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

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ABSTRACT

The present study have been designed to evaluate the *In-vitro* Anti-urolithiatic activity of herbal plant *Alpinia galanga* 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 galanga* 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 50 mg/ml,100 mg/ml,150 mg/ml,200 mg/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 lifetime1. 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 care2. 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 galanga* 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 includes Anti-tumor, Anti-microbial activity, Anti-inflammatory, Anti-rheumatoid activity3. The Rhizome has already proved anti- urolithiatic so based on the review the present study was carried out anti-urolithiatic activity of *Alpinia galanga*.

MATERIALS AND METHOD

Aggregation assay

The rate of aggregation of the calcium oxalate crystals was determined by spectrophotometric assay4. The calcium oxalate monohydrate [COM] crystals were prepared by mixing both the solution of calcium chloride and sodium oxalate of 50 mM each. Both solutions were then cooled to 370C 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 6.5and a final concentration of 1mg/ml at 620nm was recorded. The rate 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 were punctured by a glass rod in order to squeeze out the entire contents. Empty egg shells were washed thoroughly with distilled water and each egg shell was placed

in a petri-plate containing concentrated Hcl. It allows complete decalcification of eggshells forming semi-permeable membranes which were removed carefully and wash with distilled water5.

Synthesis of calcium oxalate by homogenous method

10mg of calcium chloride dihydrate mixed into 10ml of H2SO4 and 10mg of sodium oxalate into 10ml of H2SO4. Both were mixed equally into the artificial urine and dried at a 600c temperature for 2hours6.

Preparation of 0.02M KMnO4 solution

About 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 galanga* [20mg/ml]

All these groups were placed into egg's semi permeable membranes tied with thread at one end and were suspended in a conical flask containing 150ml 0.1M tris buffer each. Another end of the thread tied by a stick place on the mouth of conical flask and covered with aluminium foil. All groups were kept in an incubator, preheated at 370C for 4 hours, kept for 3 days. The entire contents of each group were removed from sutured semipermeable membranes and were transferred into test tubes individually.

4ml of 1N H2SO4 and 80 µl of 0.02M KMNO4 were added and kept aside for 2 hours. Colour change from dark pink to colourless was observed after 2 hours. Change in the colour intensity was measured against 620nm spectrophotometrically7. Concentration of undissolved calcium was determined from standard calibration curve of calcium oxalate by the measured 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 growth8. Calcium oxalate stones have classified into two types:

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

Aggregation assay

Calcium oxalate crystals begins to grow, aggregate with other crystals and retain in kidney. This is aggregation process causes renal injury9. The extract of *Alpinia galanga* demonstrated inhibitory activity on formation of COD crystals better compared to Neeri standard solution . COM has a stronger affinity with cell membranes, it may lead to become higher potential risk for renal calculi formation.

Table 1: Phytochemical Screening.

S. No.	Plant constituent	Test	Methanol and chloroform 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

Figure 2: Phytochemical Test Samples.

S. No.	Groups	Concentration [mg/ml]	Absorbance	% 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.	**CEAG**	**10**	**0.410**	**74%**
		20	**0.425**	**75%**
		30	**0.440**	**76%**
		40	**0.480**	**78%**
		50	**0.525**	**80%**

