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Article

Antilithiasic Potential of *Herniaria hirsuta* and *Plantago albicans* Extracts: Litholytic Effects and Their Use in Algerian Traditional Medicine

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Abstract: Background: Urolithiasis, or kidney stone disease, is a prevalent and recurrent condition with significant healthcare implications. Conventional treatments, including pharmacological and surgical interventions, are often costly, have side effects, and face limitations. Traditional medicine offers a complementary approach, with plants like *Herniaria hirsuta* and *Plantago albicans* historically used for their diuretic and antilithiasic properties. **Objective:** This study evaluates the antilithiasic potential of aqueous extracts from *Herniaria hirsuta* and *Plantago albicans*, focusing on their ability to dissolve kidney stones in vitro. **Methods:** The plants were harvested from the Tessala region of Algeria and identified through botanical and microscopic analyses. Aqueous extracts were prepared by decoction, and their phytochemical profiles were assessed for bioactive compounds like flavonoids, tannins, coumarins, and saponins. Dissolution tests were conducted on kidney stones collected from patients, with their composition confirmed via FT-IR spectroscopy. Weight loss of the stones after exposure to the extracts was measured to calculate dissolution rates. **Results:** Phytochemical screening revealed the presence of flavonoids, tannins, and coumarins in both extracts, with saponins predominantly abundant in *Herniaria hirsuta*. FT-IR analysis identified various stone compositions, including uric acid, calcium oxalate, calcium phosphate, cystine, and struvite. The extracts exhibited significant litholytic activity, with *Herniaria hirsuta* showing a pronounced effect on cystine, uric acid, and calcium oxalate stones, while *Plantago albicans* was most effective on cystine and struvite stones. **Conclusion:** The findings support the traditional use of *Herniaria hirsuta* and *Plantago albicans* in managing urolithiasis. Their efficacy, particularly in dissolving cystine and uric acid stones, highlights their potential as cost-effective and natural therapeutic alternatives to conventional treatments. Further in vivo studies are warranted to validate these results and explore their clinical applications.

Keywords: anti-lithiasic; ethnobotany; *Herniaria hirsuta*; kidney stones; MSA; *Plantago albicans*

I. Introduction

Plants have been used as natural remedies for centuries, with ancient civilizations utilizing them to treat a wide variety of ailments. Today, despite advancements in modern medicine, medicinal plants continue to play an essential role in healthcare, particularly in the treatment of infections and urolithiasis. Globally, the natural resources and biodiversity remain largely underexplored, making medicinal plants a rich reservoir of bioactive molecules. Algeria, renowned for its diverse flora, offers significant potential in traditional medicine through the use of these plants. Ethnobotanical research, stimulated by the increasing interest in natural therapies, aims to unlock the medicinal potential of these species. According to the World Health Organization (WHO), approximately 70% of the global population still relies on traditional medicine for healthcare needs, highlighting the importance of herbal remedies in many countries. [1,2]

Urolithiasis, also known as nephrolithiasis or kidney stone disease, is a multifactorial, non-infectious disorder characterized by the formation of mineral stones or crystals in the kidneys and urinary tract. While many stones may be asymptotically eliminated, severe cases can manifest as renal colic, a painful condition that may lead to chronic kidney damage if left untreated. Over the last five decades, the prevalence of urolithiasis has significantly increased, partly due to lifestyle changes and dietary factors. Today, it is the third most common cause of consultation in urology, surpassed only by urinary tract infections and prostate disorders. Dehydration, dietary imbalances, genetic predispositions, and certain metabolic disorders are recognized risk factors for this condition, with recurrence rates as high as 70–80% in men and 47–60% in women. [3,4]

In the treatment of kidney stones, diuretics are used for two main purposes: to accelerate the elimination of calculus and to prevent recurrence. These agents are commonly employed in the endobiogenic approach to facilitate drainage. The conventional management of urolithiasis also involves the use of anti-inflammatory drugs or surgical interventions. However, these approaches can be associated with high costs, side effects, and limited effectiveness. [5]

Consequently, there is growing interest in alternative therapies, including phytotherapy, to complement or replace conventional treatments. Medicinal plants, known for their diuretic and anti-inflammatory properties, are often used to accelerate the elimination of stones and reduce the likelihood of recurrence. Traditional Algerian medicine employs a range of plants, such as *Arenaria biflora*, *Herniaria hirsuta*, *Plantago albicans*, *Zea mays* (corn silk), *Prunus cerasus*, and *Coriandrum sativum*, to prevent and manage kidney stones. [6,7]

This study aims to evaluate the antilithiasic potential of selected medicinal plants traditionally used in the Tessala region. By conducting dissolution tests and characterizing the chemical composition of these plants, we seek to provide scientific evidence supporting their use in preventing and treating urolithiasis. Ultimately, this research may contribute to the development of cost-effective, natural therapeutic alternatives for the management of kidney stones, addressing the limitations of conventional therapies while promoting the sustainable use of Algeria's rich plant resources.

