro Anti- Urolithiac Activity with cystone as a reference

S.No.	Concentration(µg/ml)	OD at 620nm	% Inhibition
1	200	1.30	51
2	400	1.29	56
3	600	1.23	58
4	800	1.17	83
5	1000	1.15	87

Table 3: Invitro Anti-Urolithiatic activity of Achyranthes aspera by Titrimetry

S.No.	Sample	Dissolved calcium oxalate	% Dissolution
1	Ethanolic extract of *Achyranthes aspera*	0.620	62%
2	Cystone	0.715	71%

Figure 1 : Effect of ethanolic extract Achyranthes aspera on calcium oxalate crystallisation inhibition

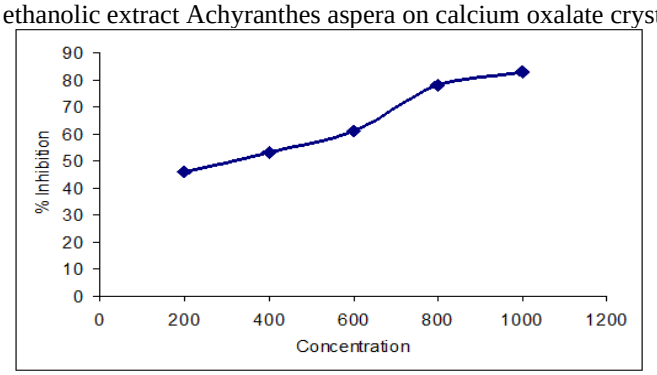


Figure 2: Effect of Cystone Standard on calcium oxalate crystallisation inhibition

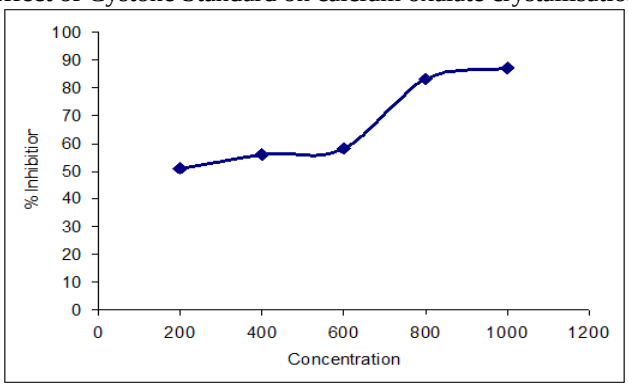


Figure.3: Comparison of Ethanolic Extract Achyranthes aspera and Cystone Standard

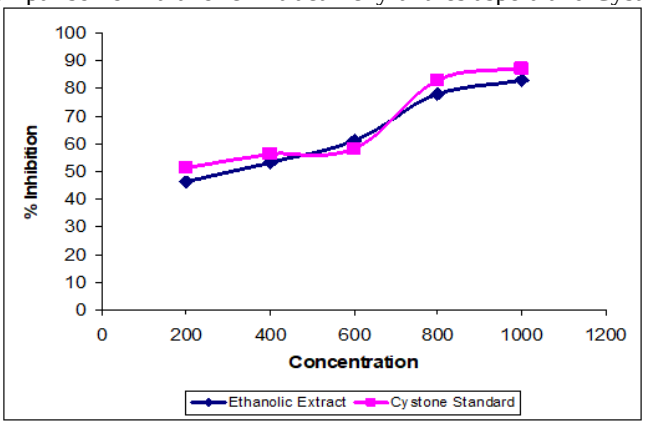
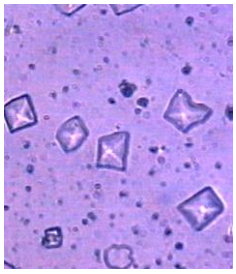
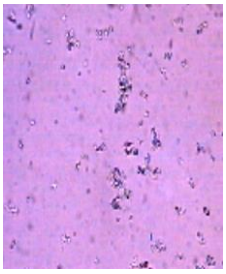


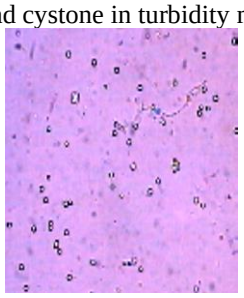
Figure.4: Shown formation of calcium oxalate nucleus and inhibition by plant extract and cystone in turbidity method.



Without inhibitor

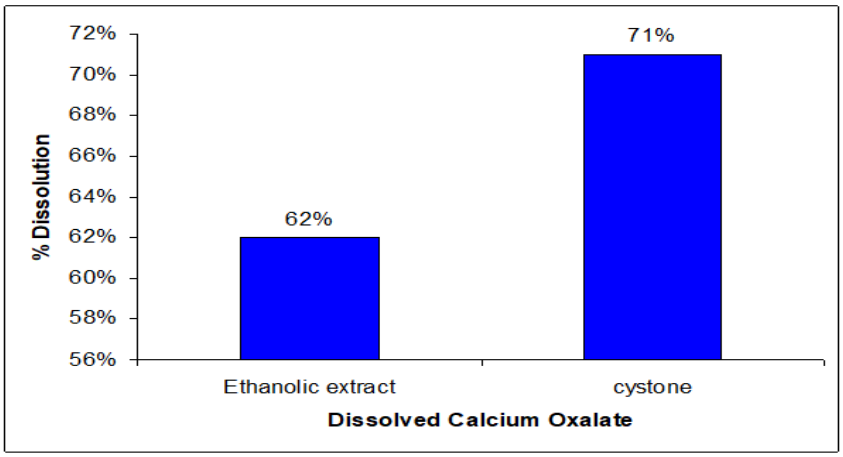


Ethanol extract of Achyranthes aspera



Cystone

Figure.5: Calcium oxalate dissolved with ethanolic extract of Achyranthes aspera and standard cystone


72%
70%
68%
66%
64%
62%
60%
58%
56%
% Dissolution
62%
71%
Ethanolic extract
cystone
Dissolved Calcium Oxalate