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Original Article

Anti-Urolithiasis Potential of Phagnaion rupestre: *In Vitro* Evaluation of Crystal Formation, Aggregation Inhibition, and Stone Dissolution

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ABSTRACT:

Purpose: Urolithiasis, commonly known as kidney stones, is a prevalent condition associated with significant discomfort. Traditional treatments often come with high costs and potential side effects. The use of medicinal plants has gained popularity as a safer, cost-effective alternative. This study aimed to evaluate the anti-urolithiasis potential of *Phagnaion rupestre*, a plant native to the arid regions of Libya, by assessing its effects on calcium oxalate crystal formation, aggregation, and dissolution.

Aim: This study aimed to determine the anti-urolithiasis activity of *Phagnaion rupestre* through in vitro assays, focusing on crystal formation inhibition, aggregation inhibition, and stone dissolution.

Materials and Methods The aqueous extract of *Phagnaion rupestre* was tested at varying concentrations (250, 500, 750, 1000 µg/mL) for its ability to inhibit calcium oxalate crystal formation, aggregation, and promote the dissolution of pre-formed stones. Synthetic urine and synthetic kidney stones were used for the assays, and the phytochemical profile of the extract was analyzed.

Results: The results indicated that *Phagnaion rupestre* significantly inhibited calcium oxalate crystal formation, with up to 68% inhibition at the highest concentration. Aggregation inhibition was also observed to be dose-dependent, with up to 70% inhibition. Additionally, the extract enhanced the dissolution of pre-formed stones, reaching a dissolution rate of 70%. The phytochemical analysis revealed the presence of bioactive compounds such as phenols, flavonoids, and alkaloids, which are likely contributing to these effects.

Conclusion: *Phagnaion rupestre* demonstrated promising anti-urolithiasis activity by inhibiting calcium oxalate crystal formation, preventing aggregation, and enhancing stone dissolution. These results suggest that *Phagnaion rupestre* could be a potential natural remedy for the prevention and management of kidney stones, offering a safer alternative to conventional treatments.

Keyword: Anti-Urolithiasis**,** Phagnaion rupestre**,** Potential**,** Dissolution**,** Stone

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INTRODUCTION:

Urolithiasis, commonly referred to as kidney stones, represents a significant public health challenge worldwide, affecting a substantial number of individuals and leading to considerable morbidity. The pathophysiology of kidney stone formation involves a complex interplay of supersaturation, nucleation, aggregation, and retention of insoluble crystals, primarily calcium oxalate (CaOx), in the renal system. This process results in the formation of crystalline stones, which are the leading cause of urinary tract obstructions [1]. The condition is multifactorial, influenced by genetic predisposition, dietary habits, and environmental factors. The management of kidney stones is complex and often includes surgical interventions, such as extracorporeal shock wave lithotripsy (ESWL) or nephrolithotomy, as well as pharmacological treatments aimed at preventing stone formation. However, recurrence rates remain high, with up to 50% of patients experiencing stone formation again within five years after treatment [2].Traditional medicine, especially the use of herbal remedies, has gained attention as an alternative or complementary approach to managing urolithiasis due to their lower cost and reduced side effects compared to conventional therapies. Numerous medicinal plants have been studied for their antilithiatic properties, including those with diuretic, antioxidant, and anti-inflammatory effects [3]. Among these, Phagnaion rupestre, a plant native to the arid regions of Libya, has been identified as a promising candidate due to its bioactive compound profile, which includes phenols, flavonoids, alkaloids, glycosides, saponins, tannins, and terpenoids. These compounds have been linked to various therapeutic effects, including the inhibition of crystal formation, aggregation, and the dissolution of pre-formed stones [4,5].This study aimed to evaluate the anti-urolithiasis potential of *Phagnaion rupestre* through a series of in vitro assays, including crystal formation inhibition, nucleation, aggregation, and dissolution assays. The primary objective was to determine the efficacy of the aqueous extract of *Phagnaion rupestre* in reducing the formation and growth of calcium oxalate crystals, a major component of kidney stones. Additionally, the phytochemical profile of the extract was analyzed to correlate the presence of specific bioactive compounds with the observed anti-urolithiasis activity. This investigation also aimed to provide a scientific basis for the use of *Phagnaion rupestre* in traditional medicine as a therapeutic agent for kidney stone prevention and treatment [6].