II. Materials and Methods

Plant Material and Study Area

The plant material used in this study includes *Herniaria hirsuta* and *Plantago albicans*, which were harvested from the Tessala region, located approximately 15 km from the city of Sidi Bel Abbès in western Algeria. The Tessala forest spans the area between Djebel Sbaa Chioukh, Ain Temouchent, and Tlemcen to the west, and Benichougrane in Mascara to the east. Known for its rich flora, this region provides diverse medicinal plants traditionally used for various therapeutic applications.

Botanical Identification

The harvested plants were initially identified using standard botanical methods, including macroscopic and microscopic examinations of the powdered material. Microscopic analysis was conducted using Gazet's reagent to reveal specific cellular structures and elements characteristic of each plant species, such as trichomes. This microscopic examination enables identification of the

different plant materials within the powder, assessment of powder quality and purity, and detection of potential adulterants or contaminants. A small quantity of plant powder was mounted on a microscope slide using glycerin or Canada balsam as a mounting fluid, covered with a coverslip, and observed under a microscope. Characteristic cellular structures were noted, including the thick cell walls and small cell size of *Herniaria hirsuta* and the large, thin-walled cells of *Plantago albicans*.

Collection and Characterization of Kidney Stones

Four kidney stones were collected for the dissolution test from the Urology Department of the University Hospital of Oran (CHU-Oran). These stones were washed and stored in physiological saline solution until further processing. Before conducting the dissolution test, each stone was rinsed with distilled water and air-dried on filter paper for two hours. The morphological characteristics, size, and weight of each stone were recorded, providing a detailed identity profile for each sample.

FT-IR Spectroscopy Analysis

To characterize the composition of the kidney stones, Fourier-transform infrared (FT-IR) spectrophotometry was performed using a SHIMADZU® FTIR-IRAffinity-1S spectrophotometer, with a spectral range from 4000 to 500 cm^{-1} . The analysis was carried out using the attenuated total reflection (ATR) technique, which allows for direct examination of crystalline and non-crystalline substances in the stones. This spectroscopic approach enables precise identification and quantification of the various crystalline species and molecular components present, preserving the structural integrity of the samples for a thorough morpho-constitutional analysis.[8]

Extraction Procedure

The extraction of bioactive compounds from the plant material was performed by decoction, a process suitable for extracting water-soluble compounds like tannins, flavonoids, and mucilages. Decoction was chosen for its efficiency in obtaining concentrated extracts and its stability over time, making it ideal for further analysis and testing. The dried and pulverized plant material was boiled in water to release the active compounds, yielding a solution ready for testing.

Phytochemical screening

The analysis of active secondary metabolites was conducted through phytochemical screening, which involves the qualitative detection of key bioactive compounds. This analysis focused on identifying the presence of polyphenols, including flavonoids, tannins, coumarins also saponin terpen according to trease and evans [9]

Dissolution Test

The dissolution activity of the plant extracts on kidney stones was evaluated using four stones. Each stone was measured for morphology, size, and weight before testing. Following the preparation of the plant decoctions, the stones were submerged in the extract solutions, and dissolution was monitored over time.

After a one-week incubation period, the kidney stones were carefully retrieved using filter paper and thoroughly rinsed with distilled water. The washed stones were then placed in a glass container and dried at 40°C for 16 hours to ensure complete dehydration. Following the drying process, each stone was weighed using a precision balance to obtain an accurate measurement of its mass. This protocol enables the assessment of any mass reduction as an indicator of the dissolution effectiveness of the tested plant extracts. This method assesses the antilithiasic potential of the plant extracts based on their ability to break down and reduce the mass of kidney stones.[10]

Evaluation of Urinary Stone Dissolution Activity

The dissolution activity of urinary stones by each plant extract was assessed by calculating the dissolution rate of the stones after exposure to the experimental medium. This was achieved by comparing the residual weight of the stones after incubation with their initial weight prior to treatment with the extract. The percentage of dissolution was calculated using the following formula:

$$A\% = \frac{W_{\text{initial}} - W_{\text{final}}}{W_{\text{initial}}} \times 100$$

where $A\%$ represents the dissolution rate of the stone, and W_{initial} and W_{final} are the weights of the stone before and after incubation with each plant extract, respectively. This formula provides a quantitative measure of the efficacy of each extract in reducing the stone mass.

