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Exploring the Anti-Urolithiatic Potential of White Seeds from *Abrus Precatorius* Via *In Vitro* Investigations

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Abstract: *Medicinal plants are a valuable aspect of local heritage with global importance, often used as remedies for various health conditions. Their therapeutic properties are linked to a wide variety of complex chemical compounds, particularly secondary metabolites, found in different plant parts. Identifying the specific components responsible for medicinal effects is essential. Phytochemicals, which are abundant in medicinal plants, are generally considered safer and less toxic compared to synthetic alternatives. In line with this understanding, the present research investigates the in vitro anti-urolithiatic potential of white seeds of Abrusprecatorius. The seeds were chosen for their possible role in treating urolithiasis. Phytochemical screening of the plant extract revealed the presence of compounds such as flavonoids, coumarins, saponins, proteins, glycosides, quinones, and tannins.Two In Vitro assays, Crystal Nucleation and Aggregation, were performed, both with and without inhibitors. The study tested various concentrations of plant extracts (50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml, and 250mg/ml) using four solvents: water, ethanol, chloroform, and petroleum ether, to assess their effect on calcium oxalate (CaOX) crystal formation, which is a primary component of kidney stones.Among the extracts tested, the water extract showed the highest inhibition of nucleation (85%) at a concentration of 250mg/ml. Aqueous, ethanol, and chloroform extracts exhibited notable anti-urolithiatic activity across different concentrations. Crystal aggregation was assessed using a spectrophotometer, and the water extract demonstrated the highest inhibition (78%) at the same concentration. Additionally, the chloroform extract showed significant anti-urolithiatic activity.When comparing extracts, the chloroform extract at 500mg/ml was the most effective in inhibiting the growth of calcium oxalate crystals. Overall, the study highlights the potential of Abrusprecatorius white seed extracts in inhibiting calcium oxalate crystal nucleation, aggregation, and growth, suggesting their potential as a treatment for urolithiasis. These results underscore the value of these extracts as promising agents in managing kidney stones.*

Keywords: Anti-urolithiasis, *Abrusprecatorius*, Crystal Growth Assay, Crystal Nucleation Assay, Crystal Aggregation Assay

I. INTRODUCTION

Urolithiasis, the formation of stones or calculi in the urinary tract, is a widespread and painful condition, affecting between 2% and 20% of the global population. Its high prevalence and frequent recurrence contribute to a significant economic burden (Johri*et al*., 2010). The term "urolith" is derived from the Greek words for urine and stone (Osborne *et al*., 1999), and historical evidence shows that urinary stones have been a common ailment for centuries. Currently, they rank as the third most common disorder of the urinary tract (Atmani, 2003).

The risk of developing urinary stones varies globally and is influenced by factors such as socio-economic status and changing dietary habits. Recent years have seen a rise in the incidence of urinary stones, along with a decrease in the age at which they first occur (Devuyst and Pirson, 2007).

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Despite advances in kidney stone treatment techniques, the issue remains unresolved. Current treatments often have serious side effects and fail to completely eliminate the possibility of recurrence. For example, shock wave therapy can lead to trauma, leave residual stone fragments, and increase the risk of infection (Atmani, 2003). Furthermore, this treatment has been associated with complications such as renal damage, hypertension, and even long-term effects like diabetes (Butterweck and Khan, 2009). Medications like thiazides may cause intracellular acidosis, leading to side effects like hypokalemia and hypocitraturia (Heilberg and Schor, 2006).

In modern medicine, effective drugs capable of dissolving kidney stones are lacking, and physicians often turn to alternative medical systems for better outcomes. Herbal medicines, in particular, are known for their efficacy and relatively fewer side effects compared to conventional treatments, which helps reduce the recurrence rate of renal stones (Prasad *et al*., 2007). However, the mechanisms behind these herbal remedies are not fully understood. While allopathic treatments target specific aspects of the stone formation process, plant-based therapies have shown effectiveness at various stages of the stone pathophysiology.

