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In vitro Antiurolithiasis potential of *Abrus precatorius* L. white seeds

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Abstract

The present research study centered on evaluating *In vitro* antilithiatic potential of *Abrus precatorius* L. White seeds. In the present study the seeds of *Abrus precatorius* have been selected for their antiurolithiatic potential. The plant extract was further subjected to phytochemicals characterizations and found to possess flavonoids, coumarins, saponins, proteins, glycoside, quinones and tannins. In the present investigation, two assays *Viz*. Crystal Nucleation and Aggregation assay were studied under *In vitro* conditions in the presence as well as absence of inhibitors. Five different concentrations (100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml and 500mg/ml) of the plant extracts were tested in each assay. The inhibitory activity of four solvents *Viz.* Water, Ethanol, Chloroform and Petroleum ether were used for the Calcium oxalate (CaOX) crystal formation, which are principally present in most of the kidney stones. Among all the extracts of *Abrus precatorius*, the highest percentage of nucleation inhibition (92%) was obtained from water extract at a concentration of 500mg/ml. The aqueous, ethanol and chloroform extracts (100mg/ml to 500 mg/ml) were found to possess significant anti-urolithiatic. The study concludes that the white seed extracts of *Abrus precatorius* have inhibitory effect on calcium oxalate (CaOX) for crystal nucleation, aggregation and crystal growth. It also found great potential in the dissolution of calcium oxalate crystals. This way, these extracts may be valuable resources for the treatment of urolithiasis.

Urolithiasis means the formation of stones or calculi in the urinary tract, is not only a painful condition affecting some 2-20% of the population worldwide but is also associated with high cost to the society because of the high prevalence of the disease and high recurrence rates[9,12]. The term urolith is derived from the Greek word meaning urine and lithos meaning stone[14]. It is clear from the historical clues, urinary stone has always been a common disease and presently it is the third most common affliction of the urinary tract[1]. The overall probability of stone formers varies in different parts of the world which depends on the socio-economic conditions and succeeding changes in the dietary habits. The occurrence of urinary stones has been increasing over the last few years at the same time as the age of onset is decreasing[6]. Despite major technical advancements in treating kidney stones, the problem still persists. Treatments available till date have serious side effects and do not eliminate the probability of recurrence completely. Traumatizing effects of shock waves, persistent residual stone fragments after ESWL and a possibility of infection pose serious problems to be taken into consideration[1]. ESWL is also reported to be associated with effects such as renal damage and hypertension[3]. Furthermore, ESWL is known to be related to long term medical effects such as diabetes mellitus[10]. Even medications like thiazides cause intracellular acidosis and can lead to hypokalemia and hypocitraturia[7]. Actually, there are no satisfactory drugs in modern medicine, which can dissolve the stone and the physicians remain to be dependent on alternative systems of medicine for better relief. Herbal medicines are very efficacious and have lesser ill effects when compared to the modern medicine also reducing the recurrence rate of renal stone[15]. The complete mechanisms of action of these remedies are lacking. The majority of the plant based therapies have been proven to be effective at diverse stages of stone pathophysiology disparate allopathic medicines which majorly targets only one aspect of urolithiatic pathophysiology.

As a result, the search for antilithiatic drugs from natural sources has assumed superior importance. They are effective with smaller amounts of side effects and are too inexpensive. That's why; the Indian plants are constantly being evaluated for their possible antilithiatic effects in a systematic manner[4]. The traditional Indian medicine, Ayurveda suggests *Abrus precatorius* (White, black and red seeds), *Tamarindus indica* Fruit Shell, *Tectona grandis* seeds, *Chenopodium album*, *Abutilon indicum* (L.) Sweet, to be antilithiatic, but scientific data supporting this statement is even now lacking. All these plants are claimed to be nontoxic, available in rural areas and culturally acceptable, and found to be effective for urinary disease and disorders.

In tune with this effort, the present research study centered on evaluating the antilithiatic potential of *Abrus precatorius* L. White seeds.

Materials :

All the chemicals employed in the present investigation were of analytical grade. $Na_2C_2O_4$ (Sodium oxalate), NaCl (Sodium chloride) and $CaCl_2$ (Calcium chloride) was

purchased from Qualigens, Thermo scientific.