III. Results



Figure 1. description of two species.

1. Microscopic Analysis of Powdered *Herniaria hirsuta* and *Plantago albicans*

The microscopic examination of the powdered aerial parts of *Herniaria hirsuta* and *Plantago albicans* revealed distinct morphological characteristics essential for identification and quality assessment.

- *Herniaria hirsuta* Powder

Under 400x magnification, the powder from *Herniaria hirsuta* showed:

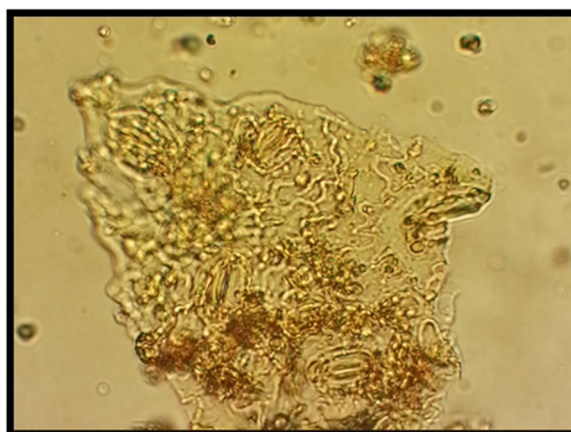
Epidermal fragments with distinctly sinuous-walled cells, contributing to its structural integrity,

Thick-walled, small cells containing rosette-shaped crystals,

A smooth cuticle layer,

Anomocytic stomata, indicative of characteristic stomatal organization in this species.

These specific features aid in distinguishing *Herniaria hirsuta* and serve as markers for its quality control.



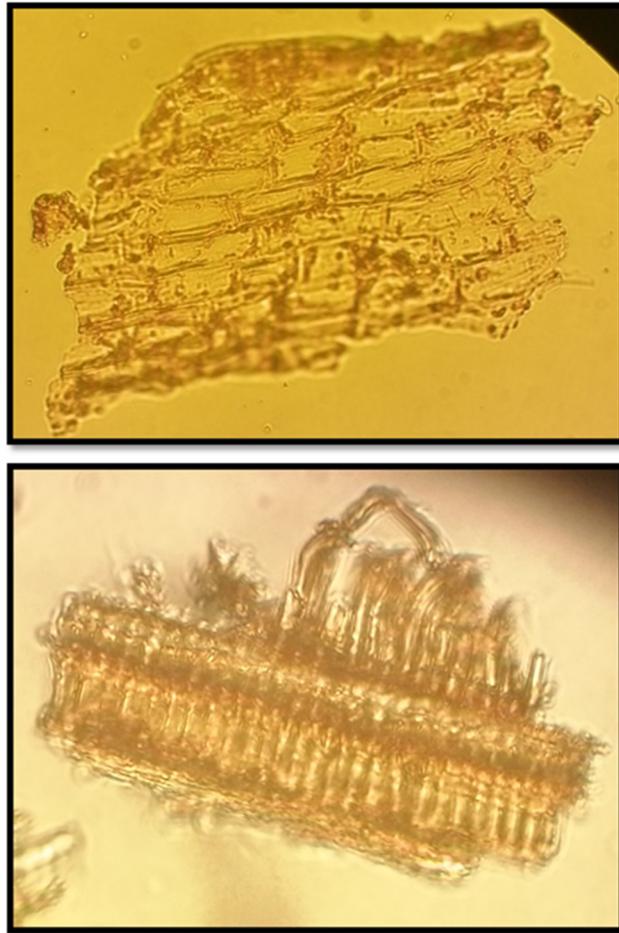


Figure 2. Microscopic Analysis of Powdered *Herniaria hirsuta*.

- ***Plantago albicans* Powder**

Microscopic evaluation of *Plantago albicans* powder at 400x magnification highlighted:

Epidermal fragments with sinuous-walled cells similar in structure,

Thin-walled, large cells with mucilage presence, which aligns with the plant's functional morphology,

A smooth cuticle layer,

Anomocytic stomata,

Uniseriate trichomes, which serve as an identifying trait of *Plantago albicans*.

These microscopic characteristics collectively support the reliable identification and purity assessment of *Plantago albicans*.

