Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 8
ISSN: 2321–3647(online)

Evaluation of Selected Medicinal Plants for Its Antilithiatic Potential under *In Vitro*

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ABSTRACT

Kidney stones are hard mineral and crystalline material formed within the kidney or urinary tract as a result of an imbalance between inhibitors and promoters of the kidney. Traditional medicinal practices have been known for centuries and are being exploited today for treatment without the risk of unwanted side effects. Extract of *Tribulus terrestris* (leaf), *Aerva lanata* (Flower), *Scoparia dulcis*(leaf), *and Tridax procumbens* (leaf), with solvents of different polarity was used to study its inhibitory effect against crystal nucleation, growth and aggregation. Based on results of the analysis, it can be concluded that there was a concentration dependent action of the extracts against the crystals. The *Aerva lanata* aqueous extract was found to be most effective in inhibiting all the three critical stages of stone formation.

Keywords: nucleation, growth, aggregation, calcium oxalate stones, lithiasis.

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Received 05 August 2014, Accepted 14 August 2014

Please cite this article in press as: Nirmaladevi R *et al.*, Evaluation of Selected Medicinal Plants for Its Antilithiatic Potential under *In Vitro* . American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Kidney stone is a ubiquitous disease. Calcium oxalate (CaOx) is one of the main components of the majority of stones formed in the urinary system [1].Lithiasis (stone formation) can cause acute and chronic renal failure, which includes nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or both) [2].Urinary stones are located in the kidney and very few are lodged in the urinary bladder and urethra. Stones start forming in the calyx and as they grow, they can block the passage of urine [3].

The medical treatment of urolithiasis requires removal of stones and prevents the development of new stones. The treatment of kidney stones involves techniques like extracorporal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PCNL) and other surgical procedures. The consequences in these procedures include renal injury, decreased renal function and increased incidence in stone recurrence along with the possibility of infection. The kidney stone forming patients are more susceptible to its recurrence and the treatment procedures are costly [4].

An alternative to these methods is the use of medicine plants. Plants have been used as traditional medicine for several thousand years. Medicinal plants as a group comprise approximately 8000 species and account for about 50% of all the higher flowering plant species in India [5]. Records of usage of medicinal plants in treatment of lithiasis are not provided through systematic and pharmacological studies [6].

Weeds are considered aggressive, troublesome and undesirable element of the world's vegetation. . Weeds are unnecessary plants drop crop yield by competing with crop plants for its development. A weed is a plant whose potential have not yet been discovered [7].

The aim of the study is to evaluate the antilithiatic potential of *Tribulus terrestris* (leaf), *Aerva lanata* (flower), *Scoparia dulcis* (leaf) and *Tridax procumbens* (leaf) extracts with solvents of increasing polarity under *in vitro* conditions.

MATERIALS AND METHOD

Collection of plant sample and preparation of extract

The plants were collected from Kalapatti, Coimbatore. The leaf and flower samples were identified and certified by the Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2014-15/Tech/19). Plants were cleaned and shade dried for extraction. The ground samples were passed through the coarse sieve (0.2mm). About 5g of the powered sample was taken in a thimble for soxhlet extraction by hot percolation method using different solvents with increasing polarity namely petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and

aqueous. The extraction was carried till the samples become colorless. The extract was collected, evaporated and refrigerated.

Preparation of aqueous extract

To about 1g of the sample, 100ml of distilled water was added and heated at 60°C for 2 hrs. The sample was filtered and centrifuged, and the supernatant was collected, evaporated and refrigerated.

Treatment groups

The treatment groups set up for the *in vitro* study is categorized as follows

T1 – Control (extract)

T2 – 50µg of extract

T3 – 100µg of extract

T4 – 200µg of extract

T5 – 400µg of extract

T6 – 800µg of extract

T7 – 1600µg of extract

T8 – 3200µg of extract

***In vitro* calcium oxalate assay**

Nucleation assay

The initial process of stone formation begins with occurrence of nuclei. Because of its simplicity and satisfactory reproducibility this classic model of oxalate crystallization was chosen. The percentage inhibition of nucleation of crystals in the presence of extract is deduced. The method used was similar to that described by Hennequin *et al* [8]

Growth assay

The crystals may combine to form a small, hard mass called as stones. The percent dissolution of calcium oxalate crystal growth induced by the presence of extract was analyzed as described by Chaudhary *et al* [9]

Aggregation assay

Aggregation occurs when the crystals in the solution stick together. The percentage dissolution of aggregated crystals induced *in vitro* by the presence of extract was determined by the method of Hess *et al* [10]

RESULTS AND DISCUSSION

Nucleation assay

of retention in the urinary tract. The present study is in accordance with the result from several scientific reports.

The results of nucleation assay were supported by Suzuki *et al.*, (1999). A good correlation between number, density and crystal diameter was observed in their study, when added to the oxalate solution. Takusha (*Alismatis rhizome*) at concentrations above 10mg/ml inhibited the nucleation rate and crystal mass significantly [11].