Drugs and Chemicals :

Water, chloroform, ethanol and petroleum ether, *etc*. chemicals used specifically.

Collection of plant :

The plant material *Abrus precatorius* White seeds was collected from the local Market of Nanded, Maharashtra. Identified parts of the plants viz. seeds of *Abrus precatorius* and then shade dried. Dried Seeds were cleansed of extraneous matter and then ground to fine powder in a grinder.

Preparation of plant extract :

The 50g powdered plant material was extracted by using Hot continuous soxhlet extraction method. The plant material was extracted with solvents like water, Ethanol (99.9% v/v), Chloroform and Petroleum ether each of 500ml for four days in a soxhlet apparatus[13].

Qualitative phytochemical characterizations of Abrus precatorius (White seeds) *:*

Qualitative phytochemical characterizations of *Abrus precatorius* (White seeds) was carried out to detect the presence of alkaloids, sterols, saponins, tannins, terpenes, carbohydrates and phenolic substances.

In vitro Antiurolithiasis activity of Abrus precatorius (White seeds) *under Study : Preparation of reagents and solution:*

All the chemicals used were of AR grade. Crystalloid forming solutions, *i.e.* solution of calcium acetate and sodium oxalate for calcium oxalate crystal formation were prepared in distilled water.

Inhibition assay :

Antilithic activity in extract of the medicinal plant *Viz*. *Abrus precatorius* (White seeds) was investigated as per the method with minor modifications[18]. The whole amount of extract solutions (50mL) was placed in the beaker in the beginning itself and the two salt forming solutions were allowed to run into it drop wise through burettes. As a result, a reservoir of the extract solutions was produced into which the salt forming solutions ran down. Lastly, the mixture was boiled on a heating mantle for a period of 10min., then allowed cooling at room temperature and the precipitate was collected into a pre-weighed centrifuge tube by centrifuging (Remi centrifuge) small volumes at a time and discarding the supernatant liquid. Further, the tube with the precipitate was dried out in a hot air oven, then cooled to room temperature and weighed till constant weight by using a weighing balance. The Weight of the precipitate was done. Concurrently blank experiments (Control) with water in place of extract were also performed for evaluating the inhibition efficiency of inhibitors when compared to water. All the experimentation was carried out at room temperature (RT).

Nucleation assay :

Solution of calcium chloride (5mmol/l) and sodium oxalate (7.5mmol/l) were prepared in a buffer containing Tris–HCl 0.05mol/l and NaCl 0.15mol/l at pH 6.5. Nine milliliter (9mm) of calcium chloride ($CaCl_2$) solution was mixed with 1ml of plant extracts under study at different concentrations

(100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml, and 500mg/ml). Crystallization was initiated after adding 950ml of sodium oxalate solution. The temperature was kept at 37°C. After 30min, the optical density (OD) of the solution was monitored at 620 nm. The degree of nucleation was estimated by comparing the induction time in the presence of extract with that of control[16].

$$\text{Percent inhibition} = \frac{(\text{Absorbance Control} - \text{Absorbance Test})}{\text{Absorbance Control}} \text{ X } 100$$

Aggregation assay :

The method used for aggregation described by[1,16] 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 crystals were utilized at a final concentration of 0.8mg/ml, buffered with sodium chloride (NaCl) 0.15mol/l, Tris–HCl 0.05mol/l and at pH 6.5. Experiments were carried out at 37°C in the presence or absence of the plant extract. The percentage aggregation inhibition was calculated by comparing the turbidity in the presence of SXS at different concentrations (100–500mg/ml) with that obtained in the control using following formula:

$$\% \text{ inhibition} = \frac{\text{1-Turbidity sample}}{\text{Turbidity control}} \text{ X } 100$$

Qualitative phytochemical characterizations of Abrus precatorius (White seeds) *:*

Abrus precatorius (White seeds) showed the presence of flavonoids, coumarins, saponins, proteins, glycoside, quinines and tannins whereas phenols, alkaloids, terpenoids, sterols, emodins and phlobatannin were absent (table-1). The phytochemical constituents present in the plant extract can be held responsible for diverse medicinal activities of the plant.