By comparing the extract's performance to established pharmacological treatments, such as Cystone, and evaluating its potential for clinical application, this study seeks to contribute to the growing body of evidence supporting the integration of phytotherapy in the management of urolithiasis [7].

MATERIALS AND METHODS:

Sample Collection

Phagnaion rupestre was collected from the Sebha region, located in the southern part of Libya, which is characterized by arid conditions [4]. The plant was harvested manually from its natural habitat. Authentication of the samples was performed by a local botanist at the Department of Biology, Sebha University, Libya.

Sample Processing

The collected Phagnaion rupestre was cleaned to remove dirt and other contaminants. The plant was then air-dried under shade to preserve its active compounds, following standard procedures for preserving medicinal plants [1]. The dried material was ground into a fine powder using a mechanical grinder. The powdered material was stored in airtight containers in a cool, dry place to prevent degradation before extraction [8].

Preparation OF Extracts

The powdered Phagnaion rupestre was subjected to extraction using an aqueous method to obtain the maximum bioactive compounds, as used in similar studies (Harshita et al., 2020). A 20 g sample of the dried powder was mixed with 100 mL of distilled water and heated at 80°C for 2 hours with stirring using a magnetic stirrer to ensure uniform mixing [11]. The resulting extract was filtered through Whatman filter paper, and the filtrate was collected for further testing [8].

Phytochemical Analysis

Phytochemical analysis was carried out to detect the presence of various bioactive compounds, using standard protocols. The methods for determining phenols, flavonoids, and alkaloids followed the well-established procedures used in similar studies [9].The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu reagent, as described in previous works [3]. The total flavonoid content was measured using the aluminum chloride colorimetric assay, as per established

protocols [8]. Alkaloids were detected using the modified method of Ferguson. Other compounds, including glycosides, saponins, tannins, and terpenoids, were also analyzed following standard methods [8].

Anti-Urolithiasis Activity

Preparation OF Synthetic Urine

conditions for in vitro testing of the anti-urolithiasis activity of Phagnaion rupestre. The urine was supersaturated with calcium chloride to promote the formation of calcium oxalate crystals [10]. The preparation was maintained at 37°C in capped vessels to simulate physiological conditions.

Preparation of Synthetic Kidney Stones

Synthetic kidney stones were prepared by mixing solutions of calcium chloride and sodium oxalate. A 1.47 g sample of calcium chloride dihydrate was dissolved in 100 mL of distilled water, while 1.34 g of sodium oxalate was dissolved in 100 mL of 1 M H_2SO_4. The two solutions were mixed and stirred to initiate calcium oxalate precipitation [6]. The crystals were washed with distilled water, dried at 60°C, and used for further experiments [10].

Inhibition Assay

was evaluated by its ability to inhibit calcium oxalate crystal formation. Various concentrations (250, 500, 750, 1000 µg/mL) of the plant extract were added to synthetic urine, as done in similar studies [8]. After stirring and maintaining the mixture at 37°C, crystals were centrifuged, dried, and weighed. The percentage inhibition was calculated using the formula:

$$\text{Percentage Inhibition} = \left(\frac{\text{WB} - \text{WS}}{\text{WB}}\right) \times 100$$

where **WB** is the weight of blank tubes, and **WS** is the weight of the sample tubes.

Nucleation Assay

For the nucleation assay, a buffer solution containing 0.05 M Tris-HCl and 0.15 M NaCl (pH 6.5) was prepared, as previously outlined by Sharma et al. (2020). The calcium chloride and sodium oxalate solutions were mixed to initiate crystallization. Various concentrations (10, 25, 50, 75, and 100 mg/mL) of the plant extract were added, and the optical density was measured at 620 nm after 30 minutes. The inhibition of nucleation was determined by comparing the optical density with the control group [8].

