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### Stones and Endourology

Original article

# In vitro litholytic activity of some medicinal plants on urinary stones



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#### KEYWORDS

Urinary stones;  
Litholytic activity;  
*Trigonella foenum-graecum*;  
*Petroselinum crispum*;  
*Opuntia ficus-indica*

#### Abstract

**Objective:** This study was designed to evaluate the effect of plant extracts used in traditional medicine on the dissolution of three types of kidney stones.

**Subjects and methods:** Kidney calculi of cystine; uric acid and pure carbapatite were incubated in vitro during 6 weeks in the presence of three of plant extracts and of 0.9% NaCl solution used as control. An extract of each plant was prepared by infusion of three grams of powdered plants during 30 min in 100 mL of a boiled NaCl 0.9% aqueous solution. Each extract was then filtered and thereafter set in a flask containing a stones. At the end of each week the stone was removed from the experimental medium and weighted after a 18 h drying at 40 °C.

**Results:** After 6 weeks of experiment and with in vitro study, we are observed that the aqueous extract of the seeds of *Trigonella foenum-graecum* has a better effect on dissolution of cystine and carbapatite stones ( $p < 0.05$ ), with mass loss of 94 mg and 73 mg respectively at the end of experiment. While with NaCl solution, the mass was small.

**Conclusion:** Our experiment failed to demonstrate a significant effect of the tested plant extracts to dissolve three types stones in vitro. However, we observed that only the extract of the seeds of *T. foenum-graecum* has a better effect on dissolution of cystine and carbapatite stones probably resulting from formation of complexes between stones and polyphenols or flavonoids present in the extracts.

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## Introduction

The renal lithiasis is well known from long time ago and it has shown to be indissociable from human history. The development of the stones is related to the decrease of urine volume or the increase of excretion of stone-forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate. Urolithiasis is a common health problem with increasing prevalence of up to 20% all over the globe. The increased prevalence of the disease is due to the lifestyle changes such as lower dietary intake of vegetables or fruit, higher consumption of animal proteins, salt, sweetened beverages, and inadequate fluid intake. Calcium oxalate stones are the most common type of nephrolithiasis [1].

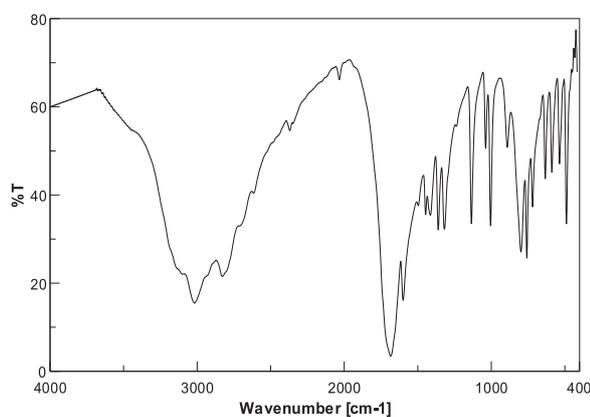
In Morocco, Few studies have been done on urolithiasis, aiming to determine the composition of urinary calculi collected in certain regions [2–7]. These studies had shown the preponderance of calcium oxalate monohydrate as the major component of urinary calculi (66.6%), followed by anhydrous uric acid (18.1%), carabapate (7.9%) and cystine (0.6%) [7].

In Morocco, more people are using traditional medicine to treat this disease, because in one hand the price of conventional medicines is relatively high, in another hand they can have a limited effect. As an example the diuretics, the anti-inflammatory and the inhibitors of some metabolites are the only medicines used to treat oxalocalcic lithiasis, though with adverse effects.

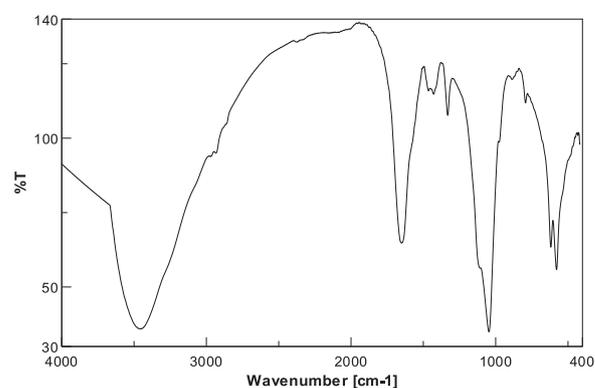
Several plants have been subject to scientific studies in Morocco and around the world to evaluate the litholytic activity in an in-vitro system like: *Herniaria hirsuta*, *Zea mays*, *Ammi visnag*, *Zizyphus lotus L.* [8,9].

