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PHYTOCHEMICAL, HPTLC STUDIES AND ANTIUROLITHIATIC *ACTIVITY* OF HYDROALCOHOLIC EXTRACT OF *IXORA COCCINEA*

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ABSTRACT

The present study was carried out to investigate the effect of ethanolic extract of *Ixoracoccinea* (Family: Rubiaceae) on calcium oxalate crystallization in urolithiasis. Calcium oxalate crystallization was induced by the addition of 0.01M sodium oxalate solutions in synthetic urine. The effect of various concentrations of extract (50, 100, 150, 200 and 250 µg/ml) was studied by measurement of turbidity in presence or absence of inhibitor (extract) at 620 nm after ten minutes by means of a spectrophotometer.Phytochemical investigation of ethanolic extract of Ixora*coccinea*leaves revealed the presence of alkaloids, glycosides, proteins, tannins, flavonoids, carbohydrates and coumarins. HPTLC analysis confirms the presence of phytoconstituents. The hydroalcoholic extract of leaves of *Ixoracoccineahas* inhibitory effect on Calcium Oxalate crystallization.

Key Words: *Ixoracoccinea,* anti urolithiatic activity, hydroalcoholic extract

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INTRODUCTION

Urinary stones affect 10–12% of the population in industrialized countries. There are only a few geographical areas in which stone disease is rare, e.g., in Greenland and in the coastal areas of Japan. Urinary calculi are the third prevalent disorder in the urinary system. The incidence of urinary stones has been increasing over the last years while the age of onset is decreasing. With a prevalence of > 10% and an expected recurrence rate of 50%, stone disease has an important effect on the healthcare system. Once recurrent, the subsequent relapse risk is raised and the interval between recurrences is shortened. Features associated with recurrence include a young age of onset, positive family history, infection stones and underlying medical conditions.In addition, the incidence of kidney stones has been increased in western societies in the last five decades. Urinary stones, in both the bladder and the kidney, have been reported in Egyptian mummies dating from 4800 bc, and urolithiasis has long been studied in an effort to understand its pathogenesis and to refine treatment strategies. Although urolithiasis has been recognized in children for centuries, the clinical picture, evaluation, and management continue to evolve. Approximately 7% of all stones occur in children younger than 16 years.Traditionally, urolithiasis was characterized by bladder calculi in children of developing countries; the incidence of upper tract calculi, occurring mainly in industrialized areas, was much lower in children than in adults. Also, in comparison with adult stone formers, children

were more likely to demonstrate risk factors outside the metabolic realm such as UTI, anatomic abnormalities, and surgical alterations in the urinary tract. Currently, the incidence of upper tract calculi in children without these predisposing factors is on the rise worldwide,and the paradigms are changing. The importance of metabolic evaluation in children with urolithiasis has been shown, even in countries thought to have primarily endemicbladder stone disease based on diet. With the introduction of smaller endoscopic instruments and the refinement of extracorporeal shock wave lithotripsy (ESWL) technology, treatment of pediatric stone disease now closely parallels stone management in adults.(1-5).

Urolithiasis affects about 10-12% of the population in the industrial countries and it accounts for third prevalent disorder in the urinary system. Urolithiasis is evident from traditional periods with a multifactorial etiology.In the management of urolithiasis, modern techniques like Extracorporeal Shock Wave Lithotripsy (ESWL), Ureteroscopy (URS), Percutaneous Nephrolithotomy (PNL), Open surgery show significant side effects such as renal damage, hypertension, Renal tissue necrosis. As the investigations proved that the alternative medicine i.e., Herbal medicine shows some potent litholytic activity and in preventing the reoccurrence with less side effects as compared with conventional urolithiatic management techniques. Hence, the present work aims in proving the *in-vitro* anti urolithiatic activity of ethanolic extract of *Ixoracoccinea with* potent litholytic action as an alternative theraphy, herbal medicine in order to alleviate the adverse effects of conventional therapy.The present study was carried out to investigate the effect of ethanolic extract of *Ixoracoccinea* (Family: Rubiaceae) on calcium oxalate crystallization in urolithiasis.

MATERIALS AND METHODS

Plant Material Collection&Identification

The leaves plant of *Ixoracoccinea*was collected from District of West Godavari, Andhra Pradesh. The plant material was dried under shade and passed through 40 mesh sieveand stored in air tight container.

Extraction

The powdered plant material was subjected to extraction with ethanol as a solvent such as in a soxhlet apparatus by continuous hot extraction for 24 hr. The extract was evaporated to dryness in a rotary flash evaporator at a temperature not exceeding 60^0C. Phytochemical tests were carried out following with the standard procedures.