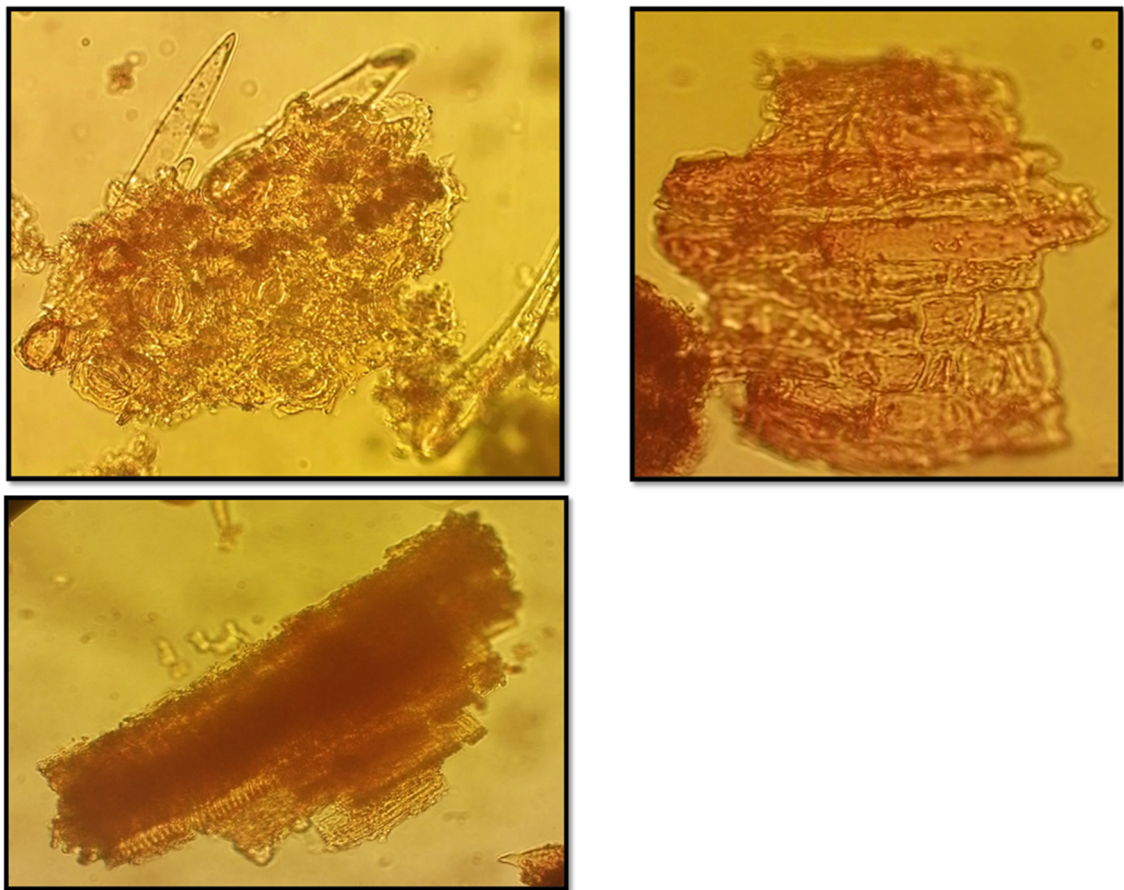


Figure 3.

- 1. Microscopic Analysis of Powder of Plantago albicans
- 2. Extraction process



Figure 4. Extraction process.

- 3. Screening Results:
The phytochemical screening revealed the presence of flavonoids, tannins, and coumarins in both plant extracts, while saponins were notably dominant in the extract of *Herniaria hirsuta*. The results are summarized in Table 1.

Table 1. Phytochemical Analysis of *Herniaria hirsuta* and *Plantago albicans* Extracts.

Secondary Metabolites	<i>Herniaria hirsuta</i> Extract	<i>Plantago albicans</i> Extract
Alkaloids	-	-
Flavonoids	+	+
Saponins	+ (I=200)	- (<100)
Tannins	+	+
Coumarins	+	+
Cardiotonic Glycosides	-	-

The foam test indicated a significant presence of saponins in *Herniaria hirsuta*, as evidenced by the formation of foam in all 10 tubes, with foam heights ranging from 0.5 to 2.0 cm. This resulted in a saponification index (Im) of 200. Conversely, *Plantago albicans* exhibited a low level of saponins, as reflected by the foam heights in the 10 tubes, which ranged from 0.2 to 0.9 cm, indicating a low saponification index (Im < 100).

Results of the Composition Analysis of kidney stones (urolithiasis):

The results of the morphological typing and calcination tests of the analyzed kidney stones are presented in Table 2.

Table 2. Morphological and Constitutional Analysis of kidney stones Subjected to Dissolution Testing.