The uses of plants extracts, essentially targeting this oxalocalcic lithiasis, have been the object of several publications [10–13]. Thus, just few studies have been interested in more rare lithiasis, often due to difficulties in social care like cystine, carabapate and uric acid lithiasis.

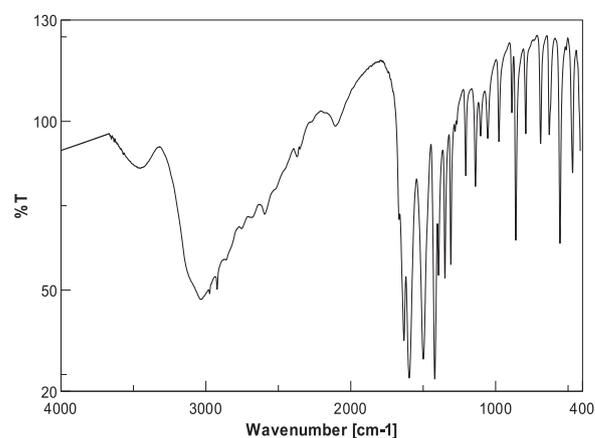
Dissolution therapy can be employed in patients with uric acid stones includes urinary alkalization, hydration, modification of diet and allopurinol [14], for cystinic patients the aim of dissolution therapy is to hydrate and alkalize the urine. A further treatment strategy is the addition of drugs that convert cystine to compounds that are more



**Figure 1** IR Spectrum of Uric Acid.



**Figure 2** IR Spectrum of Carabapate.



**Figure 3** IR Spectrum of Cystine.

soluble at lower pH values. Cystine stones are exceedingly hard and usually do not respond well to extracorporeal shockwave lithotripsy, hence if there is treatment failure with dissolution therapy then usually flexible uretero-renaloscopy or percutaneous nephrolithotomy is required [15,16].

The aim of this study was to realize a phytochemical screening of some medicinal plants, and to evaluate their dissolving effect including *P. crispum* (leaves and stalks), *O. ficus-indica* flowers, and *T. foenum-graecum* seeds already used in traditional medicine to cure renal lithiasis. To do so, we have observed in vitro the variation of calculi weight; kinetic aspects of this dissolution; (cystine, carabapate and uric acid lithiasis) during the whole experience, which last six weeks.

## Subjects and methods

### Urinary calculi

Three pure samples for each type of calculi cystine, uric acid and carabapate coming from Ibn Sina University Hospital, Rabat, Morocco have been selected, from a sample library of the Analytical Chemistry Laboratory of the Faculty of Medicine and Pharmacy of Rabat, after a spectrophotometry analysis using Fourier Transformed Infrared (FTIR) according to the protocol described by Benramdan et al. [6]. No information on the personal data of the patients has been revealed. Figs. 1–3 show the IR specters of selected kidney calculi.

**Table 1** Screening some phytochemical groups of three plants extracts.

	<i>Trigonella foenum-graecum</i>	<i>Petroselinum crispum</i>	<i>Opuntia ficus-indica</i>
Alkaloids	++	—	—
Flovonoids	+++	++	++
Coumarins	+++	+	—
Anthrasenosids	—	—	—
Saponosids	+	+	—
Condensed Tannins	+++	—	—
Polyphénols	+++	++	+++

*Phytochemical screening*

Qualitative reactions for the screening of some phytochemical groups were performed using the methods of Harbone [17], Trease and Evans [18] thus reported by Buvanewari [19,20].

*Litholytic activity*

An extract of each plant have been prepared by infusing, during 30 min, 3 g of the plant dried in 100 mL of a boiling physiological solution (9 g of NaCl per liter), this solution is also used as a witness environment to appreciate the variations of calculi weight. After filtration, the extract (60 mL) was distributed on glass Erlenmeyer. The calculi were placed in a porous bag and placed in suspension in the extract at an ambient temperature.

