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INVITRO ANTI-UROLITHIATIC ACTIVITY OF *ALPINIA GALANGA* ROOT EXTRACT

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ABSTRACT

The present study have been designed to evaluate the Invitro Anti-urolithiatic activity of herbal plant *Alpinia galangal* belonging to the family zingiberaceae. The dried powder of roots were collected and extracted by Soxhlet with solvents like methanol and chloroform. To investigate the inhibitory effect of extract on in invitro crystals through analysing aggregation assay. Materials and methods: Aqueous extraction of *Alpinia galanga* powder was prepared and arranged in the different concentrations. Homogenous precipitation methods was used to prepare artificial stones such as calcium oxalate and semi-permeable membrane of egg was used as dissolution bags. Dissolution model were incubated in 72hrs and after that, the entire content in dissolution bags was estimated spectrophotometrically. The inhibitory activity of *Alpinia galangal* powder was extracted on the aggregation assay of calcium oxalate crystals was determined by spectrophotometric assay. The percentage of Anti-urolithiatic activity is obtained as 74%,75%,76%,78%,80% for chloroform extract and 74%,75%,77%,78%,79% for methanol extract respectively at a dose of 50 mg/ml,100 mg/ml, 150 mg/ml, 200 mg/ml, 250, mg/ml. The percentage of Standard Neeri was found out to be 81%,82%,83%,84%,85% respectively at a dose of 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml. chloroform extract was more effective than methanol extract.

INTRODUCTION

Urolithiasis, formation of kidney stones presence of one or more calculi in any location within the urinary tract is one of the oldest and wide spread disease known to man.

Urolithiasis refers to the solid non-metallic minerals in the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate. Urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidney. Nephrolithiasis are renal stones disease remains a significant health problem in the adult population with serious medical consequence, throughout a patient lifetime.[1] The world wide incidence of urolithiasis is quite high, and more than 80% of urinary calculi are calcium oxalate stones alone are calcium oxalates mixed with calcium phosphate kidney stones formation is a complex process which is the outcome of several physiochemical events such as super saturation, nucleation, crystals growth, aggregation and retention.

Plant provides food, raw materials for medicine and various other requirements for the very existence of life from the origin of human being. The majority of the global population utilizers medicinal plant from their health care.[2] Even the current conventional medicine is using a lot of plant derived chemicals as therapeutic agents. Herbs and herbal drugs have created interest among the people by its clinical proven effects. *Alpinia galangal* belongs to zingiberaceae and commonly called as Rhizome plant. It is oldest Indian herbal drug, which is included in our present study is widely used by tribal people. Ayurvedic system has already noticed the importance of this plants. It has several experimentally proven pharmacological activities which include Anti tumor, Anti-microbial activity Anti inflammatory,Anti rheumatoid activity.[3] The Rhizome has already proved anti- urolithiatic so based on the review the present study was carried out anti urolithiatic activity of powder of *Alpinia galangal.*

MATERIALS AND METHOD

Aggregation assay

The rate of aggregation of the calcium oxalate crystals was determine by a spectrophotometric assay,[4] with slight modifications. The calcium oxalate monohydrate [COM] crystals were prepared by mixing both the solution of calcium chloride and sodium oxalate of 50 mM each. Both solution were then cooled to 37^{o}C and then evaporated. The COM crystals were then dissolved with 0.5ml of 0.05mM Tris buffer 0.5ml of 0.15mM NaCl solution at pH 6.5 two a final concentration of 1mg/ml absorbance at 620nm was recorded. The rete of aggregation was estimated by comparing the slope of turbidity in the presence of the extract against control.

Preparation of the semi-permeable membrane from eggs

Apex of eggs was puncture by a glass rod in order to squeeze out the entire content. Empty eggs where washed thoroughly with distilled water and an egg shell was placed in a petric plate consisting concentrated Hcl,[5] which led to the complete decalcification of semi-permeable membrane was remove carefully from eggshell and with distilled water.

Synthesis of calcium oxalate by homogenous method

10mg of calcium chloride dihydrate in 10ml of H_2 and 10mg of sodium oxalate in 10ml of H2SO4. Both were mixed equally with urea and dried at a temperature 60c for 2hours.[6]

Preparation of 0.02M KMnO4 solution

0.32gm of KMnO4 was dissolved in 100ml of distilled water. It was boiled for 30minutes. After cooling, MnO4 was removed by filtration.

METHOD

GROUP 1:1ml of calcium oxalate [1mg/ml] + 1ml of distilled water

GROUP 2:1ml of calcium oxalate [1mg/ml] + 1ml of Neeri solution [400mg/ml]

GROUP 3: 1ml of calcium oxalate [1mg/ml] + 1ml of hot aqueous extract of *Alpinia galangal* [20mg/ml]

All groups where paced in together in egg semi permeable membrane tied with thread at one end and where suspended in a conical flask containing 150ml 0.1M tris buffer each. At another end of thread tied by a stick place on the mouth of conical flask and covered with aluminium foil all group where kept in an incubator, preheated at 37^0C for 4 hours, kept for 3 days. The enter contain of each group was remove form sutured semipermeable membrane and was transfer in to test tube individually.

4ml of 1N H_2so_4 60 to 80 µg litres of 0.02M $kMnO_4$ were added and kept aside for 2 hours colour change form dark pink to colourless was observed after 2 hours change of colour intensity was measured against 620nm spectrophotometrically.[7] Concentration of undissolved calcium was determined form standard calibration curve of calcium oxalate by using the mmeasured absorbance readings.

