

## *In vitro* litholytic activity of extracts and phenolic fractions of some medicinal plants on urinary stones

Kenza Ammor<sup>1\*</sup>, Fatima Mahjoubi<sup>1</sup>, Dalila Bousta<sup>2</sup>, Radouane Elhabbani<sup>3</sup> and Abdellah Chaqroune<sup>1</sup>

<sup>1</sup>Laboratory of Materials Engineering and Environment, Sidi Mohammed Ben Abdellah University, Faculty of Sciences Dhar El Mahraz, Po Box 30003, Fez, Morocco

<sup>2</sup>Laboratory of Neuroendocrinology, Nutritional and Climatic Environment, Sidi Mohammed Ben Abdellah University, Faculty of Sciences Dhar El Mahraz, Po Box 30003, Fez, Morocco

<sup>3</sup>Laboratory of Epidemiology and Research in Health Sciences, Sidi Mohammed Ben Abdellah University, Faculty of Medicine and pharmacy, Fez, Morocco

**Abstract: Objective:** This study was carried out to evaluate the efficiency of plant extracts used in traditional medicine on the dissolution of oxalo-calcic and cystine stones. Also, the efficiency of phenolic fractions (Tannins and Flavonoids) for the plants that gave us the best stone dissolution rate.

**Subjects and methods:** Kidney stone of oxalo-calcic and cystine stones were incubated *in vitro* for 8 weeks in the presence of five plants extracts (hydro-ethanolic and aqueous extracts). NaCl solution (0,9 %) used as negative control and sodium citrate solution at 3 mM/L as a positive control. The studied plants were *Herniaria hirsuta L.* aerial parts, *Opuntia ficus-indica L.* flowers, *Zea mays L.* stigmata, *Ammi visnaga L.* seeds and *Ziziphus lotus L.* fruits. After 2, 4, 6 and 8 weeks the stones were weighed after 18 h drying at 40°C and the dissolution rate of the stones and the pH of the solution were measured.

**Results:** After eight weeks, all the plant extracts and phenolic fractions had revealed a significant effect to dissolve oxalo-calcic and cystine stones in comparison to the control solutions. The best result of cystine stones was showed with aqueous extract of *H. hirsuta* aerial parts which had a dissolution rate of 88,91 %. Concerning the dissolution rate of oxalo-calcic stones the aqueous extract of *Z. mays* had the best results with 68 % against 19 % for the sodium citrate solution. The dissolution rate of the fractions studied showed a better result for *Z. mays* flavonoids fraction toward oxalo-calcic stones, and *Z. mays* tannins fraction for cystine stones. The pH undergoes a non-negligible linear increase over the eight weeks for all extracts and phenolic fractions and both kidney stones.

**Conclusion:** The tested plants' extracts and the phenolic fractions for the plants that gave us the best stone dissolution rate were able to dissolve oxalo-calcic and cystine stones. To confirm the efficiency of these plant extracts on the treatment of oxalo-calcic and cystine stones multiple *in vivo* tests could be made.

**Keywords:** kidney stones; oxalo-calcic; cysteine; plants; phenolic fractions; dissolution.

### 1. Introduction

Renal lithiasis is a multifactorial non-infectious metabolic disease whose name derives from the Greek " lithos " which means stone<sup>1</sup>. The formation of kidney stones is a complex process that results from a succession of several physicochemical events, including supersaturation, nucleation, growth, aggregation, and retention within renal tubules<sup>2</sup>. 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<sup>3</sup>.

Urolithiasis prevalence is increasing up to 20 % of the

population all over the world. In most cases, the increase of the prevalence is associated with nutritional disorders as lower dietary intake of vegetables or fruit, higher consumption of animal proteins, salt, sweetened beverages, and inadequate fluid intake<sup>3</sup>.

Some studies had shown the preponderance of calcium oxalate as the major component of kidney stone with a proportion of 80 %; it has a recurrence rate of 70 to 81 % in man and 47 to 60 % in women<sup>4</sup>.

In Morocco, many studies are mainly directed against oxalo-calcic lithiasis<sup>5-7</sup>. Contrariwise few works have been done on rare lithiasis, which often poses more

\*Corresponding author: Kenza Ammor  
Email address: [kenza.ammor1@gmail.com](mailto:kenza.ammor1@gmail.com)  
DOI: <http://dx.doi.org/10.13171/mjc9602001101135ka>

Received October 27, 2019  
Accepted September 7, 2019  
Published January 10, 2020

problems, such as cystine lithiasis<sup>8</sup>. Cystine stones account for 1 % of urinary lithiasis observed in adults and about 10 % of those observed in children<sup>9</sup>. It is a hereditary disease, characterized by a defect of renal tubular reabsorption of cystine and essential amino acids (ornithine, lysine, arginine). Cystine is poorly soluble in water, and its excessive concentration in the urine leads to repeated formation of stones<sup>10</sup>.

The recurrence of cystine stones represents a severe problem. Therefore, stone treatment is highly recommended. The use of extracorporeal shock-wave lithotripsy (ESWL) method may cause acute renal injury, a decrease in renal function, haemorrhage, hypertension and an increase in stone recurrence. In addition, persistent residual stone fragments and possibility of infection after ESWL represent a severe problem in the treatment of stones<sup>11</sup>. Thus, alternative treatment using phytotherapy has been suggested<sup>5-8</sup>.

In Morocco, the use of phytotherapy is well known from several centuries. Nowadays, more people are using traditional medicine to treat urolithiasis, because in one hand the price of conventional medicines is relatively high, in another hand they can have a limited effect. For example, diuretics, anti-inflammatories and inhibitors of specific metabolites are the only drugs used in the treatment of oxalocalcic lithiasis with unavoidable side effects<sup>12</sup>.

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 *P. crispum*, *O. ficus-indica*, *T. foenum-graecum*, *Parietaria officinalis*, *Arenaria ammophila*, *Paronychia argentea*, *Herniaria hirsuta* L., *Zea mays* and *Ammi visnaga* L.<sup>8,13-15</sup>.

To value our plant heritage, we have chosen five plants known by the Moroccan population in the treatment of urolithiasis to evaluate their dissolving effect including *Herniaria hirsuta* L. aerial parts, *Opuntia ficus-indica* L. flowers, *Zea mays* L. stigmata, *Ammi visnaga* L. seeds and *Ziziphus lotus* L. fruits.