For each experience, the weight loss of kidney calculi have been evaluated by weighting the calculus after letting it dry in an oven at 40 °C during 18 h. Each experience was performed in triplicate.

The activity of the extract has been evaluated by calculating the rate of calculi dissolution after the time spent in the experimental environment by comparing their residual weight with their initial one before the incubation of the extract. The percentage of dissolution was calculated by the following formula [13]:

$$a\% = (W_{\text{initial}} - W_{\text{final}}) \times 100 / W_{\text{initial}}$$

a%: is the rate of calculi dissolution; W initial and W final are the weights of the calculus before and after the incubation with the plants extracts.

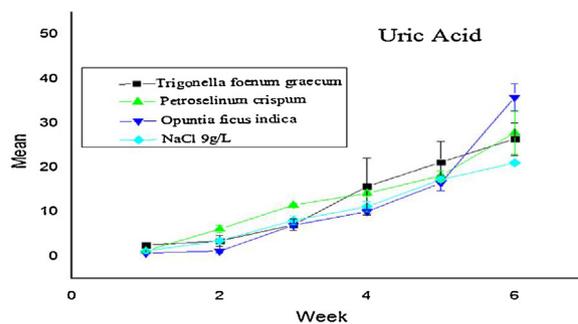
*Data analysis*

Data were expressed as mean ± standard deviation (SD). Statistical comparisons between all groups were performed by a one-way ANOVA with Bonferroni's posttest. The difference is considered as significant for  $p \leq 0.05$ .

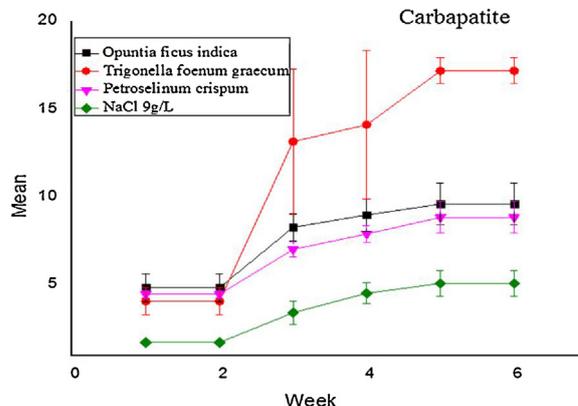
**Results**

*Phytochemical characterization*

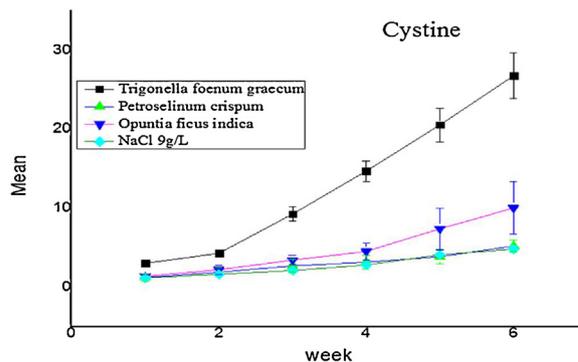
The qualitative characterization of extracts is summed up in the Table 1.



**Figure 4** Dissolution of uric acid calculi.



**Figure 5** Dissolution of carbapatitecalculi.



**Figure 6** Dissolution of cystine calculi.

Many phytochemical groups have been looked for like: alkaloids, flavonoids, condensed tannins, coumarins, anthrasenosids, saponosids and polyphenols.

*Litholytic activity*

The dissolution capacity of different types of calculi by different extracts during 6 weeks is illustrated in Figs. 4–6.

*Study of the effect of plants extracts on cystine calculi*

The extract of *T. foenum-graecum* had a more notable effect starting from the second week of the experience ( $p < 0.05$ ) than other extracts and control solution of NaCl ( $p = 0.11$ ).

At the end of the experience, we have noticed a weight loss of  $94 \text{ mg} \pm 1.5 \text{ mg}$  (27%) while leaves extracts of *O. ficus-indica* and

*P. crispum* have caused a weight loss of 40 mg ± 12 mg (10%) and 21 mg ± 5 mg (5%). The NaCl solution gives a calculus weight reduction of 20 mg ± 2.2 mg (4%).