The search for natural antilithiatic drugs has gained traction due to their effectiveness, fewer side effects, and lower cost. As a result, Indian plants are being systematically studied for their potential in preventing and treating urolithiasis (Christina *et al*., 2005). According to traditional Indian medicine, particularly Ayurveda, plants like *Abrusprecatorius* (with white, black, and red seeds), *Tamarindusindica* fruit shell, *Tectonagrandis* seeds, *Chenopodium album*, and *Abutilon indicum* (L.) Sweet are believed to possess antilithiatic properties. Despite their cultural acceptance, non-toxicity, and availability in rural areas, scientific validation for these claims is still limited.

In alignment with this knowledge, the current study focuses on evaluating the antilithiatic potential of the white seeds of *Abrusprecatorius*.

II. MATERIALS

All chemicals used in this study were of analytical grade. Sodium oxalate ($Na_2C_2O_4$), sodium chloride (NaCl), and calcium chloride ($CaCl_2$) were sourced from Qualigens, Thermo Scientific.

2.1 Drugs and Chemicals

The chemicals used specifically in this study included water, chloroform, ethanol, and petroleum ether.

2.2 Collection of Plant Material

The white seeds of *Abrusprecatorius* were collected from the local region of Nanded, Maharashtra.

2.3 Preparation of Plant Extract

For the preparation of the plant extract, 50g of powdered *Abrusprecatorius* white seeds were subjected to hot continuous Soxhlet extraction. This process involved using 500ml of solvents, including water, ethanol (99.9% v/v), chloroform, and petroleum ether, for a period of four days. The extraction was conducted using a Soxhlet apparatus as described by Muthusamy *et al*., (2009).

The Soxhlet extraction is a continuous process where the solvent is repeatedly cycled through the plant material. Vapors from the heated solvent rise into the condenser, condense back into liquid, and flow over the plant material, allowing for efficient extraction. The apparatus consists of an extractor body (thimble) connected to a distillation flask and a condenser, with standard joints to secure the setup.

2.4 Qualitative Phytochemical Characterization of *Abrusprecatorius* White Seeds

The qualitative phytochemical characterization of the *Abrusprecatorius* white seeds was carried out to detect the presence of various compounds, including alkaloids, sterols, saponins, tannins, terpenes, carbohydrates, and phenolic substances.

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2.5 *In Vitro* Antiurolithiasis Activity of *Abrusprecatorius* White seedsunder Study

2.5.1 Preparation of Reagents and Solutions

All chemicals used in this study were of analytical reagent (AR) grade. The solutions required for calcium oxalate crystal formation, including calcium acetate and sodium oxalate, were prepared using distilled water.

2.5.2 Inhibition Assay

The antilithiatic potential of the *Abrusprecatorius* white seed extract was evaluated following the method described by Farrook*et al*., with modifications based on the work of Sangeetha and Muniyandi (2004). In this modified protocol, 50 mL of the extract solution was placed in a beaker as a reservoir. Two solutions, calcium acetate and sodium oxalate, were added dropwise through burettes into the extract solution. This mixture was then heated on a heating mantle for 10 minutes and allowed to cool to room temperature.

The resulting precipitate was collected by centrifuging small volumes of the mixture, with the supernatant being discarded. The precipitate was then transferred to pre-weighed centrifuge tubes, dried in a hot air oven, cooled to room temperature, and weighed until a constant weight was achieved using a precision balance. The weight of the precipitate was recorded for further analysis.

Control experiments were conducted using water instead of the plant extract to evaluate the inhibition efficiency of the extracts in comparison to water. All experiments were performed at room temperature (RT).