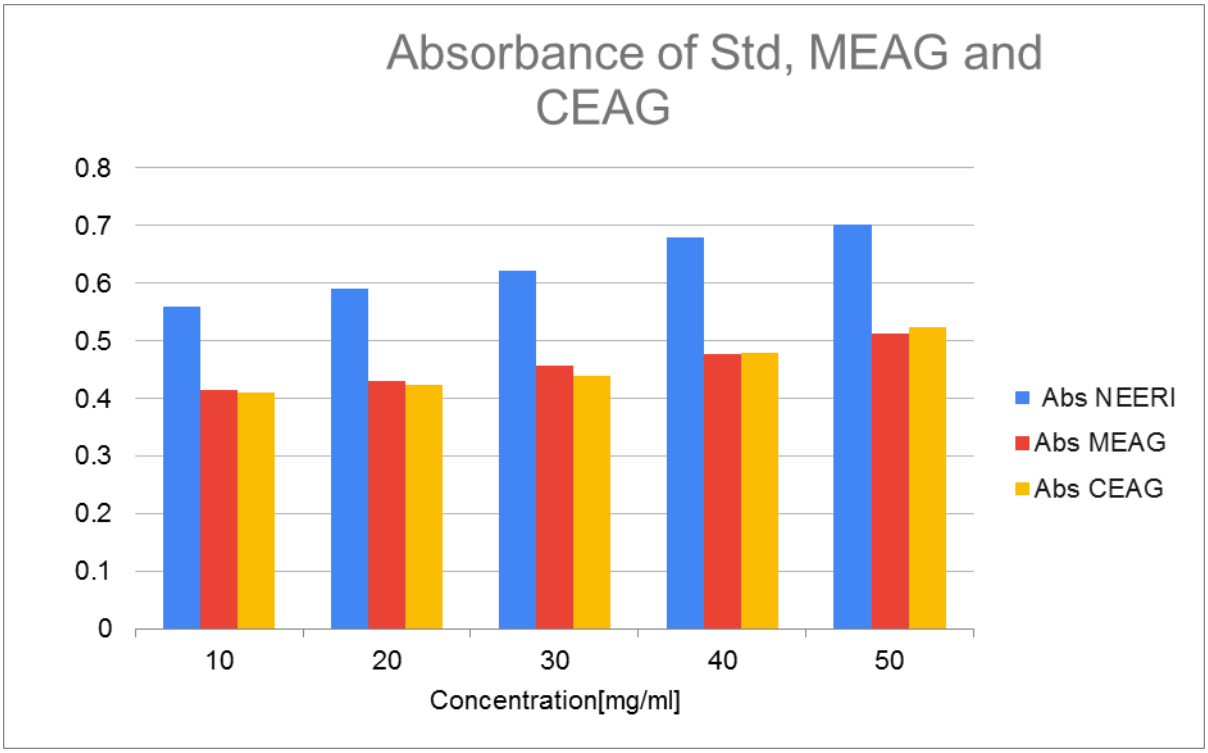

Figure 3: Percentage inhibition calcium oxalate crystals.

X-AXIS: Concentration of standard and sample extracts.

Y-AXIS: Inhibition of calcium oxalate crystals

MEAG: Methanol extract of *Alpinia galanga* rhizome extract

CEAG: Chloroform extract of *Alpinia galanga rhizome extract*

CONCLUSION

Alpinia galanga extract exhibited significant *in-vitro* anti-urolithiatic activity. Therefore, our present *in-vitro* 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.

REFERENCES

1. Mital SK, Gopumodhavan S, Venkataranganna MV, Sundaran R. Effect of Cystone, a herbal formulation glycolic acid-induced urolithiasis in rats. Phytother Res., 1998; 12: 372-4
2. Kirtikar KR, Basu BD. Indian Medical plants. Dehradun: International Book Distributors. [2nd ed], 1983; 1615-7.

3. Sathish CJ, Sharma RA, Jain R, MascaloN,Capasso F, vijayvergia R, et al.Ethnopharmacological Evaluation of Pergulariadaemia [Forsk.]Chiov. Phytother Res., 1998; 1: 378-80.
4. Sumayyasikandari, Prathima Mathad. Invito anti-urolithiatic activity of *Buteamonosperma Lam.* and *Nigella Sativa Linn.* Seeds. Ukaaz-Ann Phytomed, 2015; 4: 105-7.
5. Finkielstein VA, Goldfarb DS. Strategies for preventing calcium oxalate stones. CMAJ, 2006; 174[10]: 1407-1409.
6. Huges J, Norman RW. Diet and Calcium stones. Can *Med Asso J* 1992; 146: 137-143.
7. Verma P, Gauttam V, Kalia AN. Comparative Pharmacognosy of Pashanbhed. *J AyuverdaIntege Med,* 2014; 5[2]: 104-108.
8. Saha S, Verma RJ. Inhibition of calcium oxalate crystallization in *vitro* by an extract of *Bergenia ciliate. Arab Journal of Urology, 2013*; 11: 187-192.
9. Manish A. Patel, paras K. Patel, Avinash K Seth. Inhibition of calcium oxalate crystallization fruit extracts of *Piper nigrum L. Pharmacologyonline,* 2011; 2: 1169-1177.