Calculus Type	External Shape	Surface	Color	Section	Hardness	Calcination
Uric Acid	Rounded Heteromorphic	Slightly Irregular, Budded	Light Brownish Yellow	Unorganized Amorphous Crystals	Brittle - Fragile	Charred - Burns without flame - > 80% black residue
Calcium Phosphate	Cylindrical with Buds	Irregular with Buds	Light Brown to Creamy	More or less Solid Prismatic Crystals	Brittle - Fragile	No charring - Non-burning - Abundant white residue
Calcium Oxalate	Flattened Oval	Smooth - Homogeneous, Budded	Brown - Black	Compact Concentric with Radial Crystallization	Hard - Non-fragile	No charring - Non-burning - Abundant grayish residue

Cystine	Rounded	Smooth Irregular	Creamy Yellow	-	Hard - Fragile	Burns without flame - Charred with no residue
Struvite	Rounded Heteromorp hic	Slightly Irregular, Budded	Light Brown	Unorganize d Amorphous Crystals	Brittle - Fragile	Charred - Burns without flame - > 80% black residue

Results of Infrared Composition Analysis:

The results of the FT-IR (ATR Diamond) analysis for these five calculi are presented in Figures 5 and 6

IR Spectrum of Struvite Calculus

This spectrum displays a broad band between 2300 and 3800 cm⁻¹, along with multiple medium-intensity peaks within the 570–1670 cm⁻¹ range and another prominent peak at 570 cm⁻¹ in the 1005–570 cm⁻¹ region. Comparison with reference spectra (Appendix 1, Figure 01) confirms similarity to the reference spectrum for STRUVITE (hexahydrated ammonium magnesium phosphate).

IR Spectrum of Cystine Calculus

This spectrum features two primary bands: one with a single maximum-intensity peak at 996 cm⁻¹, and another with dual peaks at 570 cm⁻¹ and 500 cm⁻¹. Additionally, there are broad bands with a maximum intensity at 3010 cm⁻¹ and lower-intensity peaks between 1410 and 1680 cm⁻¹. These findings closely match the reference spectrum for cystine.

IR Spectrum of Calcium Oxalate Calculus

The spectrum displays two major bands: a single peak at 1010 cm⁻¹ and a second at 570 cm⁻¹. Broader bands appear with maximum intensity at 2878 cm⁻¹ and additional lower-intensity peaks between 1430 and 1680 cm⁻¹. Comparison with the reference spectrum confirms similarity to calcium oxalate.

IR Spectrum of Uric Acid Calculus

This spectrum includes a broad band from 2600 to 3010 cm⁻¹ and several medium-intensity peaks within the 520–1660 cm⁻¹ range. Another notable peak appears at 740 cm⁻¹ within the 740–520 cm⁻¹ range. This spectrum matches the reference spectrum for uric acid dihydrate crystals

IR Spectrum of Calcium Phosphate Calculus

The spectrum displays four primary bands with peak intensities at 560 cm⁻¹, 870 cm⁻¹, 1030 cm⁻¹, and 1410 cm⁻¹. A broad band also appears between 3460 and 3580 cm⁻¹ with lower intensities. Comparison with reference spectra (Figure 5) shows similarity to weddellite and carbapatite, indicating a type Ia (Whewellite) + type IVa (Carbapatite) composition. Thus, this kidney stone consists of calcium oxalate with calcium and/or magnesium phosphate.