Aggregation Assay

The aggregation of calcium oxalate crystals was assessed by measuring the turbidity of the solution after the addition of different concentrations (10–100 mg/mL) of Phagnaion rupestre extract, as done in similar studies [9]. A decrease in turbidity indicated inhibition of crystal aggregation. The percentage inhibition was calculated by comparing turbidity changes between the sample and control groups as follows:

$$\text{Percentage Inhibition} = \left(1 - \frac{\text{Turbidity of Sample}}{\text{Turbidity of Control}}\right) \times 100$$

Egg Membrane Assay

To simulate the dissolution of kidney stones, synthetic calcium oxalate stones were placed inside semi-permeable egg membranes. The membranes were suspended in 100 mL of 0.1 M Tris buffer, and the plant extract was added. After 2 hours of incubation at 37°C, the solution was titrated with 0.2 M potassium permanganate to determine the amount of calcium oxalate dissolved [10]. The percentage dissolution was calculated as described in previous studies [11].

Statistical Analysis

Pearson's Correlation was performed to assess the linear relationship between the concentration of Phagnaion rupestre extract and the percentage of inhibition, aggregation, and dissolution. A correlation coefficient close to 1 indicates a strong positive relationship between the concentration and the observed effect. Linear Regression was used to determine the slope and intercept for each test, providing insight into how the activity (inhibition, aggregation, and dissolution) changes as the concentration of the extract increases. Statistical significance was determined using a p-value threshold of 0.05, where a p-value less than 0.05 indicates a statistically significant result.

RESULTS:

1. Inhibition of Crystal Formation (Nucleation and Inhibition Assays)

The inhibitory effect of Phagnaion rupestre on calcium oxalate crystal formation was evaluated through nucleation and inhibition assays at different concentrations of the extract. As shown in Table 1, increasing concentrations of the extract resulted in a dose-dependent increase in the inhibition of crystal

formation. At the lowest concentration (250 µg/mL), the inhibition of crystal formation was 30%, which increased to 45%, 55%, and 68% at concentrations of 500, 750, and 1000 µg/mL, respectively.The Pearson's correlation for the inhibition activity was found to be 0.997, indicating a very strong positive linear relationship between the concentration of Phagnaion rupestre extract and the inhibition percentage. The slope value of 0.0496 suggests that the inhibition effect increases linearly with the extract concentration, while the intercept (18.5) corresponds to the baseline inhibition at zero concentration.

Table 1 Inhibition of Crystal Formation in Nucleation and Inhibition Assays at Different Concentrations of Phagnaion rupestre Extract

Concentration (µg/mL)	Inhibition of Crystal Formation (%)	Pearson's Correlation	Slope	Intercept
250	30.0	0.997	0.0496	18.5
500	45.0			
750	55.0			
1000	68.0			

2. Crystal Aggregation

The effect of Phagnaion rupestre extract on the inhibition of calcium oxalate crystal aggregation was evaluated at varying concentrations. As summarized in Table 2, the inhibition of crystal aggregation increased with higher concentrations of the extract. At 250 µg/mL, the inhibition was 35%, which progressively increased to 50%, 60%, and 70% at concentrations of 500, 750, and 1000 µg/mL, respectively.The Pearson's correlation value of 0.994 indicates a strong positive linear relationship between the concentration of the extract and the percentage inhibition of crystal aggregation. The slope of 0.0460 suggests a steady increase in inhibition with increasing extract concentration, and the intercept (25.0) represents the baseline inhibition when no extract is present.

Table 2 Inhibition of Calcium Oxalate Crystal Aggregation by Phagnaion rupestre Extract at Varying Concentrations

Concentration (µg/mL)	Crystal Aggregation Inhibition (%)	Pearson's Correlation	Slope	Intercept
250	35.0	0.994	0.0460	25.0
500	50.0			
750	60.0			
1000	70.0			

3. Dissolution of Formed Stones (Egg Membrane Model)

The dissolution of calcium oxalate (CaOx) stones was assessed using the egg membrane model at various concentrations of Phagnaion rupestre extract. As indicated in Table 3, the dissolution of the stones increased in a concentration-dependent manner. At a concentration of 250 µg/mL, 40% of the CaOx stones were dissolved, and this dissolution increased to 55%, 60%, and 70% at concentrations of 500, 750, and 1000 µg/mL, respectively.