*Herniaria hirsuta* is known throughout the Maghreb. It is found on sandy soils, at the edge of paths, in uncultivated places, dry lawns, pastures, olivettes and on places of passage because they resist trampling. This species is traditionally indicated in fluid retention, edema, oliguria, renal lithiasis, catarrh of the bladder and whenever it is necessary to stimulate the renal functions for the elimination of water<sup>16</sup>.

The stigmas of *Zea mays* have diuretic, cholagogues, sedative properties on the urinary system, antihemorrhagic (vitamin K) properties. The oil has anti-atheromatous properties due to its richness in unsaturated fatty acids<sup>16</sup>. The stigmata have been used in many parts of the world for the treatment of

edema, cystitis, gout, kidney stones, kidney disease and prostatitis<sup>17,18</sup>.

*Opuntia ficus indica* represents a vital food resource for the Moroccan populations. It is sometimes dried to be consumed throughout the year after rehydration. Flowers are indicated as a diuretic (edema, urinary retention, weight loss treatments, etc.) and in the treatment of non-infectious diarrhea<sup>16</sup>.

*Ammi visnaga* (khela) species is answered in Morocco; people use it for several treatments. Orally, khella is used for the treatment of colic and abdominal cramps, kidney stones, respiratory problems such as asthma, bronchitis, cough and whooping cough<sup>19</sup>.

*Ziziphus lotus* (L.) is a Mediterranean species resistant to moderate cold and hot weather, able to vegetate in a dry atmosphere and be content with low rainfall<sup>16</sup>. The different species of *Ziziphus* are widely used in the treatment of certain diseases such as inflammatory diseases, digestive disorders, weakness, liver diseases, obesity, urinary disorders, diabetes, skin infections, fever, diarrhea and insomnia<sup>20,21</sup>.

The different plants studied are already used in traditional medicine to eliminate kidney stones. To do so, we studied the efficiency of plant extracts and phenolic fractions of the plants with the best dissolution rate of oxalo-calcic and cystine stones.

The study was done by following *in vitro* the mass variation of the stones during an eight-week incubation period; we also evaluate the solvent effects. These observations were supplemented by pH measurements of the solution to discuss the effect of this parameter on eventual dissolution.

## 2. Material and methods

### 2.1. Urinary stones

Samples of each type, oxalo-calcic and cystine stones were collected from the University Hospital Centre Hassan II Fez, Morocco. From different patients, kidney stones were collected by surgery, percutaneous nephrolithotomy or spontaneous expulsion. They were selected by binocular microscopy examination and their chemical composition was authenticated using Fourier Transformed Infrared (FTIR) according to the protocol described by *Benramdan et al.*<sup>4,16,17</sup>. The identification of the different kidney stones was carried out in a previous work<sup>24</sup>. No information on the personal data of the patients has been revealed.

The values of the initial average mass ( $\pm$  standard deviation) of the stone fragments and the stones in the solution loaded with plant extracts and in the control solutions are summarized in [Table 1](#) below:

**Table 1.** Initial average mass of calculations and fragments of calculations.

Plants	Kidney stones	
	Oxalo-calcic (mg)	Cystine (mg)
<i>A. visnaga</i>	140,2 ± 36,6	137,65 ± 2,15
<i>Z. lotus</i>	98,7 ± 17,3	66,65 ± 6,95
<i>O. ficus indica</i>	125,6 ± 24,2	50,55 ± 3,85
<i>Z. mays</i>	247,8 ± 15	41,05 ± 0,05
<i>H. hirsuta</i>	156,4 ± 35,3	39,3 ± 2,2
NaCl Solution	260 ± 15	203,42 ± 6
Citrate Solution	245 ± 21	240,45 ± 5

## 2.2. Plant material

The choice of plants selected for this study was based on an ethnopharmacological survey carried out in the Sidi Hrazem spa (where people do a cure for removing kidney stones), the CHU Hassan II of Fes and the old medina of Fez (at herbalists).

The selected plants are: *Herniaria hirsuta* L. (aerial parts) collected in the Middle Atlas mountain in Morocco, *Zea mays* L. (stigmata), *Opuntia ficus indica* L.(flowers), *Ammi visnaga* L.(seeds) and *Ziziphus lotus* L.(fruits) that came from Taounate (north-east of Fez). These plants were harvested during the flowering period.

These plants, freshly collected, were dried in the shade and protected from light in a dry and ventilated place. Then have been identified. Samples were deposited at the herbarium of the Biotechnology and Natural Resources Preservation Laboratory of the Dhar El Mehraz Faculty of Science in Fes (RAB 76986 for *A. visnaga*, RAB 1264407 for *O. ficus indica*, *H. hirsuta* for RAB 090814. RAB 1108434 For *Z. mays* and RAB 357564 for *Z. lotus*).

## 2.3. Preparation of the plant extracts

### 2.3.1. Decoction

We opted for the use of decoction in order to get as close as possible to the most traditionally used method<sup>25,26</sup>. The extraction method was performed by taking 20 g of a dried and pulverized plant with 200 mL of distilled water. The mixture was heated (reflux system) for 30 minutes. The mixture was filtered using Whatman filter paper and concentrated under reduced pressure.

### 2.3.2. Soxhlet extraction

The Soxhlet extraction was chosen because it allowed having the highest yield, which can be explained by the heating applied during the operation as well as the long extraction time<sup>27</sup>. The extraction was realized by using a Soxhlet system, 25 g of dried plant powder was put in a cellulose cartridge and extracted with 250 mL of ethanol-water (70/30). The extraction process continues until the solvent in the siphon tube of an extractor become colourless. After that, the extract was filtered and been concentrated under reduced pressure. We realized in previous studies on these

plants a phytochemical screening as well as the dosage made of the flavonoids and the total phenols<sup>28-30</sup>.

## 2.4. Preparation of polyphenolic fractions

### 2.4.1. Extraction of tannin content

The first step of extraction of tannins content consists of taking 30 g of plant powder, allowing them to macerate in 100 mL of petroleum ether for 24 hours to be defatted. After filtration, the marc is recovered while chlorophyll and lipids are removed.

The recovered marc was taken up in 50 mL of diethyl ether then it was filtered to remove phenols, catechins and oxy butyric acid.