HPTLC analysis of the crude extract

Preparation of the Sample solution

1gm of freeze dried hydroalcoholic extract powder was mixed with 10ml of methanol and completely dissolved and filtered using whatman filter paper of grade 1. The filtrate was used for the HPTLC analysis.

Preparation of the Standard solution

1gm of Quercetin powder was dissolved in 10ml of methanol and filtered using whatman filter paper of grade 1. The filtrate was used for further studies.

Procedure (6)

TLC was performed on Silica gel 60F 254 TLC plates (E Merck, Germany) with Toluene: Ethyl acetate: Methanol: Formic acid in the ratio of 5.5:3:1:0.5(v/v) as mobile phase. The standard and the prepared samples were applied to the plates as 6mm wide from the bottom, by means of pressurized nitrogen gas (150 kg/cm^2) through CAMAG Linomat V fiited with 100µl syringe. Ascending development was performed in a twin–trough glass chamber (10x10cm) obtained from CAMAG which is previously saturated with the mobile phase for 30 minutes at room temperature (25±2ºC) and relatively humidity (60±5%) for a distance of 10cm. The bands were visualized in CAMAG UV Cabinet at 254nm and 366nm and scanned through WINCATS 4 software. The results of the HPTLC analysis were given in Table-1.

ANTI UROLITHIATIC ACTIVITY

Preparation of artificial urine (7,8)

The artificial urine (AU) was prepared according to the method Burns and Finlayson and had the following composition: sodium chloride 105.5 mmol/l, sodium phosphate 32.3 mmol/1, sodium citrate 3.21 mmol/l, magnesium sulfate 3.85 mmol/1, sodium sulfate 16.95 mmol/1, potassium chloride 63.7 mmol/1, calcium chloride 4.5 mmol/1, sodium oxalate 0.32 mmol/1, ammonium hydroxide 17.9 mmol/1, and ammoniumchloride 0.0028mmol/1. The AU was prepared fresh each time and pH 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. The 0.5 ml of 0.01M sodium oxalate was added, to the previous volume, and the measurement is made after a period of ten minutes. For each experiment, six replicates were taken.

Study with inhibitor

The extract was dissolved in distilled water, filtered through membrane filter and the concentration of 50, 100, 150, 200 and 250µg/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 0.01M sodium oxalate solution was added and immediately the absorbance was measured after a period of ten minutes at 620nm. For each experiment, six replicates were taken

The percentage of inhibition was calculated using the following formula:

%inhibition = {1-[Ai / Ac]} X 100

Where; Ai: Absorbance in the presence of inhibitor(Extract), Ac: Absorbance without inhibitor (Control).

RESULTS AND DISCUSSION

HPTLCAnalysis of HydroalcoholicExtractof*Ixoracoccinea*

The finger print of the constituents present in hydroalcoholic extract was recorded using CAMAG and TLC visualiser and WinCats Software. The phytocompound isolated at various peaks, Rf value, peak height and peak area were identified and presented in the Table-1 and Fig-1, 2. Hence, hydroalcoholic extract was chosen for the pharmacological evaluation. Those phytocompound were responsible for the specified pharmacological activity.

Table-1HPTLC analysis of hydro alcoholic extract of *Ixoracoccinea*

Peaks	Rf	Peak Height	Peak Area
Peak 1	0.52	172.69	2466.66
Peak 2	0.52	314.77	4755.46
Peak 3	0.52	397.00	6355.07
Peak 4	0.52	457.34	7620.33
Peak 5	0.52	505.28	8801.24
Peak 6	0.52	523.01	9542.78
Peak 7	0.51	60.76	736.37