Figure 1: Incubated Samples.

RESULTS AND DISCUSSION

In kidney stone formation, calcium oxalate in the urine form crystals on the inner surface of kidneys. This stage is called as initial mineral phase formation. Over the period of time crystals may combined to form a small, hard mass called as stones and stage is referred as crystal growth.[8] Calcium oxalate stones have classified into two types:

1. Calcium oxalate monohydrate stones (COM)
2. Calcium oxalate dihydrate (COD)

Aggregation assay

Calcium oxalate crystals begin grow; aggregate with other crystals and retained in the kidney. This is aggregation process which causes renal injury.[9] The extract of *Alpinia galangal* demonstrated slightly better compared to Neeri standard solution to inhibit promoted the formation of COD crystals. COM has a stronger affinity with cell membranes; it may lead to become higher potential risk for renal ca lculi formation.

S. No.	Plant constituent	Test	Methanol and chloroform rhizome extract of *Alpinia galanga*
1	Alkaloids	Mayers test	-ve
2	Glycosides	Killer killiani test	++ ve
3	Steroids	Libermannburchards test	++ve
4	Flavanoids	Shinoda test	++ve
5	Saponins	Foam test	+ve
6	Carbohydrates	Molish's test	+ve

Where +ve = positive, -ve = negative

Figure 2: Phytochemical Test Samples.

S. No.	Groups	Concentration [mg/ml]	Absorbance [nm]	% inhibition
1.	Control	-	0.105	-
2.	Neeri	10	0.560	81%
		20	0.590	82%
		30	0.622	83%
		40	0.680	84%
		50	0.702	85%
3.	MEAG	10	0.415	74%
		20	0.430	75%
		30	0.457	77%
		40	0.478	78%
		50	0.512	79%
4.	CFAG	10	0.410	74%
		20	0.425	75%
		30	0.440	76%
		40	0.480	78%
		50	0.525	80%

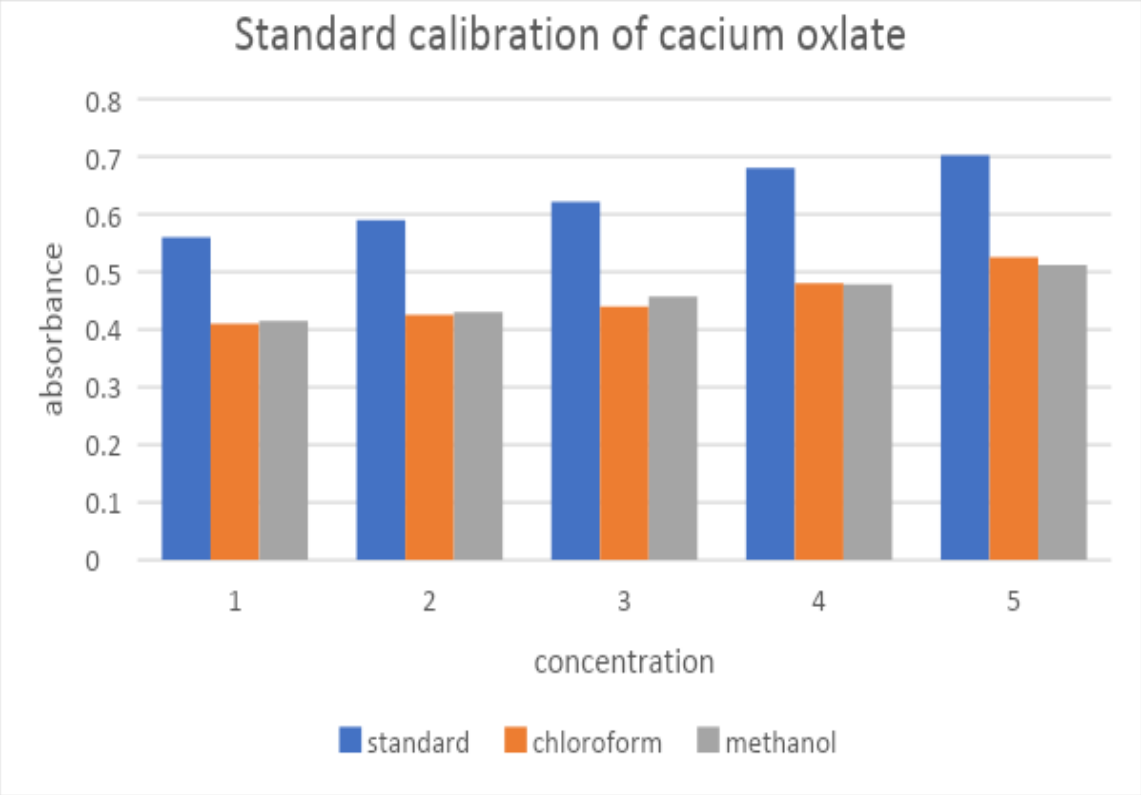

Figure 3: Percentage inhibition calcium oxalate crystals.

X-AXIS: Concentration of standard and sample extracts.

Y-AXIS: Inhibition of calcium oxalate crystals

MEAG: Methanol extract of *Alpinia galanga*rhizomeextract

CFAG: Chloroform extract of *Alpinia galanga rhizome extract*

CONCLUSION

Alpinia galangal extract exhibited significant *in-vitro* anti-urolithiaticactivity. Therefore, our present invitro studies on methanol and chloroform extracts of *Alpinia galanga*root demonstrated the significant anti -urolithiatic. Due to the presence of active principles such as alkaloids, glycosides, flavonoids may responsible for this activity.

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