The marc was taken up a second time with 100 mL of methanol. Then filtered and the methanolic filtrate was evaporated under vacuum to obtain a dry residue. A pure tannin extract has been weighed<sup>31</sup>.

### 2.4.2. Extraction of flavonoid content

A quantity of 30 g of plant powder was macerated in 100 mL of methanol for 72 h. After filtration, the methanol was evaporated by a rotary evaporator at a temperature of 40°C. under vacuum. The dry residue obtained was treated with 50 mL of warm water to obtain an aqueous extract.

We carried out a series of liquid-liquid extraction into a separating funnel with non-miscible solvents with the aqueous extract. This operation allowed the separation of one or more constituents by using of their unequal distribution in two non-miscible liquids. It consisted of the addition of 3x30 mL of chloroform which removed chlorophyll and lipids. Then 3x30 mL of diethyl ether was added to extract the free genomes and flavonoids.

Finally, the addition of 3x30 mL of ethyl acetate made the elimination of monosids possible and caused the majority of the flavone glycosides. During these different stages, we recovered the aqueous phase. During the last aqueous phase, we added 3x30 mL of butanol to recover the alcoholic phase. This last phase containing the flavonoids was recovered in a previously weighed balloon<sup>31</sup>. It was then subjected to evaporation of butanol under vacuum at 55°C to

obtain the dry residue. The flavonoids extract has been weighed.

## 2.5. Control

The effect of plant extracts was compared to a positive control of an aqueous solution of sodium citrate at 3 mM/L, which corresponds to the average concentration of urinary citrate obtained during the treatment of cystinuria by alkalinisation of the urine (2.91 mM/L in our experiment). The aqueous solution of NaCl at 9 g / L was used as a negative control.

## 2.6. Effect of plant extracts on kidney stones

The first experiment consisted in comparing the effects of the five plants extracts by placing the stones (cystine and oxalo-calcic) in an Erlenmeyer in the presence of 0,5 % of plant extracts in physiological solution (9 g of NaCl /L) at room temperature.

The exhibition of the stones to the extracts did not result in perceptible changes either after 24 hours or after three days, and the stones were left in contact with the extracts for eight weeks.

Every 15 days, the stones were taken out of the experimental medium, washed with distilled water, dried at 40°C for 16 hours, then weighed using a precision balance to evaluate the loss mass. The weighing was carried out after two, four, six and eight weeks (S<sub>2</sub> to S<sub>8</sub>), also for each experiment, the pH of the solution was measured. Each experiment was performed in triplicate under the same conditions, and the results were expressed by calculating the mean and standard deviation of the values obtained.

## 2.7. Effect of phenolic fractions on kidney stones

Phenolic fractions (Tannins and Flavonoids) of *O. ficus-indica* flowers, *Z. mays* stigmata and *H. hirsuta* aerial parts have been tested in the presence of cystine and oxalo-calcic stones under the same conditions as those described previously. The choice to realise fractions of these plants was because they gave us the best stone dissolution rate.

## 2.8. Evaluation of the ability of extracts and phenolic fractions to dissolve the urinary stone

The activity of the extracts was evaluated by calculating the dissolution rate of the stones after residence in the experimental medium by comparing the residual weight of the stones with respect to their initial weight before the incubation with the extract. The percentage of dissolution was calculated by the following formula <sup>32</sup>.

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

$\alpha$  %: is the Dissolution Rate of the calculation (DR)

$W_{\text{initial}}$ : the stone weight before incubation.

$W_{\text{final}}$ : the stone weigh after incubation.

## 2.9. Data analysis

Data were expressed as mean  $\pm$  standard deviation (SD). Statistical comparisons between all groups were performed by a one-way ANOVA. The difference is considered significant for  $p \leq 0,05$ .

## 3. Results

### 3.1. Effect of hydro-ethanolic and aqueous extracts on oxalo-calcic stones

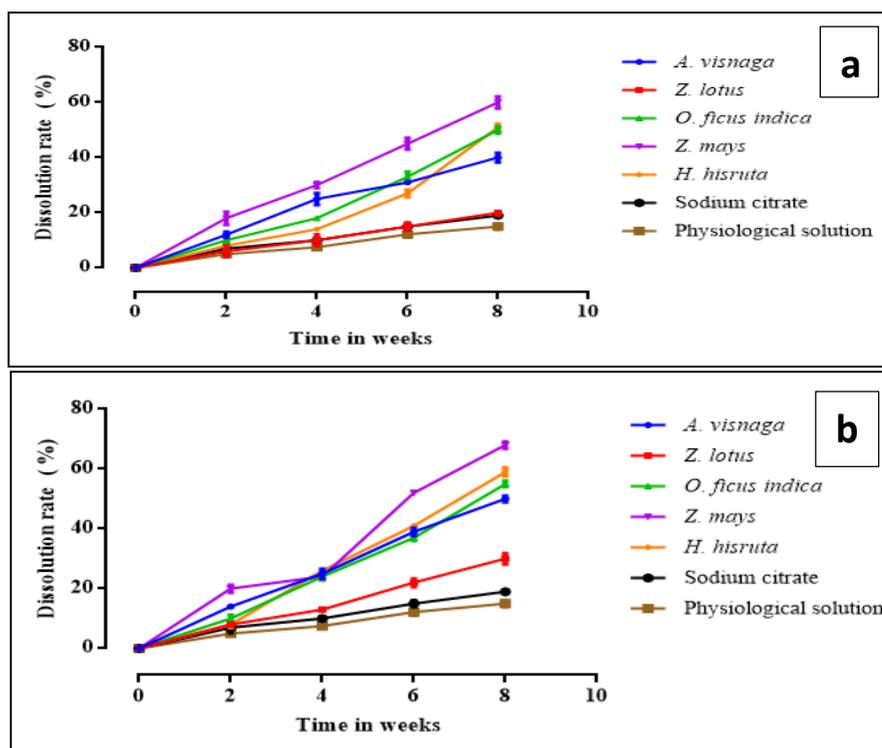
#### 3.1.1. Temporal evolution of the dissolution rate (DR)

The kinetic evolution of the dissolution rate (DR) of oxalo-calcium stones for different extracts of the plants studied is presented in Fig. 1 (a, b).