Table-1. Showing presence or absence of phytoconstituents in white seeds *A. precatorius*

Sr. No.	Test	*Abrus precatorius* White seeds			
		Water	Ethanol	Chloroform	Petroleum ether
1.	Phenols	-	-	-	-
2.	Flavonoid	+	+	+	+
3.	Tannins	+	-	-	+
4.	Coumarins	+	-	+	+
5.	Alkaloid	-	-	-	-
6.	Terpenoids	-	-	-	-
7.	Saponins	+	-	+	-
8.	Sterols	-	-	-	-
9.	Protein	+	+	+	+
10.	Glycoside	+	-	-	-
11.	Emodins	-	-	-	-
12.	Phlobatannin	-	-	-	-
13.	Quinones	-	-	+	-

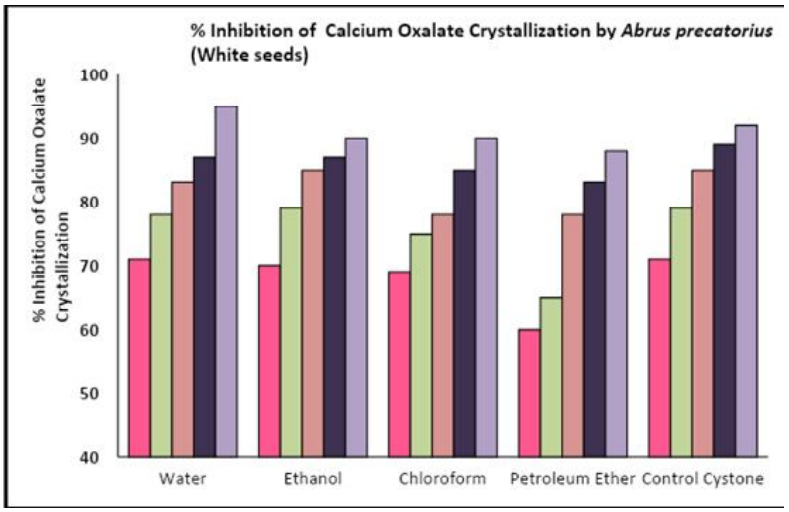

Figure 1: % Inhibition Potential of Calcium Oxalate Crystallization by *Abrus precatorius* (White seeds)

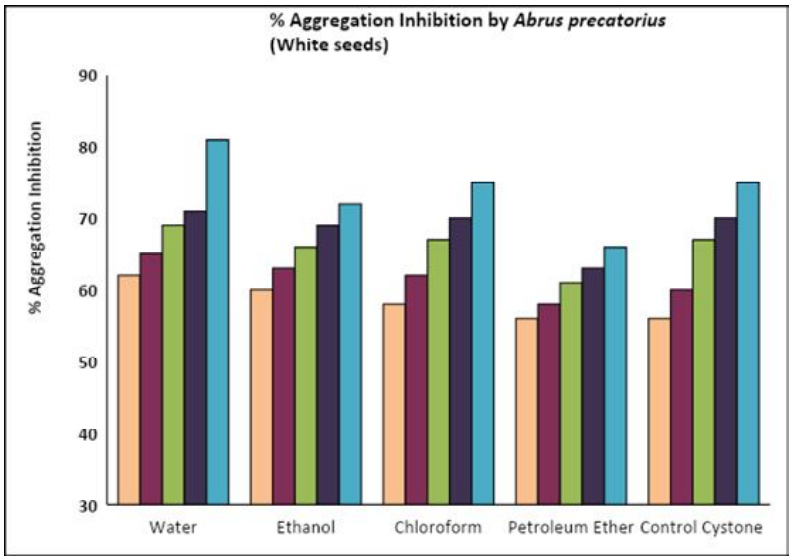

Figure 2: % Inhibition Potential of Calcium Oxalate crystal Aggregation by *Abrus precatorius* (White seeds)

In vitro Antiurolithiasis activity of Abrus precatorius (White seeds) *:*

An earlier increase in the turbidity (growth) was suggestive of the nucleation event, while the decrease in the later part indicated the aggregation phenomenon. These two phenomena corresponded to the complete process of *In-Vitro* crystallization which was observed previously[8]. The extract from *Abrus precatorius* White seeds inhibited the crystallization of Calcium oxalate (CaOx) in solution.