2.5.3 Nucleation Assay

A nucleation assay was performed by preparing a solution of calcium chloride (5mmol/L) and sodium oxalate (7.5mmol/L) in a buffer containing Tris–HCl (0.05 mol/L) and NaCl (0.15mol/L) at a pH of 6.5. To this, 9 mL of the calcium chloride solution was mixed with 1 mL of the *Abrusprecatorius* extract at different concentrations (100mg/mL, 200mg/mL, 300mg/mL, 400 mg/mL, and 500mg/mL). Crystallization was initiated by adding 950 µL of the sodium oxalate solution to the mixture. The reaction was maintained at a constant temperature of 37^0C.

After 30 minutes, the optical density (OD) of the mixture was measured at 620 nm to assess the degree of nucleation. The results were compared to a control to evaluate the induction time and the effectiveness of the extract in inhibiting nucleation (Paras *et al*., 2012).

Top of Form

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

2.5.4 Aggregation Assay

The aggregation assay method, based on the protocols by Atmani*et al*., (2003) and Paras *et al*., (2012), was modified for this study to assess the anti-urolithiatic potential of *Abrusprecatorius* white seed extracts. Calcium oxalate (CaOX) crystals were prepared by mixing sodium oxalate and calcium chloride at a concentration of 50mmol/L. The two solutions were equilibrated in a water bath at 60^0C for 1 hour, followed by cooling to 37^0C overnight. The crystals that formed were harvested via centrifugation and dried by evaporation at 37^0C.

The prepared calcium oxalate crystals were used at a final concentration of 0.8 mg/mL in a buffer containing sodium chloride (0.15mol/L), Tris–HCl (0.05mol/L), and adjusted to pH 6.5. The aggregation assay was performed at 37^0C, in the presence or absence of the *Abrusprecatorius* plant extract at varying concentrations (100–500 mg/mL).

To evaluate the inhibition of crystal aggregation, the turbidity of the solution was measured, and the percentage inhibition of aggregation was calculated using the following formula:

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

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III. RESULTS AND DISCUSSIONS

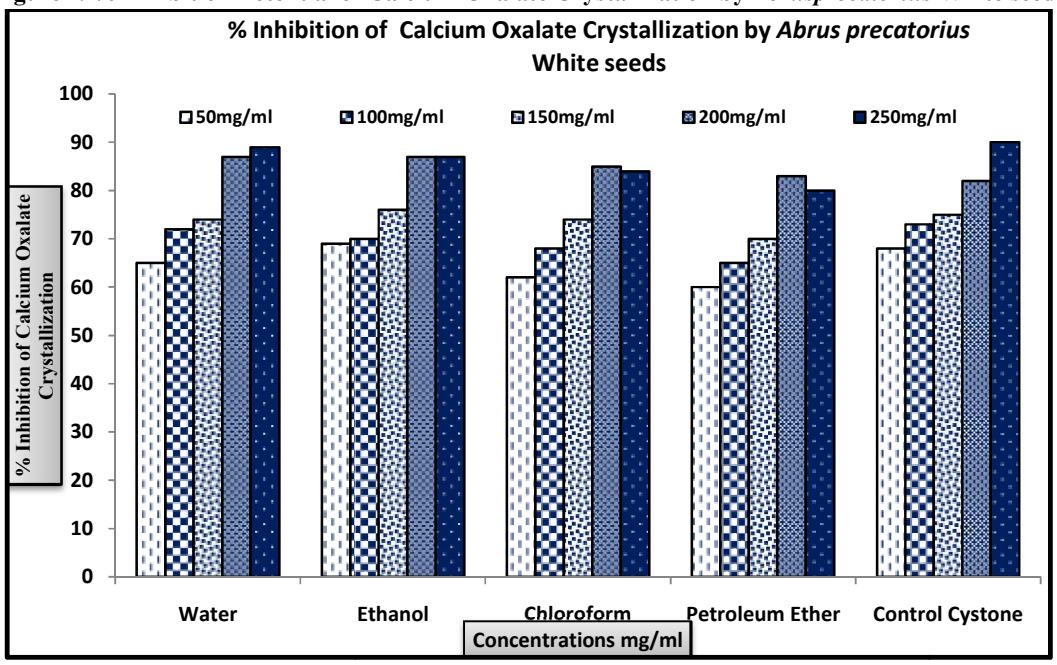
3.1 Qualitative Phytochemical Characterizations of *Abrusprecatorius*(White seeds)