#### Study of the effect of plants extracts on carapatite calculi

For carapatite calculi, the three medicinal plants extracts had effects on the dissolution of this type of calculi starting from the second week of the experience ( $p < 0.05$ ) and had provoked a weight loss of 17% (73 mg ± 18 mg) for the aqueous extract of *T. foenum-graecum*, and 10% for the two extracts of *O. ficus-indica* and *P. crispum*.

The control solution of NaCl produces a weight loss in the calculus of 4% (18 mg ± 2 mg).

#### Study of the effect of plants extracts on uric acid calculi

The extracts of *O. ficus-indica* had a more notable effect, at the end of the experience, it provoked 36% of weight loss for uric acid calculi while *P. crispum* and *Trigonella foenum-graecum* seeds extracts caused a weight loss of 27% and 26%, the NaCl solution caused a weight loss of 20%.

### Discussion

In this work, we have studied in vitro the effect of three plants extracts *T. foenum-graecum*, *O. ficus-indica* and *P. crispum* on the dissolution of three types of kidney calculi cystine; carapatite and uric acid well characteristic and we had evaluated the effect of each extract by calculating the rate of calculi dissolution after a period of time in the experimental environment by comparing the residual weight of calculi with their initial weight.

Our results had shown no considerable dissolution effect for the plants tested in the control solution nor in the extracts and this for the three types of kidney calculi during the six weeks period of the experimentation.

The works that have been realized and published, and have tested the medicinal plants extracts on urinal calculi were often interested in cystinic lithiasis [21–23]. The study of Moroccan plants brought by Meiouet et al. [21]; had shown a very notable dissolving effect (dissolution was total) on cystine calculi with all the tested extracts.

Although, In our study, Fig. 6 shows the line slope of cystine calculi dissolution obtained for *T. foenum-graecum* extract got bigger in the last weeks compared to the observed lines of the other extracts, but needs without a doubt more time than the plants of Meiouet study.

Regarding carapatite calculi, we had observed that the *T. foenum-graecum* seeds extract has a more notable effect (17%) compared to the other extracts and to the NaCl solution (4%), for uric acid-type stones, our experiment did not show any significant efficacy of the extracts tested to dissolve uric acid stones.

The analysis of chemical composition shows that *T. foenum-graecum* seeds are rich of polyphenols and flavonoids, which suggests the formation of a complex calculus-principle active; the complexes formed would be a lot more soluble than the calculus itself.

Our experimental protocol was similar to the one described by the Algerian plants study of Hannache et al, in the way that the

calculi were contained in porous bags; this protocol has been chosen to test only the chemical effect of the tested solution. This Algerian study had not allowed also proving a significant efficiency of tested extracts in dissolving cystine calculi: *Arenaria ammophila*, *Parietaria officinalis*, *paronychia argentea*.

### Conclusion

The effect of several medicinal plants used in the treatment of urolithiasis in traditional medicine has been studied in vitro. Our experiment failed to demonstrate a significant effect of the tested plant extracts to dissolve three types stones in vitro. However, we observed that only the extract of the seeds of *T. foenum-graecum* has a better effect on dissolution of cystine and carapatite stones probably resulting from formation of complexes between stones and polyphenols or flavonoids present in the extracts. Further tests will be necessary to objectify a scientifically proven benefit of these extracts tested on the solubilization of such calculi. Given the time required to observe a beneficial effect in vitro, it seems difficult to recommend these plants in patients to dissolve in situ calculi. However, these plants may have an interest, which remains to be assessed, for the prevention of recurrence.

### Conflicts of interest

The authors declare that they have no competing interests.

### Ethics committee approval

Since this is a retrospective and non-interventional study, it is not mandatory to seek the advice of an ethics committee according to Moroccan regulations.

### Authors' contributions

LY, SB, ZA and MB carried out the study, designed and conducted all laboratory experiments; analyzed and interpreted experimental results. AC, MOB and MD supervised the study and involved in manuscript preparations. All authors read and approved the final manuscript.

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