

***In Vitro* Percent Aggregation Inhibition Potential of Selected Medicinal Plants**

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Introduction

Kidney stone formation depends on urinary volume, concentrations of calcium, phosphate, oxalate, sodium cystine and uric acid ions, concentrations of natural calculi inhibitors and urine pH (Menon and Resnick 2002). Kidney stones occur in gout, hypercalciuria, hyperparathyroidism, renal tubular acidosis and certain inherited metabolic condition like cystinuria and hyperoxaluria (Gilhotra *et al.*, 2013). Stone formation is one of the painful urologic disorders which occur in approximately 12% of the global population and its re-occurrence rate in males is 70%-81% and 47%-60% in female (Soundararajan *et al.*, 2006). It is reported that the level of contribution by different risk factors to stones formation varies among different populations. Many patients reportedly had more than one risk factor (Mute V.S., 2012).

Herbal medicines are in vogue (Isaacs, 2010). Herbalists narrated that the patients having kidney stones generally experience sudden acute pain. Acute pain is due to the blocking in the urinary tract; the symptom starts with incessant vomiting (Stamatelou *et al.*, 2003; Schoenstadt, 2008). The incidence of urinary stones has been increasing over the last years, while the age of onset is decreasing (Moe, 2006).

The medical administration of urolithiasis includes the surgical removal of stones. Among the treatments exercised are Extracorporeal Shock Wave Lithotripsy and drug treatment and surgical procedures such as ureteronoscopy (URS) or percutaneous nephrolithotomy (PCNL) and the combination of these techniques (Gurocak and Kupeli, 2006). The high cost, compelling data now a days suggest that the exposure to shock waves in therapeutic doses may cause decrease in renal function.

Management of stone disease depends on the size of calculi, severity of symptoms, degree of obstruction, kidney function, location of the stones and the presence or absence of associated infection influence the choice of one type of intervention over the other. Currently this serious problem can be treated with increased fluid intake, medications like thiazides, allopurinol (Atmani, 2003) and potassium citrate (Tracy and Pearle, 2009) for reducing urinary saturation of stone forming calcium salts, diuretics for facilitating urine flow.

In spite of substantial improvement in the pathophysiology and treatment of urolithiasis, there is no satisfactory drug offered for use in clinical therapy.

Indian medicine recommends numerous medicinal plants and exploits large number of plant drugs as ancient times to treat urinary stones. Ayurveda offers vast scope for the successful treatment of urolithiasis (Satish *et al.*, 2009). Herbal remedy may help to prevent, dissolve and expel kidney stones naturally. The Ayurvedic literature claims countless plants to be useful in urinary stones; even many plants need to be exploited for their pharmacological actions (Prasad, 2007). As a results, the search for antilithiatic drugs from natural sources has assumed superior importance.

Material and Methods

Selection of Plant Parts

The medicinal plant *Viz.* *Abrus precatorius* L. (White seeds), *Abrus precatorius* L. (Black seeds) *Abrus precatorius* L. (Red seeds), *Tamarindus indica* L. (Fruit shell), *Tectona grandis* L. (Seeds), *Chenopodium album* L. (Leaf) and *Abutilon indicum* L. (Leaf) were selected for inhibition of calcium oxalate crystallization activity based on the literature survey.

Drugs and Chemicals

Water, ethanol, chloroform and petroleum ether, Methanol all Chemicals used were of analytical grade. Sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$), Calcium chloride dehydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), Sodium chloride (NaCl), Gallic acid, Rutin, DPPH. etc

Preparation of Plant Extract by using Soxhlet method.

The 50g powdered plant material were extracted by using soxhlet extraction method. The plant material were extracted in solvents water, Ethanol, Chloroform and Petroleum ether in a soxhlet apparatus for 9 hours. The extract were concentrated by using rotavapor. Were each extract stored at 4 °C for further use.

In Vitro Calcium Oxalate Crystallization Inhibition

The precipitation of calcium oxalate (CaOx) at pH 6.5 and 37°C has been examined by the measurement of optical density (Turbidity) at 620nm. A UV/V is (SHIMADZU 1800) spectrophotometer were employed to measure the developed turbidity as a result of formation of calcium oxalate as per the procedures of Bensatal A., Ouahrani M.R., (2008); Sathya M., Kokilavani R. (2012)