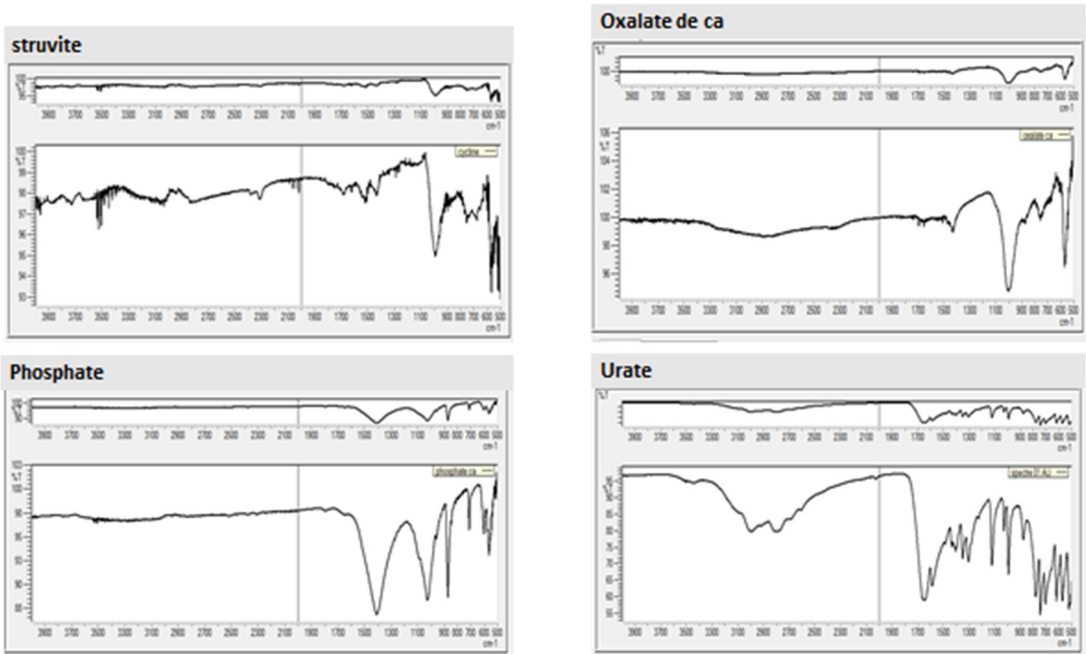


Figure 5.

1. In Vitro Evaluation of Plant Extracts’ Activity on Kidney Stones:.

Weight Measurements During Experiments:

Figures 7 and 8 illustrate the weight changes in five types of kidney stones when exposed to alcoholic extracts from *Plantago albicans* (for three stone types: cystine, struvite, and uric acid) and *Herniaria hirsuta* (for five stone types: cystine, struvite, uric acid, calcium oxalate, and calcium phosphate). Control samples were incubated in physiological saline for each stone type. Stone weights were measured with a precision electronic scale (in grams).

Note:

- S0: represents the week before the dissolution test began; S1, S2, ... S8 are the weeks of the experiment.

The weight evolution graphs demonstrate a significant reduction in the weight of the cystine stone in the alcoholic extract of *Plantago albicans*, with an initial weight of 0.74 g reduced to 0.56 g over the eight-week experiment, accompanied by a decrease in size from 1.20 x 0.46 cm to 0.90 x 0.40 cm.

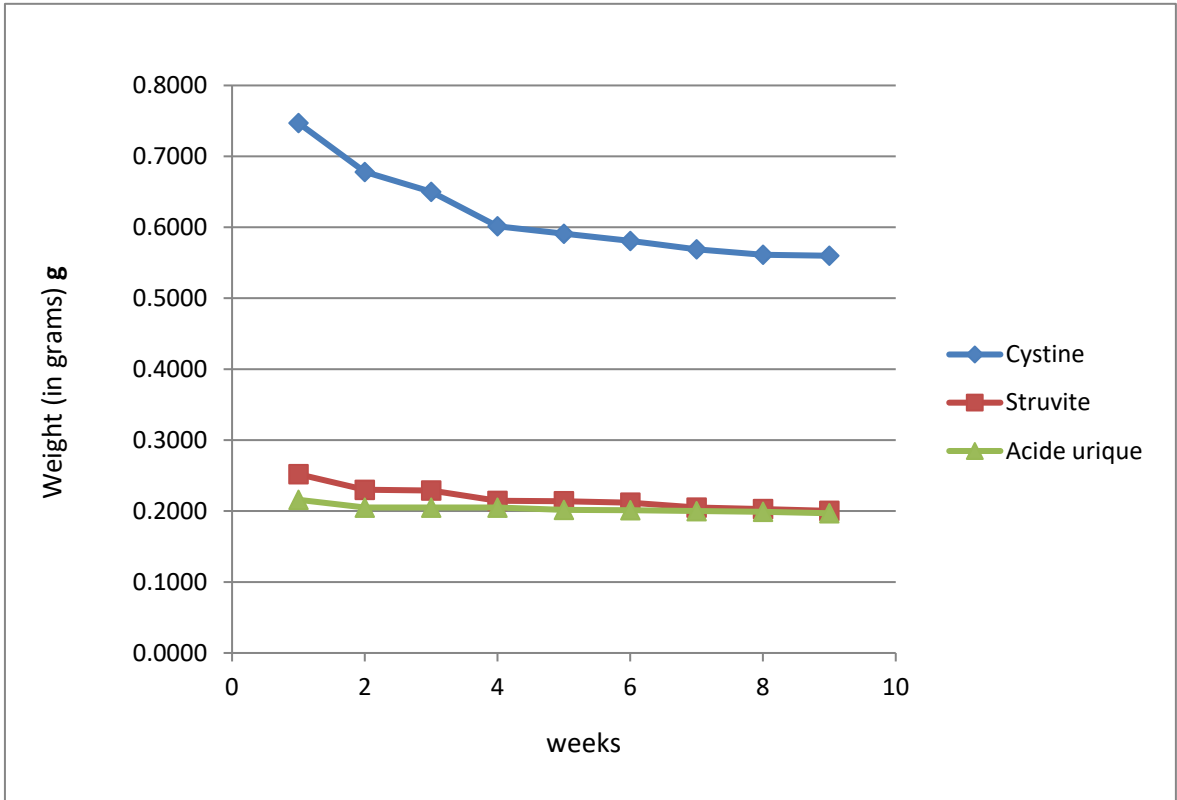


Figure 7. Weight evolution of three stones in *Plantago albicans* extract over eight weeks (in grams).