Sathya and Kokilavani (2012) had reported that ethanolic extract of *Saccharum spontaneum Linn.* Induced concentration dependent trend of inhibition in oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization *in vitro* [12].

Growth assay

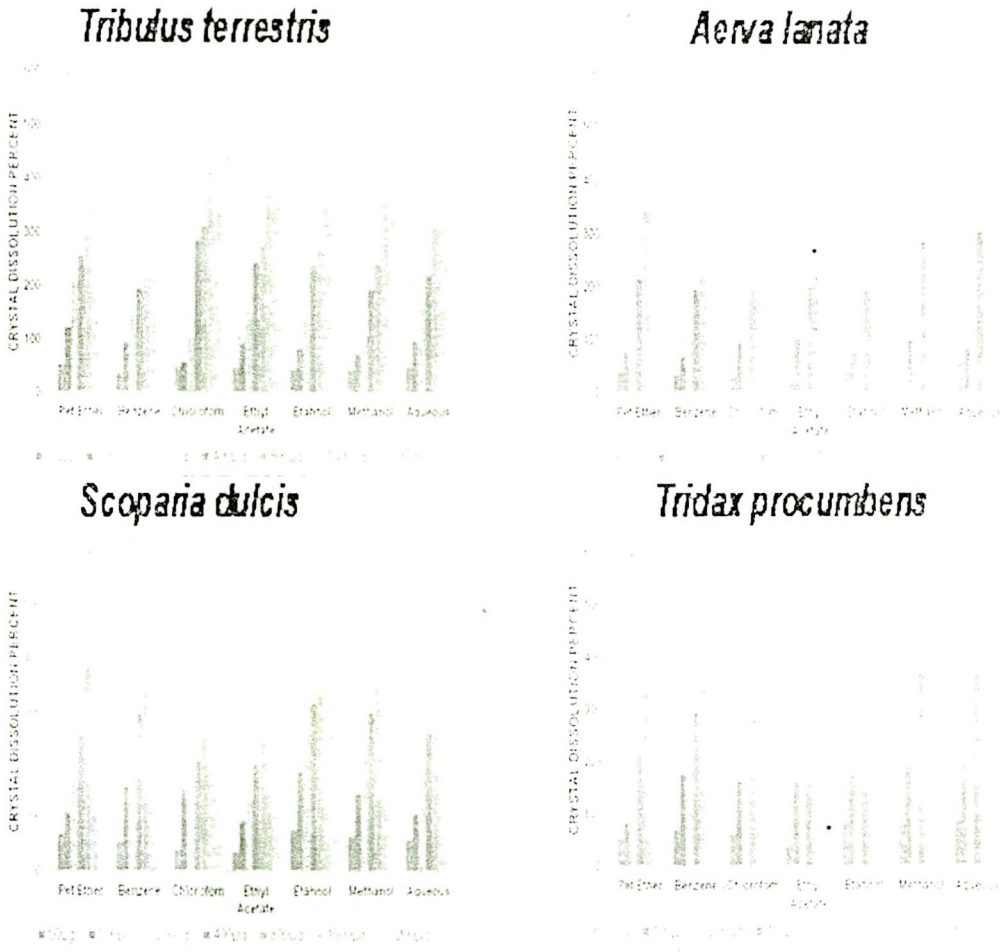

Figure 2 Effect of medicinal plant extracts on growth of calcium oxalate crystals

The aggregated clusters of calcium oxalate particles grow further. The inhibition of the growth of the crystals by the presence of the extracts was determined and presented in Figure 2 and plate 2.

All the extracts were able to inhibit growth of the crystals and that the aqueous flower extract of *Aerva lanata* at concentration (1600µg) showed a significant inhibitory effect against the crystals. As observed in plate 2, the large number of crystals formed in the control group were maximally diminished and reduced in the presence of extract.

Mayee and Thosar (2011) reported that ethanolic extracts of *Lantana camara Linn.* promoted calcium oxalate growth inhibition significantly by increasing more crystals in urine, there by reduced supersaturation and the size of the particles [13]. Khare *et al.*, (2014) reported that methanolic extract *Coleus Aromaticus Benth* has the potential to inhibit the formation of both calcium phosphate and calcium oxalate crystals *in vitro*[14]. Manjula *et al.* (2012) reported that COM and COD were commonly found in urinary calculi (stones). The studies on the inhibitory effect of aqueous *Costus igneus* stem extract on the growth of COM crystals indicated that there was a concentration dependant action against the crystals growth[15].