The effect of different concentrations of *Abrus precatorius* white seeds on percent inhibition of calcium oxalate crystallization were studied and the results depicted in Figure 1. Cystone was taken as a positive control. The extracts of *Abrus precatorius* white seeds were prepared in water, ethanol, chloroform and petroleum ether. It is revealed from the Figure I that the highest inhibition of nucleation (95%) activity was observed at 500mg/ml concentration of aqueous extract of *Abrus precatorius* white seeds higher than the positive control whereas 90% inhibition was observed in extract prepared in ethanol and chloroform. The extract prepared in petroleum ether showed 88% inhibition of nucleation at 500mg/ml. The second highest inhibition of nucleation (90%) activity was seen by the ethanol and chloroform extract of *Abrus precatorius* white seeds. All the four extracts of *Abrus precatorius* white seeds showed increasing trends in inhibition activities from 100mg/ml to 500mg/ml.

The aqueous extract of *Abrus precatorius* white seeds resulted in fewer numbers of crystals in solution, in that way reduced the supersaturation and the size of the CaOX particles. This property of the extract is thus advantageous in preventing a urinary stone formation by inducing the excretion of small CaOX particles from the kidney and also reduces the risk of retention in the urinary tract.

In another research study, an administration of a drug having *T. terrestris* to sodium glycolate fed rats produced a significant decrease in urinary oxalate excretion and a significant increase in urinary glyoxylate excretion[18]. Recently different herbal plants *Viz*. *Flos carthami*, *Tribulus terrestris Costus igneus* and *Scoparia dulcis have* successfully proved as prophylactic and remedial medicine for urolithiasis[11,17].

Another recent *In vitro* investigation showed that leaf extracts of *I. eriocarpa* contains potent antiurolithiatic potential[5]. So the results of the present investigation agree with the findings of the above investigations.

The effect of different concentrations of *Abrus precatorius* white seeds on percent inhibition of calcium oxalate aggregation was studied and the results depicted in Figure 2. Cystone was taken as a positive control. The extracts of *Abrus precatorius* white seeds were prepared in water, ethanol, chloroform and petroleum ether. It is revealed from the Figure 2 that the highest inhibition of aggregation (81%) activity was observed at 500mg/ml concentration of aqueous extract of *Abrus precatorius* white seeds higher than the positive control whereas the second highest inhibition of aggregation (75%) was observed in extract prepared in chloroform. The extract prepared in ethanol showed 72% inhibition of aggregation whereas 66% inhibition of aggregation was seen in extract prepared in petroleum ether at 500mg/ml. All the four extracts of *Abrus precatorius* white seeds also showed increasing trends in inhibition activities from 100mg/ml to 500mg/ml.

The % inhibition of turbidity (aggregation) in the presence of *Abrus precatorius* White seeds extracts was lower than in the control, which reveals that crystals were less aggregated. The aggregation inhibition associated with the plant extract increased with concentration. The extract of *Abrus precatorius* White seeds

prepared in different solvents (100mg/ml-500mg/ml) and cystone (500mg/ml) inhibited both the rate of nucleation and aggregation. An extract of *Ficus religiosa* was used to study the growth behavior of CHPD crystals. *F. religiosa* inhibits the growth of CHPD crystals. This can be further verified by the results of the CHPD crystals formation[2].

Results of the present study clearly indicate *In vitro* anti-urolithiatic potential of *Abrus precatorius* White seeds against calcium oxalate (CaOX) urolithiasis. The extracts of *Abrus precatorius* White seeds prepared in all four solvents such as water, ethanol, chloroform and petroleum ether showed significant inhibition of all the stages of calcium oxalate (CaOX) stone formation including aggregation, nucleation and growth. It also showed great potential in the dissolution of crystals of calcium oxalate. The plant used in the present study has ethnomedical application in the treatment of urinary problems and renal stones. The study has given crucial scientific evidence for the traditional utilization of the plant in the prevention and treatment of urolithiasis. As a result, this plant could be a potential source of novel drug molecules having anti-urolithiatic activity.

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