The results show an increasing kinetic evolution of the DR. This evolution seems more important for hydro-ethanolic and aqueous extracts compared to the control solutions (physiological solution and sodium citrate).

The comparison of the dissolution rates obtained with the five hydro-ethanolic extracts (Figure 1, a) shows that the extract of *Z. mays* had a more notable effect than the other extracts after two weeks of contact with the stone. Thus, the DR of *Z. mays* is 18 % while it is only 12 % for the extract of *A. visnaga*, 10 % for the extracts of *O. ficus-indica*, 8 % for *H. hirsuta* and 6 % for the extract of *Z. lotus*. The sodium citrate, as well as the physiological solution of NaCl, give a DR of 7 % and 5 % respectively. After eight weeks of contact with hydro-ethanolic extracts and the stones, the DR is 60 % for *Z. mays*, 51 %, 50 %, 40 % and 20 % for *H. hirsuta*, *O. ficus indica*, *A. visnaga* and *Z. lotus* respectively. This DR value does not exceed 20 % for Witness solutions.

The evolution of the dissolution rate of the aqueous extracts of the plants studied is displayed in Figure 1 b, after two weeks of contact with the stones, the DR of *Z. mays* is 20 %, while it is only 14 % for *A. visnaga* and that *O. ficus indica* is 10 %. *H. hirsuta* and *Z. lotus* have the same RD, which is 8 %. Sodium citrate and physiological NaCl solution have a DR of 7 % and 5 % respectively. After eight weeks, the DR is 68 % for the extract of *Z. mays*, while it is 59 %, 55 %, 50 % and 30 % with *H. hirsuta*, *O. ficus indica*, *A. visnaga* and *Z. lotus* respectively. The control solutions have a DR which does not exceed 20 %.

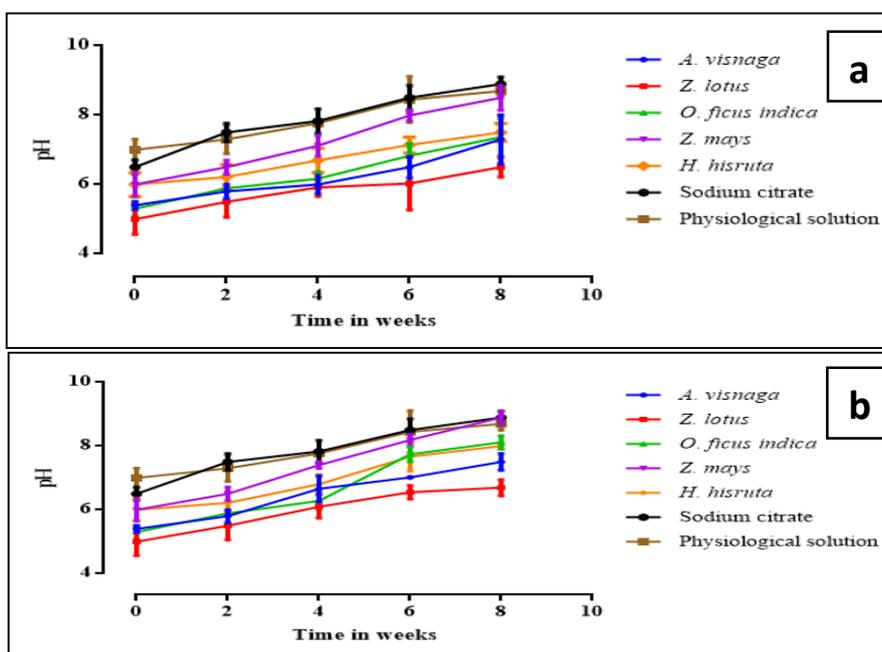


**Figure 1.** Dissolution rate of oxalo-calcic stones for hydro-ethanolic extracts (a); Dissolution rate of oxalo-calcic stones for aqueous extracts (b)

### 3.1.2. Temporal evolution of pH

The evolution of the pH is shown in Fig. 2 (a, b). These results indicate that the initial pH of the

solutions is slightly acidic, to undergo a slight linear increase which is not negligible over the eight weeks.



**Figure 2.** pH Evolution in the function of time in the presence of oxalo-calcic stones for hydro-ethanolic extracts (a); pH evolution in the function of time in the presence of oxalo-calcic stones for aqueous extracts (b)

## 3.2. Effect of hydro-ethanolic and aqueous extracts on cystine stones

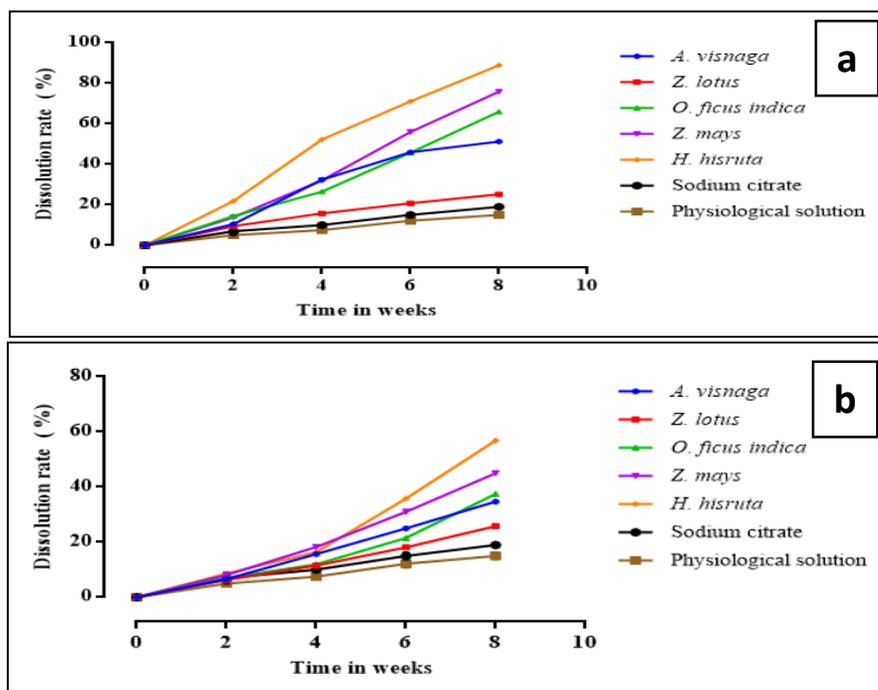
### 3.2.1. Temporal evolution of the dissolution rate

The kinetic study of the dissolution rate of cystine stones, for the different extracts for 8 weeks is presented in Fig. 3 (a, b).