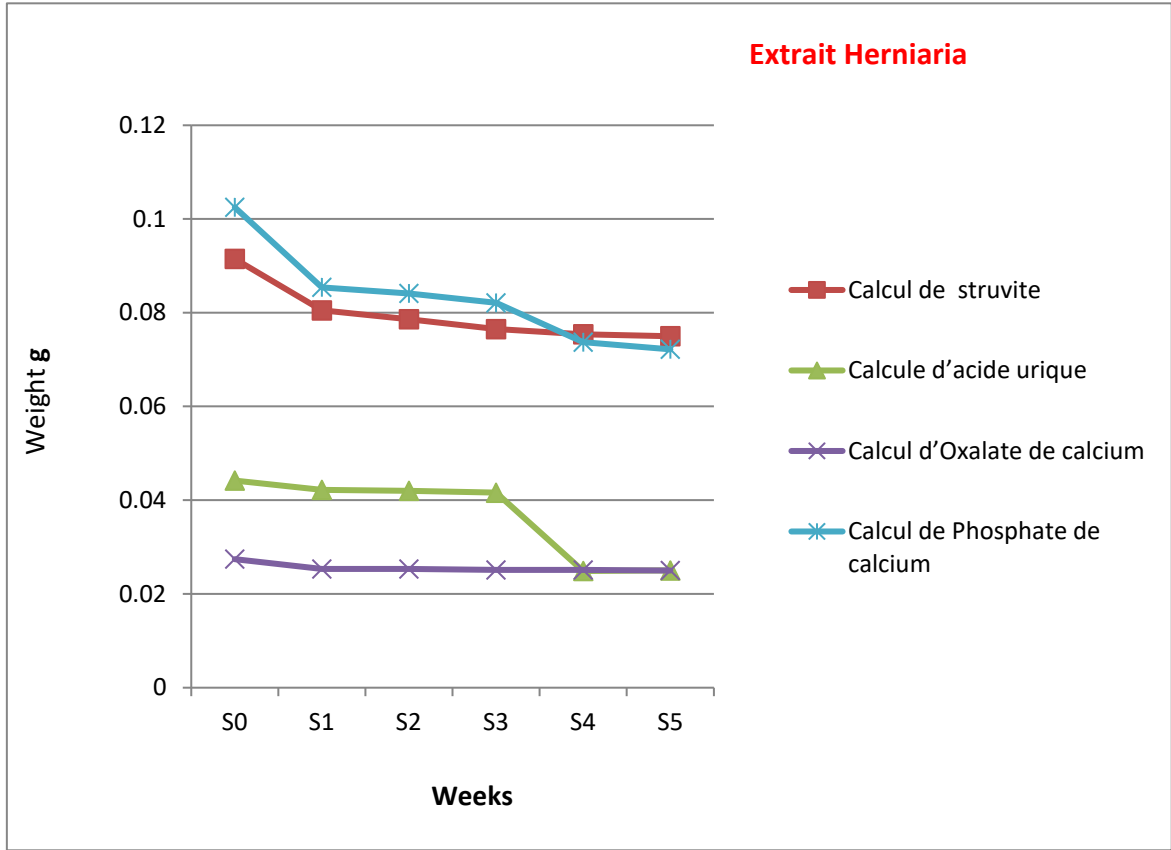


Figure 8. Weight evolution of five stones over four weeks (in grams).

• Discussion

The current study demonstrates a significant litholytic effect of *Herniaria hirsuta* extract, particularly on cystine, uric acid, and, to a lesser extent, calcium oxalate stones. These findings corroborate previous studies that report the potential of various medicinal plants in the management and dissolution of urinary stones. For instance, Crescenti et al. observed an antiurolithiasic effect using a combination of *Herniaria glabra*, *Agropyron repens*, *Equisetum arvense*, and *Sambucus nigra*. Their findings, similar to ours, highlighted *Herniaria* species as effective in the dissolution of certain kidney stone types, particularly those soluble in aqueous solutions.[11]

Moreover, Hannache et al. demonstrated that certain medicinal plant extracts can effectively dissolve cystine stones in vitro. This aligns closely with our observations of *Herniaria hirsuta*'s strong effect on cystine stones, supporting its traditional use for urinary health. Additionally, Meiouet et al. reported that multiple medicinal plants show litholytic activity against cystine stones, underscoring *Herniaria hirsuta*'s promising role within this group.[6,12]