Aggregation assay

The method used for aggregation described by (Atmani *et al.*, 2003; Paras *et al.*, 2012) was modified. Calcium oxalate (CaOx) crystals were made by mixing sodium oxalate and calcium chloride (CaOx) at 50mmol/l. Both solutions were equilibrated to 60°C in a water bath for 1 hour and then cooled to 37°C overnight. The crystals formed were harvested by using centrifugation and then evaporated at a temperature of 37°C. Calcium oxalate (CaOx) crystals were used at a final concentration of 0.8mg/ml, buffered with NaCl 0.15mol/l, Tris-HCl 0.05mol/l and at pH 6.5. Experiments were carried out at 37°C in the presence and absence of the plant extract. The percentage aggregation inhibition were calculated by comparing the turbidity in the presence of Plant extract at different concentrations.

with that obtained in the control using following formula:

$$1 - \text{Turbidity sample} \times 100$$

$$\% \text{ inhibition} = \frac{\text{Turbidity control} - \text{Turbidity sample}}{\text{Turbidity control}} \times 100$$

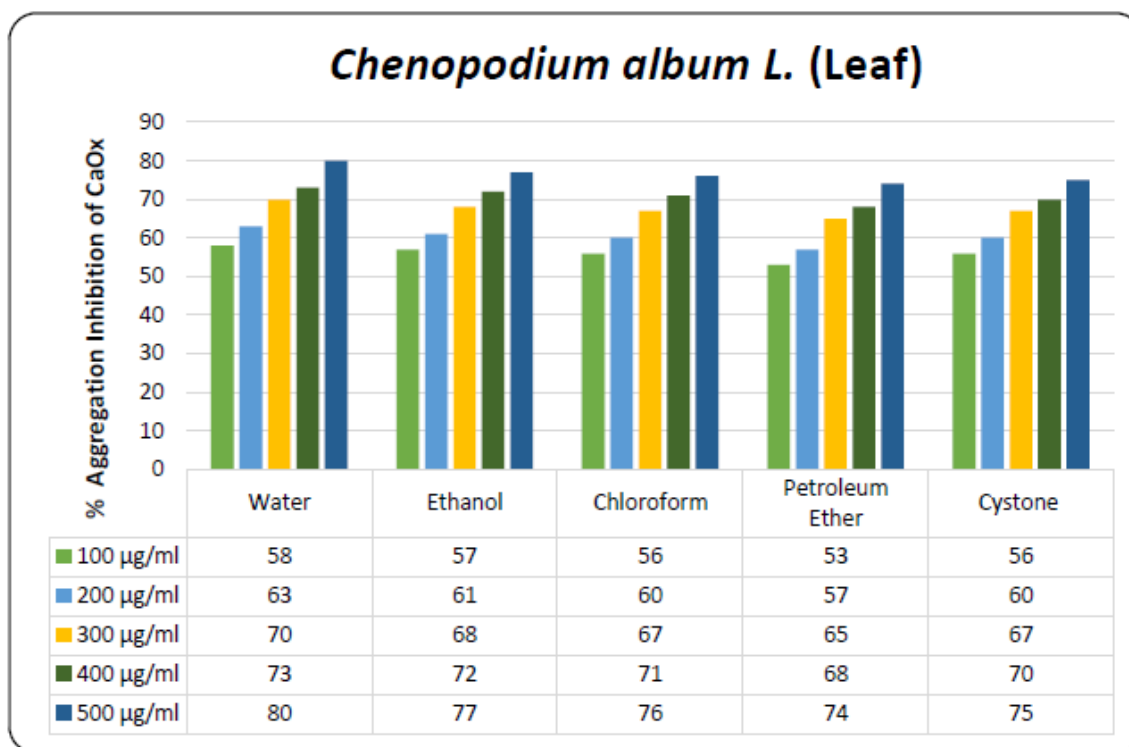
Result and Discussion

Aggregation Inhibition CaOx Crystallization by *Chenopodium album* L. (Leaf)

The Percent Inhibition of Calcium Oxalate Crystal aggregation by *Chenopodium album* L. (Leaf) was studied and the results represented in Figure 1. The leaf extract inhibited the crystal aggregation while in absence of these leaf extract the crystals were extremely aggregated.

The petroleum ether extract of all tested concentrations showed lesser action of percent aggregation of inhibition as compared to standard. The chloroform extract from 100 to 300 µg/ml concentration showed similar potential in percent aggregation of inhibition as compared to standard and the concentration of 400 and 500 µg/ml showed more activity of percent aggregation of inhibition as compared to standard and shoed similar potential in the action. The ethanolic extract of 100 to 300 µg/ml concentration have the similar potential and showed more activity in percent aggregation of inhibition as compared to standard drug. The ethanolic extract of 400 and 500 µg/ml concentration showed similar potential and as compwere to standard these showed more percent aggregation of inhibition. The aqueous extract of all the test concentrations showed positive results of percent aggregation of inhibition as compared to standard. The 200 and 400 µg/ml concentrations showed the similar potential of percent aggregation of inhibition.