The curve of Figure 3a shows no detectable difference during the first two weeks for the hydro-ethanolic

extracts. After eight weeks, the hydro-ethanolic extracts present some differences with *H. hirsuta* which has the best dissolution rate which reaches 57 %, 45 %, 38 %, 35 % and 26 % for *Z. mays*, *O. ficus-indica*, *A. visnaga* and *Z. lotus*, respectively. Control solutions, including sodium citrate and physiological solution, have a DR less than 20 %.

Similarly, after two weeks, the aqueous extracts show no significant difference. However, after eight weeks the DR is 89 % for the extract of *H. hirsuta* followed by 76 %, 66 %, 51 % and 25 % for *Z. mays*, *O. ficus-indica*, *A. visnaga* and *Z. lotus*, respectively. The controls (sodium citrate and physiological solution) show a similar development but do not exceed a DR of 20 %.

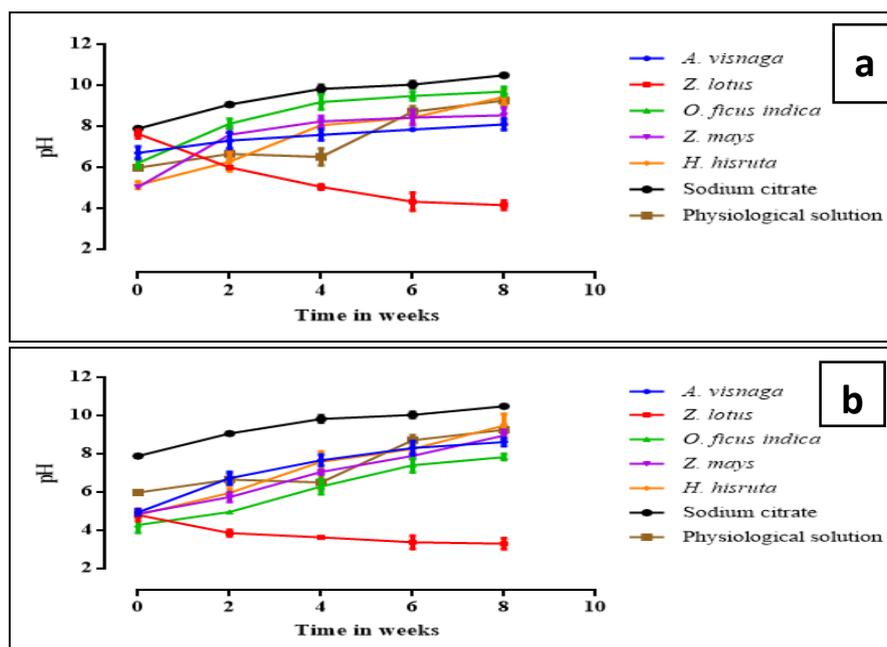


**Figure 3.** Dissolution rate of cystine stones for hydro-ethanolic extracts (a); Dissolution rate of cystine stones for aqueous extracts (b)

### 3.2.2. Temporal evolution of pH

Fig. 4 (a, b) show the pH evolution, indicating that the solutions initial pH is slightly acid, ranging

from 4,3 for *O. ficus indica* flowers to 7,9 for citrate solution.



**Figure 4.** pH evolution in the function of time in the presence of cystine for hydro-ethanolic extracts (a); pH evolution in the function of time in the presence of cystine for aqueous extracts (b)

The pH undergoes a non-negligible linear increase after eight weeks, ranging from 7,9 for *O. ficus indica* to 10,5 for sodium citrate. On the other hand, *Z. lotus* is the only plant that has a pH effect decreasing throughout the 8 weeks beginning with pH 7,65 and ending around pH 3,3.

### 3.3. Effect of flavonoid and tannin fractions on kidney stones

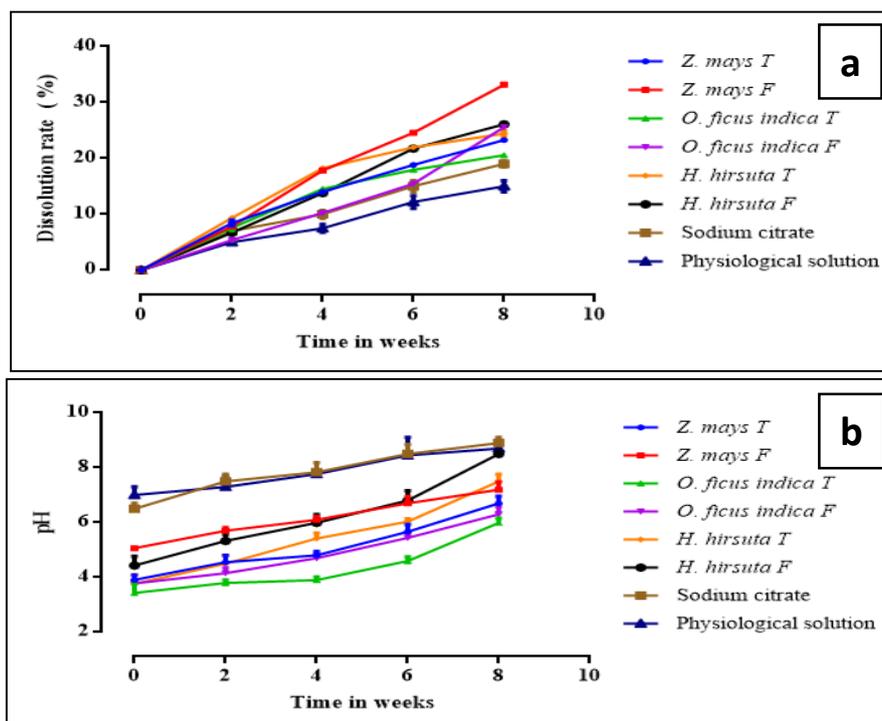
In this study, we have chosen to make a phenolic fraction of the three plants which have the best dissolution rate: *Zea mays stigmata*, *Opuntia ficus indica* flowers and *Herniaria hirsuta* aerial parts was made, for each plant we carried out a fractionation to

obtain tannins and flavonoids to test their *in vitro* activity.