The qualitative phytochemical analysis of *Abrusprecatorius* white seeds revealed the presence of various bioactive compounds. Notably, flavonoids, coumarins, saponins, proteins, glycosides, quinones, and tannins were detected in the plant extract. However, the analysis indicated the absence of phenols, alkaloids, terpenoids, sterols, emodins, and phlobatannins. The presence of these phytochemicals may explain the medicinal properties attributed to *Abrusprecatorius* white seeds, particularly their potential anti-urolithiatic activity.

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

3.2 *In Vitro*Antiurolithiasis Activity of *Abrusprecatorius*White seeds

Figure I: % Inhibition Potential of Calcium Oxalate Crystallization by *Abrusprecatorius* White seeds



An early increase in turbidity suggested the occurrence of a nucleation event, while a subsequent decrease indicated the onset of the aggregation process. These two distinct phases reflect the entire sequence of in vitro crystallization, as

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previously described by Hess *et al*., (2000). The inhibition of calcium oxalate (CaOx) crystallization in solution by *Abrusprecatorius* white seed extract was observed during this process. The extract's ability to hinder both nucleation and aggregation indicates its potential in preventing the formation and growth of kidney stones, contributing to its anti-urolithiatic efficacy.

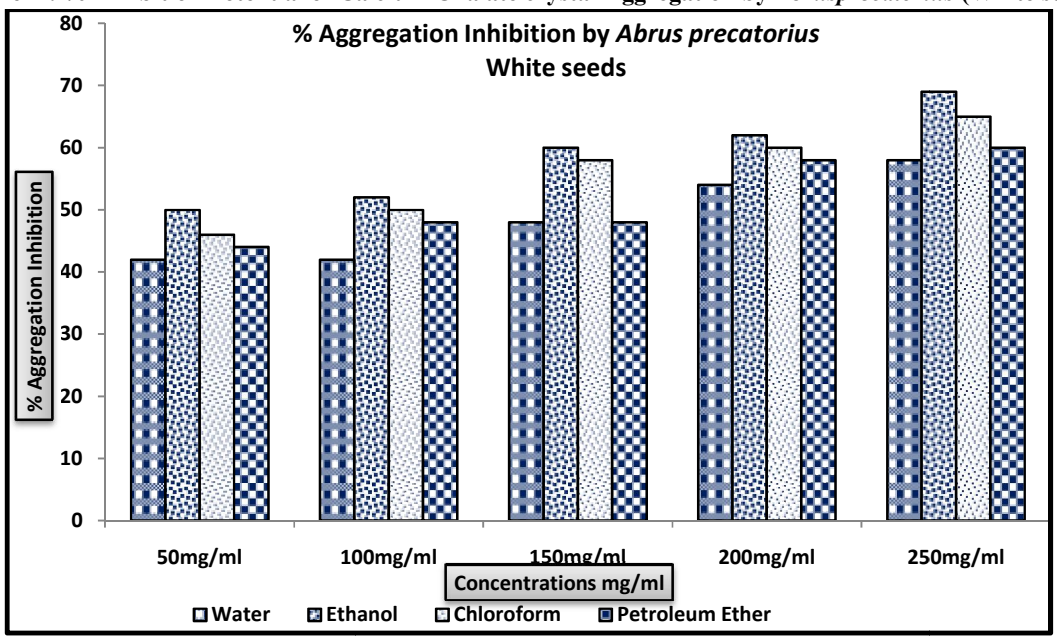
The study investigated the effect of various concentrations of *Abrusprecatorius* white seed extracts on the percentage inhibition of calcium oxalate crystallization, with Cystone serving as the positive control. The extracts were prepared using water, ethanol, chloroform, and petroleum ether solvents.