The Pearson's correlation for the dissolution data was 0.981, reflecting a strong positive correlation between the concentration of the extract and the percentage of stone dissolution. The slope of 0.0380 suggests a gradual but consistent increase in dissolution with higher extract concentrations, while the intercept (32.5) represents the baseline dissolution observed in the absence of the extract.

Table 3 Dissolution of Formed Calcium Oxalate Stones Using Phagnaion rupestre Extract in the Egg Membrane Model at Different Concentrations

Concentration (µg/mL)	Dissolution of CaOx Stones (%)	Pearson's Correlation	Slope	Intercept
250	40.0	0.981	0.0380	32.5
500	55.0			
750	60.0			
1000	70.0			

4. Phytochemical Analysis Results:

The phytochemical analysis of Phagnaion rupestre revealed the presence of several bioactive compounds with varying concentrations. The total phenolic content was quantified at 4.2 mg of gallic acid equivalent (GAE) per gram of extract, while the total flavonoid content was 2.5 mg of quercetin equivalent (QE) per gram of extract. Alkaloids were present at 1.8 mg per gram of extract.

Glycosides were found in trace amounts, and saponins were present in moderate levels. Tannins were detected at significant levels, indicating a high concentration of this compound. The presence of terpenoids ranged from moderate to high, reflecting a broad spectrum of bioactive compounds within the plant.

Table 4 Phytochemical Composition of Phagnaion rupestre Extract, Including Concentrations of Bioactive Compounds (Phenols, Flavonoids, Alkaloids, Glycosides, Saponins, Tannins, and Terpenoids)

Compound	Content (mg/g or qualitative)
Phenols	4.2 mg GAE/g
Flavonoids	2.5 mg QE/g
Alkaloids	1.8 mg/g
Glycosides	Traces
Saponins	Moderate
Tannins	Significant levels
Terpenoids	Moderate to high presence

DISCUSSION:

Phagnaion rupestre, a plant species native to the arid regions of southern Libya, has been studied for its potential anti-urolithiasis activity due to its rich phytochemical profile. The primary aim of this study was to evaluate the inhibitory effects of Phagnaion rupestre extract on calcium oxalate (CaOx) crystal formation, aggregation, and dissolution using in vitro assays. Furthermore, this study aimed to analyze the bioactive compounds in the plant extract and explore their potential clinical relevance in the prevention and treatment of kidney stones, a prevalent and painful condition caused by the crystallization of calcium oxalate.

Urolithiasis, particularly the formation of calcium oxalate kidney stones, remains a major health problem worldwide, leading to significant pain, urinary complications, and in severe cases, renal failure. Current treatment options, including surgical removal and medication, primarily focus on managing symptoms rather than addressing the root cause of stone formation. Furthermore, medications used to treat urolithiasis may have side effects and do not always prevent stone recurrence. As a result, there is growing interest in natural compounds that may prevent stone formation, reduce the recurrence of kidney stones, and offer a safer alternative to conventional therapies. Phagnaion rupestre, with its range of bioactive compounds, has demonstrated promising anti-urolithiasis activity in this study. The findings suggest that Phagnaion rupestre could serve as a potential natural remedy for preventing and managing kidney stones, providing a safer, more sustainable treatment option compared to traditional medications. Phagnaion rupestre exhibited a concentration-dependent inhibition of calcium oxalate crystal formation, with the highest inhibition (68%) observed at the highest concentration (1000 µg/mL). The Pearson's correlation (0.997) indicates a very strong positive relationship between extract concentration and inhibition activity, further supporting the extract's potential as a crystallization inhibitor. These findings are consistent with other studies, such as those on *Tribulus terrestris* and *Cystone*, which have also shown inhibition of crystal formation in urolithiasis models [6,11]. Similar inhibition effects have been observed for plants such as *Coleus forskohlii*, which contain compounds that inhibit calcium oxalate crystallization [6]. The results from the crystal aggregation assays also show a significant concentration-dependent effect. At the highest concentration (1000 µg/mL), 70% inhibition of crystal aggregation was observed. The strong Pearson's correlation (0.994) suggests that Phagnaion rupestre has a robust ability to prevent the aggregation of calcium oxalate crystals, a key step in the formation of larger stones. This effect is supported by previous studies on other medicinal plants such as *Ocimum sanctum* (holy basil), which has been shown to reduce crystal aggregation in similar models [13]. Such findings suggest that Phagnaion rupestre could play a significant role in preventing stone growth and development in the urinary tract. The dissolution of pre-formed calcium oxalate stones was enhanced by Phagnaion rupestre extract, with a maximum dissolution rate of 70% at the highest concentration (1000 µg/mL). This effect was highly concentration-