Aggregation assay

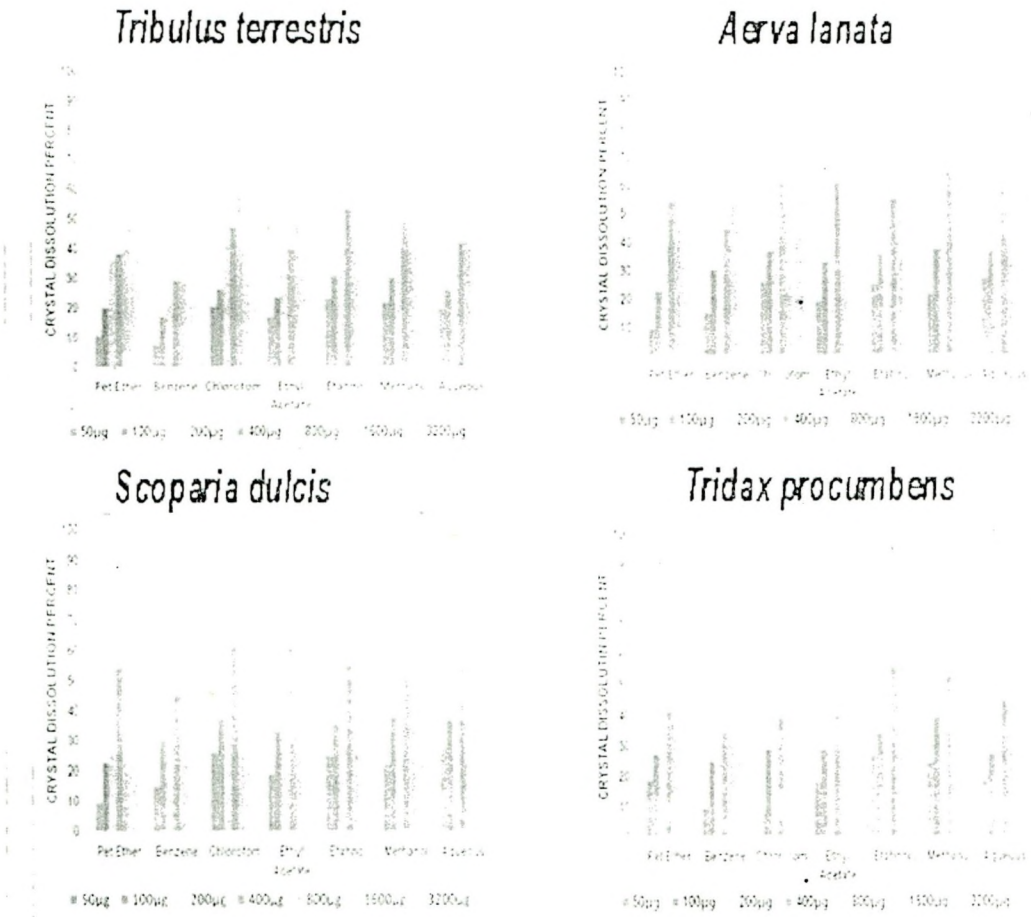

Figure 3 Effect of medicinal plant extracts on aggregation of calcium oxalate crystals

Plate 1 Nucleation

a. Monohydrate crystal

b. Dihydrate crystals

Plate 2 Growth

Control

Extract

Plate 3 Aggregation

Control

Extract

The next step to crystal nucleation is crystal growth, which is a critical step in the urinary stone formation due to agglomeration of particles. Figure 3 and plate 3 depicts the results of aggregation of the calcium oxalate crystal in the presence of the flower extract. The results

confirmed that calcium oxalate monohydrate crystal aggregation was steadily decreased by the increased concentration of flower extract which were in accordance to nucleation and growth. Microscopic view of calcium oxalate crystals showed that the crystal size was bigger and aggregated in the control. However, the size of the crystal was considerably reduced and very well dispersed by the presence of the extract. It was evident that the aqueous extract reduced the crystal growth to a greater extent due to the inhibitory nature of the components present in the plant against the aggregation of the COM crystals which could ultimately prevent the stone formation. This property of plant extract could be important in preventing kidney stone formation. Kumar *et al.* (2013) revealed that the aqueous Vaishvanara churna causes inhibition of crystal formation and aggregation[16]. In a study conducted by Pareta *et al.*, (2011) the hydroalcoholic extract of *Achyranthes indica* Linn inhibited the Calcium Oxalate crystal nucleation and aggregation in a concentration dependent manner. Ability of extract to reduce the nucleation increases the limit of oxalate in urine and preventing precipitation of the Calcium oxalate crystal [17].

CONCLUSION

The results of the *in vitro* assays (nucleation, aggregation and growth) performed revealed that the nucleation of the crystals initiated under *in vitro* condition is readily inhibited and further crystal growth is prevented by the presence of the different solvent extracts. The number and morphology of the COM crystals were observed under light microscope (400X magnification). Among the different solvent extracts analysed, aqueous flower extract of *Aerva lanata* at concentration (1600µg/ ml) showed a greater potential towards crystal growth inhibition when compared to all other extracts tested. Appearance of dendritic like crystals rather than regular hexagonal shape indicates the inhibitory potential of the extracts.

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