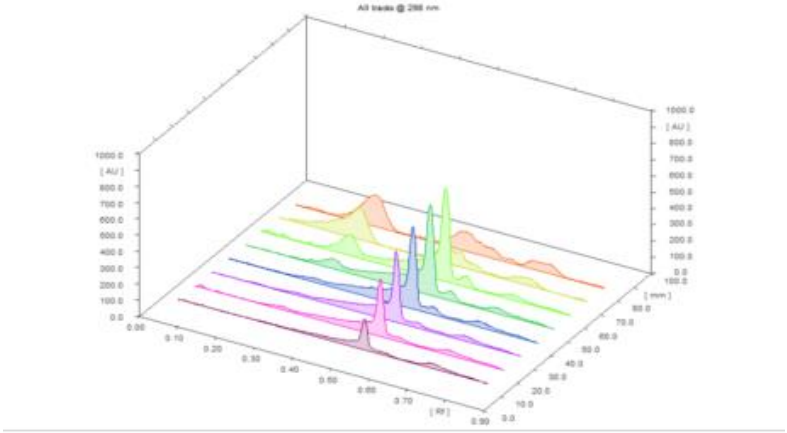

Fig-1 HPTLC 3D chromatogram of Hydroalcoholic extract of *Ixoracoccinea*

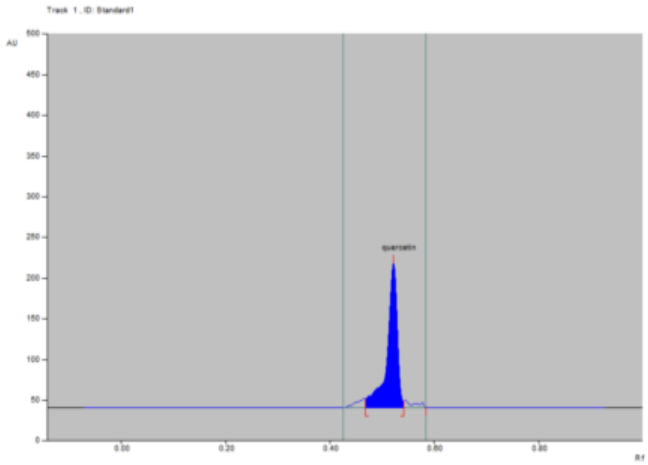

Fig-2 HPTLC Chromatogram of Standard Quercetin

IN-VITRO ANTIUROLITHIATIC ACTIVITY

*In-vitro*antiurolithiatic activity of ethanolic extract of leaves of *Ixoracoccinea* plant was studied by using UV spectrophotometric method. UV spectrophotometric method uses the turbidity measurement in the presence of inhibitor(extract) and without inhibitor at 620nm by using artifificial urine in which the calcium oxalate crystals were induced by adding sodium oxalate solution.

Table-2 Percentage of inhibition of calcium oxalate crystals by ethanolic extract of *Ixoracoccinea*

S.NO	Extractof*Ixoracoccinea (Linn)*	% Inhibition of Calcium Oxalate Crystals*				
		50Mgm/ml	100Mgm/ml	150Mgm/ml	200Mgm/ml	250Mgm/ml
1.	Ethanolic Extract	27.51%	32.61%	40.28%	52.9%	63.1%

***All values are triplicate.**

So, by the above spectroscopic observations it has been evident that the ethanolic extract of *Ixoracoccinea* shows significant in-vitro anti urolithiatic activity by inhibiting the calcium oxalate crystals formation. It has been noticed that the ethanolic extract shows significant inhibition in calcium oxalate crystals upto 63.1% with 250µg/ml concentration than the other concentrations of extract (Table-2 and fig-3).

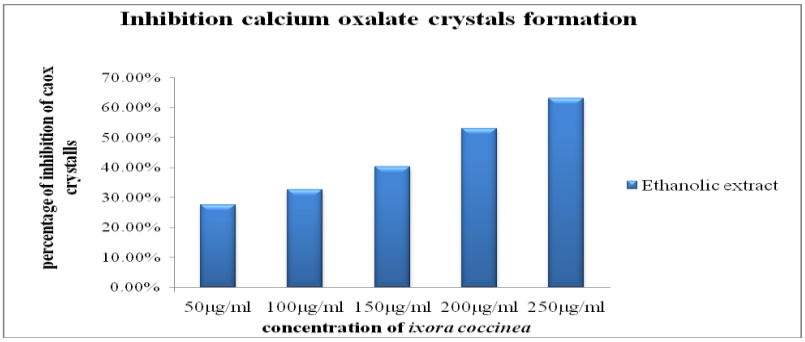

Fig-3 Effect of ethanolic extract of Ixora*coccinea*(leaves) on inhibition calcium oxalate crystals formation

CONCLUSION

Phytochemical investigation of ethanolic extract of Ixora*coccinea* leaves revealed the presence of alkaloids, glycosides, proteins, tannins, flavonoids, carbohydrates and coumarins. HPTLC analysis confirms the presence of phytoconstituents. The

hydroalcoholicextract of leaves of *Ixoracoccineahas* inhibitory effect on Calcium Oxalate crystallization. But further studies are needed to evaluate, the *in-vivo* anti-urolithiatic activity and also to evaluate the exact component is responsible for the potent anti-urolithiatic activity.

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