#### 3.3.1. Temporal evolution of the dissolution rate and the pH on the oxalo-calcium calculation stones

The results present in Fig. 5 (a, b) show an increase in the dissolution rate for the different fractions on the oxalo-calcium stones. The dissolution rate is negligible after two weeks of incubation. After eight weeks, the DR is between 15 and 33 % for all the fractions (tannins and flavonoids).

The evolution of the pH is presented in Figure 5b. It indicates that the initial pH of the solutions is between 3,3 and 8. The pH undergoes a slight increase during the eight weeks.



**Figure 5.** Dissolution rate of oxalo-calcic stones for the extracts of tannins and flavonoids fractions (a); pH evolution in the function of time in the presence of oxalo-calcic stones for the extracts of tannins and flavonoids fractions (b), T: Tannins; F: Flavonoids

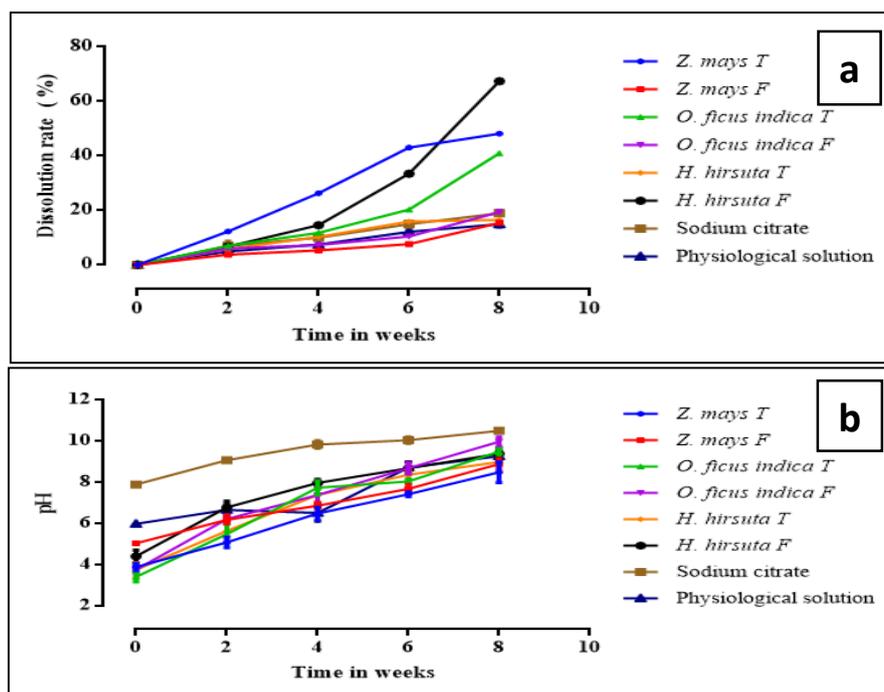
#### 3.3.2. Temporal evolution of the dissolution rate and the pH on the cystine stones

The results in Fig.6 (a, b) show an increasing dissolution rate for the different fractions during the eight weeks on the cystine stones. The comparison of the results obtained after two weeks shows that all fractions do not have the same effect and kinetic.

The DR for tannins was 12 % for *Z. mays*, 7 % for *O. ficus indica* and 6 % for *H. hirsuta*. After eight weeks it was respectively 48 %, 41 %, and 16 %. For the Flavonoids fractions results, it was 4 % for *Zea mays*, 6 % for *O. ficus indica* and 7 % for *H. hirsuta*. After

eight weeks, the percentages increased to 15 %, 19 % and 64 % respectively. The sodium citrate, as well as the physiological solution of NaCl, gave a 7 % and a 5 % DR result after the first two weeks, the eighth week the DR value could not go beyond 20 % when considering the control solutions.

The pH evolution showed in Fig. 6b, indicates that the initial pH of the solutions going from 3.3 for a fraction of tannins of *O. ficus indica* to a pH of 8 for the citrate solution. The pH increases linearly during the eight weeks of incubation.



**Figure 6.** Cystine stones dissolution rate for the extracts of tannins and flavonoids fractions (a); pH evolution in the function of time in the presence of cystine stones for the extracts of tannins and flavonoids fraction (b)  
T: Tannins; F: Flavonoids

#### 4. Discussion

The *in vitro* tests carried out on oxalo-calcium, and cystine stones in the presence of the different extracts showed better kinetics of solubilization of the plants studied than that of the controls (physiological solution and sodium citrate).

Other studies on the dissolution of cystine stones by the plants traditionally used in Algeria against lithiasis were carried out by Hannache<sup>15</sup>. This study is focused on *Arenaria ammophila* (leaves and stems), *Parietaria officinalis* (leaves and flowers separately), *Paronychia argentea* (flowers). None of the extracts tested had any tangible effect in dissolving cystine stones. The only one that appeared to have a solvent effect unrelated to pH was the extract of *A. ammophila* with a dissolution rate of 21 % at the end of the experiment versus 9 % for the NaCl solution.

The study of the dissolution of cystine stones by aqueous extracts (infusion) of *Herniaria hirsuta*, *Opuntia ficus-indica*, *Zea mays* and *Ammi visnaga* was carried out by Meiou<sup>8</sup>. These cystine stones were studied with magnetic stirring. After eight weeks, the dissolution was complete with all the extracts, but faster with *Z. mays* and *A. visnaga* while the citrate and NaCl reduced the mass of the stones by 18 % and 20 % respectively. From these results, it can be said that agitation played a role in the dissolution of cystine stones during this study.

A similar study was made by R. El Habbani<sup>24</sup> who tested the effect of the same plants on the dissolution of cystine and oxalo-calcic stones (without agitation). After eight weeks of contact, the aqueous extract of

the stigma of *Z. mays* shows a dissolution rate of 72 % for cystine stones and 55 % for calcium oxalate stones. The extracts of *A. visnaga* solubilize 67 % of cystine stones and 47 % of calcium oxalate stones. *O. ficus-indica* extract can reach a DR of 64 % and 45 % of the total mass of cystine stones and calcium oxalate stones, respectively. The DR of *H. hirsuta* extracts gave 61 % for cystine stones, whereas it is 50 % when using calcium oxalate stones.