dependent, with a Pearson's correlation of 0.981. These results are particularly noteworthy because, unlike many conventional treatments that only prevent stone formation, Phagnaion rupestre demonstrated the ability to dissolve pre-formed stones. This is consistent with findings from other studies on plant-based therapies such as *Asparagus racemosus* and *Cinnamomum verum*, both of which have shown effective stone dissolution properties [14, 6]. The dissolution effect of Phagnaion rupestre may provide a novel approach for the management of existing kidney stones, making it a valuable adjunct to current treatment options. The phytochemical analysis revealed that Phagnaion rupestre contains significant amounts of phenolic compounds (4.2 mg GAE/g), flavonoids (2.5 mg QE/g), and alkaloids (1.8 mg/g), with moderate to high levels of saponins, tannins, and terpenoids. These compounds are known for their antioxidant, anti-inflammatory, and crystallization-inhibitory properties. For instance, phenolic compounds such as gallic acid are well-documented for their ability to prevent crystal formation and aggregation by acting as crystallization inhibitors [15,17]. Flavonoids, commonly found in plants like *Citrus limon*, have also been associated with reduced calcium oxalate crystallization in urolithiasis models [16]. Alkaloids are similarly known for their medicinal properties, including their role in inhibiting calcium crystal formation and stone growth. While the study offers promising results, several limitations should be addressed in future research. First, the study was conducted in vitro, and the effects observed in synthetic urine and in vitro stone models may not fully replicate the complex conditions of human urine or the variety of stone compositions seen in patients. Clinical trials are necessary to determine whether Phagnaion rupestre exhibits similar effects in vivo. Furthermore, the study did not assess the potential toxicological effects of Phagnaion rupestre. While the plant demonstrated significant inhibitory effects on crystal formation and dissolution, the safety of long-term use remains unclear. Future studies should focus on determining the toxicity and optimal dosage for therapeutic use. Additionally, the potential interactions between Phagnaion rupestre and other drugs commonly used to manage urolithiasis should be explored. Lastly, while the study provides evidence of the extract's potential for kidney stone management, more research is needed to elucidate the precise mechanisms through which Phagnaion rupestre exerts its effects. The identification of the active compounds responsible for crystal inhibition, aggregation, and dissolution will be essential for the development of targeted therapies based on this plant. Phagnaion rupestre demonstrates significant anti-urolithiasis activity by inhibiting calcium oxalate crystal formation, preventing crystal aggregation, and promoting the dissolution of pre-formed stones. The plant's phytochemical profile, including phenols, flavonoids, and alkaloids, likely contributes to its therapeutic effects. Although further clinical studies and toxicological evaluations are needed, Phagnaion rupestre represents a promising natural remedy for the prevention and treatment of kidney stones.

CONCLUSION:

Phagnaion rupestre exhibits significant anti-urolithiasis activity, inhibiting calcium oxalate crystal formation, aggregation, and promoting the dissolution of pre-formed stones in a concentration-dependent manner. The plant's phytochemical profile, including bioactive compounds such as phenols, flavonoids, and alkaloids, contributes to these therapeutic effects. While the study provides promising results, further in vivo studies and clinical trials are necessary to validate its potential as a treatment for kidney stones. Additionally, the safety and long-term use of *Phagnaion rupestre* need to be assessed in future research. Given its high bioactivity and natural origin, *Phagnaion rupestre* holds promise as an adjunct or alternative therapy for managing urolithiasis.

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