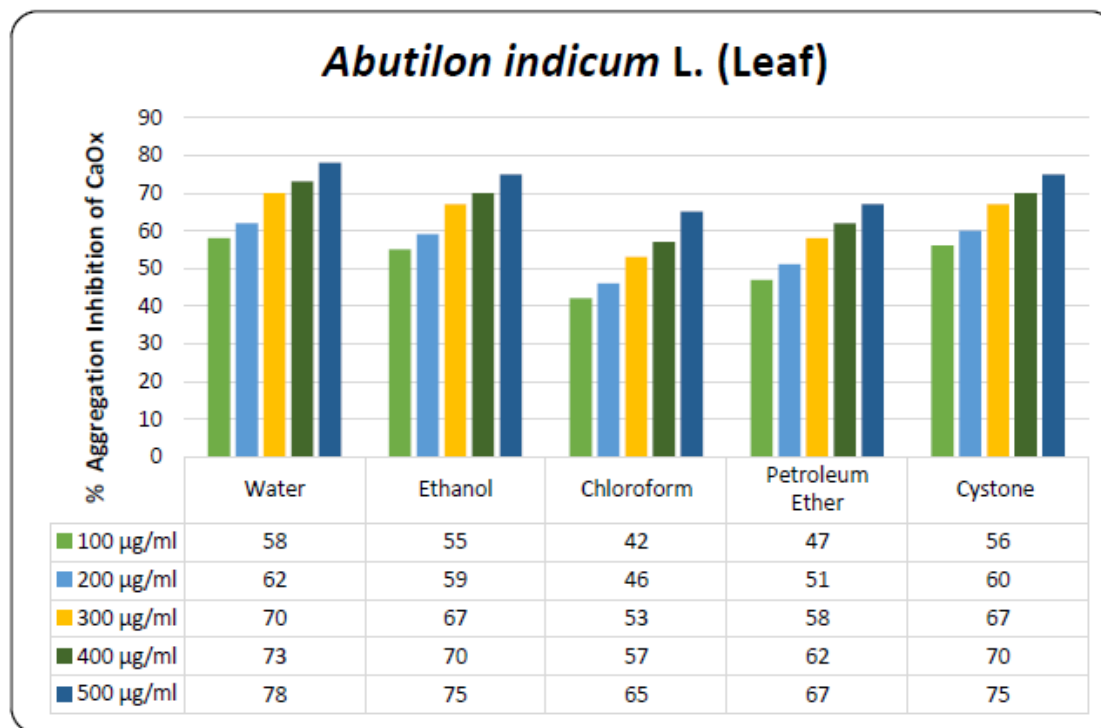
It is interesting to note that water and ethanolic extracts showed positive results in the percent aggregation of inhibition and 400 and 500 µg/ml concentrations of chloroform extracts also showed the positive results. 500 µg/ml concentration of aqueous extract showed highest in the percent aggregation of inhibition whereas the petroleum ether extract showed the negative results in the percent aggregation of inhibition as compared to standard.



Aggregation Inhibition CaOx Crystallization by *Abutilon indicum* L. (Leaf)

The Percent Inhibition of Calcium Oxalate Crystal aggregation by *Abutilon indicum* L. (Leaf) was studied and the results represented in Figure 2. The leaf extract inhibited the crystal aggregation while in absence of these leaf extract the crystals were extremely aggregated

The chloroform and petroleum ether extract showed the negative results of percent aggregation of inhibition in all the test range of concentrations as compared to standard but the minimum percent aggregation of inhibition is observed in the chloroform extract. The ethanolic extract of 100 and 200 µg/ml concentrations showed the negative action in the percent aggregation of inhibition as compared to standard. The 300 to 500 µg/ml concentrations of ethanolic extract showed the similar potential in the percent aggregation of initial as compared to standard. The aqueous extract s in all the test concentrations showed the positive results in percent aggregation of inhibition. The 300 to 500 µg/ml concentrations of aqueous extract showed the similar potential in percent aggregation of inhibition and this extract have the maximum potential in percent of inhibition aggregation as compared to standard.



Conclusion

The % Inhibition of Calcium Oxalate Crystal aggregation by *Chenopodium album* L. (Leaf) was studied and It is interesting to note that water and ethanolic extracts showed positive results in the percent aggregation of inhibition and 400 and 500 µg/ml concentrations of chloroform extracts also showed the positive results. 500 µg/ml concentration of water extract showed highest in the percent aggregation of inhibition

The % Inhibition of Calcium Oxalate Crystal aggregation by *Abutilon indicum* L. (Leaf) was studied and the water extracts in all the test concentrations showed the positive results in percent aggregation of inhibition.

Referances

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