The pH during the eight weeks of incubation of the oxalo-calcic and cystine stones shows a passage of the solutions from an acid state to a basic state. Thus, for oxalo-calcic stones, the pH of the extracts does not exceed 7,5. On the other hand, for the cystine stones, the pH of the extracts reached 9,5. This may explain why the pH has a more practical effect on the reduction of cystine stones than on oxalo-calcic stones.

The study made by R. El Habbani<sup>24</sup>, showed for cystine stones that the initial pH of all the solutions is slightly acid varying between 5,8 for the extract of flowers of *Opuntia ficus-indica* to 7,2 for the citrate solution. This pH increases slightly in a linear fashion during the eight weeks varying from 6,6 for *Herniaria hirsuta* to 7,9 for potassium citrate. Regarding calcium oxalate calculations, the initial pH of the solutions is more or less neutral or slightly basic, varying between 6 for the extract of *Opuntia ficus-indica* flowers and 7,8 for the citrate solution. The latter undergoes a slight linear increase over the eight weeks ranging from 7,5 for *Herniaria hirsuta* to 9 for potassium citrate and physiological solution.

The urinary solubility of cystine remains closely linked to the change in pH because it does not exceed 250 mg / L at acid pH but can reach 500 mg / L from pH 7,5<sup>33</sup>.

One of the first treatments proposed for cystinuria is a diuretic treatment based on a diet limiting the consumption of methionine and salt. This result shows that the objective is to maintain the cystine in a soluble form by acting in particular on the urine concentration as well as on the pH value. In case of persistence of cystine, further treatment with pharmacological agents based on sulfhydryl would be proposed. However, this treatment has many side effects that limit its long-term use<sup>34</sup>.

The examination of the hydro-ethanolic and aqueous extracts of the plants studied suggests that a mechanism of action independent of pH may be responsible for the dissolution of cystine stones and calcium oxalate. This effect could be linked to the formation of cystine-molecules (tannins or flavonoids) or oxalate- molecules (tannins or flavonoids), the stability of which would be ensured by hydrogen bonds and hydrophilic bonds between the functions of the active molecules and the carboxylic functions or amines of the cystine molecule and a calcium oxalate molecule.

The dissolution rate results for the tannin and flavonoid fractions showed a lower DR than that obtained by the hydro-ethanolic, and aqueous extracts of the plants studied.

The results of the dissolution of the oxalo-calcium stones by the fractions of tannins and flavonoids enabled us to note that the extract of *Z. mays* gave a DR of tannins and flavonoids of 23 % and 33 %, which we give a total of 56 %. This value is close to that of the hydro-ethanolic extract of *Z. mays* with a DR of 60 %. Concerning the extract of *H. hirsuta* the DR of tannins and flavonoids is 24 % and 26 %, which gives us a total of 50 % close to that of hydro-ethanolic extract of *H. hirsuta* with a value of 51 %. For the extract of *O. ficus indica* the DR of tannins and flavonoids is 20 % and 26 % successively. The total obtained is 46 % which is almost equivalent to that of hydro-ethanolic extract of *O. ficus indica* with a TD of 50 %.

Regarding the results of the dissolution of cystine stones by the fractions of tannins and flavonoids, the extract of *H. hirsuta* gave a DR of tannins and flavonoids of 16 % and 67 %, which gives us a total of 83 % close to that of *H. hirsuta* hydro-ethanolic extract with a value of 89 %. For the extract of *Z. mays* we obtained a DR of 48 % and 15 % successively. These results give us a total of 63 %, which is close to that of hydro-ethanolic extract of *Z. mays* with a DR of 75 %. Regarding the extract from the *O. ficus indica*, the DR of tannins and flavonoids is 41 % and 19 % successively. This total obtained (60 %) is close to that of hydro-ethanolic extract of *O. ficus indica* with a DR of 65 %.

According to these results, it is found that the presence alone of tannins and flavonoids has given a less dissolving effect than that of their presence together in the hydro-ethanolic and aqueous extracts of the plants studied. It can be said that the dissolution of stones, whether cystine or calcium oxalate, is due to the presence of several compounds and not of an active principle alone.

## 5. Conclusion

The results obtained showed the effectiveness of the five plants extracts on the stones dissolution.

These extracts could, therefore, constitute a curative and/or prophylactic treatment for lithiasis patients, especially cystinuric patients. To confirm the plant's effect, complementary investigations are needed, and other factors should be considered, such as the diet composition and the quality and quantity of water consumed.

## References

- 1- M. Daudon, RJ Reveillaud, Whewillite and weddellite: toward a different etiopathogenesis the significance of morphological typing of calculi, *Nephrol.* **1984**, 5, 195-201.
- 2 - A. Laroubi, M. Touhami, L. Farouk, IRA Zrara, A. Benharref, A. Chait, Prophylaxis effect of *Trigonella foenum graecum* L. seeds on renal stone formation in rats, *Phytother. Res.* **2007**, 21, 921-925.
- 3 - F. Assadi, M. Moghtaderi, Preventive kidney stones. Continue medical education, *Int J Prev Med.* **2017**, 8, 67.
- 4 - M. Daudon, CA. Bader, P. Jungers, Urinary calculi: review of classification methods and correlations with etiology, *Scanning Microsc.* **1993**, 7, 1081-106.
- 5- S. Bouanani, C. Henchiri, E. Migianu-Griffoni, et al., Pharmacological and toxicological effects of *Paronychia argentea* in experimental calcium oxalate nephrolithiasis in rats, *J Ethnopharmacol.* **2010**, 129, 38-45.
- 6- F. Atmani, Y. Slimani, AN. Mbark, M. Bnouham, A. Ramdani, in vitro and in vivo anti-lithiasic effect of saponin rich fraction isolated from *Herniaria hirsuta*, *J Bras Nefrol.* **2006**, 28, 199-203.
- 7- A. Khouchlaa, A. Talbaoui, AEY. El idrissi, A. Bouyahya, S. Ait lahsen, A. Kahouadji, M. Tijane, Determination of phenol content and evaluation of in vitro litholytic effects on urolithiasis of moroccan *zizyphus lotus* L. extract, *Phytothérapie*, **2018**, 16, 14-9.
- 8- F. Meiouet, S. El Kabbaj, M. Daudon, In vitro study of the litholytic effects of herbal extracts on cystine urinary calculi, *Prog Urol.*, **2011**, 21, 40-7.
- 9- P. Jungers, D. Joly, MF. Gagnadoux, M. Daudon, cystine lithiasis: physiopathology and medical treatment, *Prog urol.*, **2001**, 11, 122-126.
- 10-P. Jungers, M. Daudon, P. Conort, (b) cystine lithiasis. In: renal lithiasis, diagnosis and treatment, Paris Flammarion Médecine Sciences, **1999**, 149-161.
- 11-R. Selvam, P. Kalaiselvi, A. Govindaraj, V. Bala Murugan, AS. Sathish Kumar, Effect of *A. lanata*