The high saponoside content in *Herniaria hirsuta*, confirmed by a saponification index (Im) of 200, may be a crucial factor in its litholytic action. Vincken et al noted that oleanane-type saponins—prevalent in the Caryophyllaceae family—contribute to foaming properties that may facilitate the breakdown of stone components. In contrast, *Plantago albicans*, with a lower saponification index (<100), exhibited only moderate activity, highlighting the importance of saponosides in stone dissolution. This difference could explain why *Herniaria hirsuta* demonstrates greater efficacy in vitro. [13,14]

In addition to their saponoside content, both plants' bioactive compounds, including flavonoids, tannins, and coumarins, may contribute to their antilithiasic activity. These metabolites are known for antioxidant and anti-inflammatory properties, which may mitigate factors that exacerbate kidney stone formation. Comparative studies suggest that the synergistic effects of these compounds, particularly in *Herniaria hirsuta*, enhance its litholytic potential.[15,16]

For struvite stones incubated in *Plantago albicans* methanolic extract and uric acid stones dissolved in the same extract, a weight reduction of 0.05 g and 0.019 g was noted during the first week, after which weight stabilized over the following seven weeks. The uric acid stone showed a minimal weight reduction over eight weeks, suggesting that *Plantago albicans* has a stronger dissolving effect on cystine stones compared to uric acid stones.

Conversely, *Herniaria hirsuta* exhibited a more potent dissolving effect on cystine and uric acid stones, but no effect on calcium oxalate stones. Therefore, *Plantago albicans* appears more effective on cystine stones than uric acid stones, while *Herniaria hirsuta* showed limited efficacy on calcium phosphate and struvite stones and no effect on calcium oxalate stones.

The average mass loss of cystine stones in *Plantago albicans* extract was significant over eight weeks, at 0.17 g (74.96%). For uric acid stones, a 0.05 g mass loss (83.33%) was observed in the first week. In contrast, average mass losses in *Herniaria hirsuta* extract were 34.14% for cystine stones, 56.56% for uric acid stones, and 70.43% for calcium phosphate stones, with negligible mass loss observed for struvite and calcium oxalate stones throughout the experiment.

Figures 7 and 8 depict the dissolution rate of stones in aqueous extracts of *Plantago albicans* L. and *Herniaria hirsuta* L. over the eight-week experiment.

The mass loss results correlated strongly with the dissolution rates observed for *Plantago albicans* extract. For cystine stones, a high dissolution rate (25.04%) was recorded. Struvite stones showed a dissolution rate of 20.44%, while uric acid stones had a low rate of 8.8% over the eight weeks. This suggests that *Plantago albicans* methanolic extract has a greater capacity for dissolving cystine stones, with negligible effect on uric acid stones. In contrast, *Herniaria hirsuta* demonstrated a remarkably high dissolution rate for cystine stones (65.86%) and uric acid stones (43.44%) compared to struvite stones (18.04%). This greater effect on cystine and uric acid stones may be attributed to the higher saponoside content in *Herniaria hirsuta* compared to *Plantago albicans*. The Saponin test results reveal a notably high presence of saponosides in *Herniaria hirsuta*, with a saponification index (Im) of 200, indicating a strong foaming capability. In contrast, *Plantago albicans* exhibits a much lower saponoside

content, evidenced by the modest moss height in the 10 test tubes (0.2–0.9 cm), and a saponification index below 100. [17,18]

These findings align with those of Vincken et al., who identified oleanane-type saponins as the predominant saponin class in the Caryophyllaceae family, to which *Herniaria* belongs. This high saponoside content in *Herniaria hirsuta* contributes to its dissolution efficacy in vitro by facilitating the formation of foaming solutions that interact with the crystalline components of water-soluble kidney stones [10,13]. Consequently, *Herniaria hirsuta* demonstrates a stronger potential as a natural litholytic agent compared to *Plantago albicans*, due to its elevated saponoside levels.

Conclusion

This study underscores the efficacy of *Herniaria hirsuta* as a promising natural agent in dissolving cystine and uric acid kidney stones, with moderate activity observed for calcium oxalate stones. The plant's rich saponoside content is likely central to its dissolution capabilities, reinforcing findings from prior studies on the Caryophyllaceae family's antilithiasic activity.

In comparison, *Plantago albicans* demonstrated a weaker effect, suggesting it may be less effective alone but still potentially beneficial as part of a combined treatment with other litholytic agents. The study's strength lies in its detailed comparative analysis of two plants with different saponoside profiles and in demonstrating a potential application of *Herniaria hirsuta* in alternative therapies for urolithiasis.

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