As shown in Figure I, the highest inhibition of nucleation (69%) was observed at a concentration of 250 mg/mL in the ethanol extract of *Abrusprecatorius* white seeds, exceeding the positive control's effectiveness. Additionally, the aqueous and chloroform extracts showed 58% and 65% inhibition, respectively, while the petroleum ether extract exhibited 60% inhibition of nucleation at the same concentration.

The aqueous extract of *Abrusprecatorius* white seeds were particularly effective in reducing the number of crystals in solution. This reduction led to decreased supersaturation and smaller calcium oxalate (CaOx) particles. This property is significant in preventing the formation of urinary stones, as smaller CaOx particles are more easily excreted from the kidneys, reducing the risk of retention in the urinary tract.

These findings align with previous studies, including one where rats treated with *Tribulusterrestris* showed reduced urinary oxalate excretion and increased glyoxylate excretion (Sangeeta*et al*., 1994). Additionally, several herbal plants, such as *Floscarthami*, *Tribulusterrestris*, *Costusigneus*, and *Scopariadulcis*, have demonstrated efficacy in preventing and treating urolithiasis (Rajan*et al*., 2014; Lin *et al*., 2012). Moreover, *Indigoferaeriocarpa* leaf extracts have shown strong antiurolithiatic potential in recent *In Vitro* studies (Das *et al*., 2016), further supporting the results of this investigation.

Figure II: % Inhibition Potential of Calcium Oxalate crystal Aggregation by *Abrusprecatorius* (White seeds)



The study examined the effects of various concentrations of *Abrusprecatorius* white seed extracts on the percentage inhibition of calcium oxalate (CaOX) aggregation, with results shown in Figure II. Cystone, a known antiurolithiatic agent, served as the positive control. Extracts were prepared using water, ethanol, chloroform, and petroleum ether.

As depicted in Figure II, the ethanol extract of *Abrusprecatorius* white seeds demonstrated the highest inhibition of aggregation (69%) at a concentration of 250 mg/mL, surpassing the control. The chloroform extract followed closely with 65% inhibition, while the aqueous and petroleum ether extracts exhibited 58% and 60% inhibition, respectively, at the same concentration.

Notably, all extracts showed a positive trend, with increasing inhibition activities as concentrations rose from 50mg/mL to 250mg/mL. The inhibition of turbidity (aggregation) in the presence of the extracts was lower than in the control, indicating reduced crystal aggregation. The extracts' efficacy in inhibiting both nucleation and aggregation increased with concentration, demonstrating a concentration-dependent inhibition of calcium oxalate crystal formation.

This result is consistent with previous research, such as the study by Baskaraboopathy*et al*., (2017), where an extract of *Ficusreligiosa* was shown to inhibit the growth of calcium hydrogen phosphate dihydrate (CHPD) crystals. The findings from the present study similarly highlight the potential of *Abrusprecatorius* white seed extracts in inhibiting calcium oxalate crystal growth, suggesting their utility in preventing urolithiasis.

IV. CONCLUSION

Abrusprecatorius white seeds, prepared using four solvents—water, ethanol, chloroform, and petroleum ether—demonstrated significant inhibition across all stages of calcium oxalate (CaOX) stone formation, including nucleation, aggregation, and growth. Additionally, the extracts exhibited promising potential in dissolving calcium oxalate crystals.This study builds on the plant's long-standing ethnomedical use in treating urinary ailments and renal stones, offering critical scientific validation for its traditional applications. The results suggest that *Abrusprecatorius* could be a valuable source of novel therapeutic agents with potent anti-urolithiatic properties, reinforcing its relevance in both modern and traditional medicine for the prevention and treatment of urolithiasis.

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