- leaf extract and vediuppu chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria, *Pharmacol Res.*, **2001**, 43, 89-93.
- 12-Lipkin M, Shah O, The use of alpha-blockers for the treatment of nephrolithiasis, *Rev Urol.*, **2006**, 8, 35-42.
- 13 - L. Yachi et al., In vitro litholytic activity of some medicinal plants, *African Journal of Urology*, **2018**, 24, 197-201.
- 14 - A. Amar, D. Harrache, F. Atmani, G. Bassou, F. Grillon. Effect of *Parietaria officinalis* on the crystallization of calcium oxalate in urine, *Phytothérapie*, **2010**, 8, 342-347.
- 15 - B. Hannache, D. Bazin, A. Boutefnouchet, M. grass, Effect of medicinal plant extracts on the dissolution of cystine kidney stones in vitro: a mesoscopic study, *Progres en urologie*, **2012**, 22, 577-582.
- 16-J. Bellakhdar, Plantes médicinales au Maghreb et soins de base précis de phytothérapie moderne. Ed le Fennec, **2006**, 13-23.
- 17- CA. Newal, LA. Anderson, A Guide for Health-care Professionals, *Herbal Medicine*, **1996**, London.
- 18-F. Grases, J.G. March, M. Ramis, and A. Costa-Bauza, The influence of *Zea mays* on urinary risk factors for kidney stones in rats, *Phytotherapy Research*, **1993**, 7, 146-149.
- 19-R. Bencheraïet, H. Kherrab, A. Kabouche, Z. Kabouche and M. Jay, Flavonols and antioxidant activity of *Ammi visnaga* L. (Apiaceae), *Rec Nat Prod*, **2011**, 5, 52-55.
- 20-AO. Abdel-Zaher, SY. Salim, MH. Assaf et RH. Abdel-Hady, Antidiabetic activity and toxicity of *Zizyphus spina-christi* leaves, *J. Ethnopharmacol*, **2005**, 101,129-138.
- 21-S. Suksamrarn, N. Suwannapoch, N. Aunchai, M. Kuno, P. Ratananukul, R. Haritakum, C. Jansakul et S. Ruchirawat, Ziziphine N, O, P, new antiplasmodial cyclopeptides alkaloids from *Zizyphus oenoplia* var. *brunoniana*. *Tetrahedron*, **2005**, 61, 1175-1180.
- 22-L. Benramdane, M. Bouatia, MOB. Idrissi, M. Draoui, Infrared analysis of urinary stones, using a single reflection accessory and KBr pallettransmission, *Spectrosc Lett.*, **2008**, 41,72-80.
- 23-L. Estepa, M. Daudon, Contribution of Fourier transform infrared spectroscopy to the identification of urinary stones and kidney crystal deposits, *Biospectroscopy* **1998**, 3, 347-69.
- 24-R. El Habbani, A. Chaqroune, TS. Houssaini, M. Arrayhani, A. Lahrichi, Contribution to the study of the mass reduction of stones by some medicinal plants, *Int J Innov Res Sci Eng Technol*, **2015**, 04(04), 1867-75.
- 25-TE. Grønhaug, S. Glaeserud, M. Skogsrud, et al., Ethno- pharmacological survey of six medicinal plants from Mali, West-Africa, *J Ethnobiol Ethnomed*, **2008**, 4, 26.
- 26-DJ. Simbo, An ethnobotanical survey of medicinal plants in Babungo, Northwest Region, Cameroon, *J Ethnobiol Eth- nomed*, **2010**, 6, 8.
- 27-S. Jennan, R. Fouad, A. Nordine, A. Farah, B. Bennani, S. Moja, H. Greche & F. Mahjoubi, Chemical Composition and Antibacterial Screening of Aerial Parts of Essential Oils of Three *Satureja* species (*Satureja briquetii*, *Satureja atlantica* and *Satureja alpina*) Growing Wild in the Middle Atlas Mountains of Morocco, *Journal of Essential Oil Bearing Plants*, **2018**, 21(3), 741-748.
- 28 - K. Ammor, D. Bousta, S. Jennan, FE. Amarti, R. Lagzizir A. Chaqroune and F. Mahjoubi, Study of Antioxidant, Anti-inflammatory, anti-antinociceptive activities and toxicity of stigmata of *Zea mays* extracts from Morocco, *Phytothérapie*, **2019**, 10.3166/phyto-2019-0209.
- 29 - K. Ammor, D. Bousta, S. Jennan, B. Bennani, A. Chaqroune, and F. Mahjoubi, Phytochemical Screening, Polyphenols Content, Antioxidant Power, and Antibacterial Activity of *Herniaria hirsuta* from Morocco, *The Scientific World Journal*, **2018**, 7.
- 30 - K. Ammor, B. Dalila, J. Sanae, A. Chaqroune, F. Mahjoubi Fatima, Total Polyphenol Content and Antioxidant Power of *Ammi visnaga* from Morocco, *Der Pharma Chemica*, **2017**, 9, 73-78.
- 31-J. Bruneton, Pharmacognosy, phytochemistry, medicinal plants, Tec et doc édition Lavoisier, **1999**, 1120.
- 32-L. Saso, G. Valentini, MG. Leone, E. Grippa, B. Silvestrini, Development of an *in vitro* assay for the screening of substances capable of dissolving calcium oxalate crystals, *Urol Int*, **1998**, 61, 210-214.
- 33-P. Jungers, Cystine lithiasis, In: Dore B, editor, *Les lithiases rénales*. Springer-Verlag, **2004**, 137-44.
- 34-M. Daudon, Morphoconstitutional analysis of stones in the etiological diagnosis of urinary lithiasis in children, *Arch PCdiatr*, **2000**, 7, 855-65.