

## Inhibiting Effect of Moroccan Medicinal Plants on Crystallization of Oxalo-calcic Calculations in vitro

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**Abstract-** The aim of this work is to study the inhibitory effect of some Moroccan medicinal plants: parsley, nettle, oregano and corn beard on the crystallization of oxalocalcium urinary stones under experimental conditions which simulate the urinary environment (physiological concentrations in calcium and oxalate, temperature and pH). The experimental tests were followed by the turbidimetric method using UV-Visible Model SP8-400 spectrophotometry, the response of which restores the concentration of calcium oxalate. The results showed that the potassium and magnesium ions which constitute the main elements of these plants compete with the calcium ions in order to combine with the oxalate ions. All the competitive reactions reflecting the affinities of the different ions towards each other contribute to the observed overall inhibition of the crystallization of calcium oxalate.

**Keywords:** Crystallization, Urinary Calculus, Calcium oxalate, Inhibition, Moroccan Medicinal Plants

### Introduction

Urolithiasis is a common pathology affecting nearly 20% of the population in industrialized and developing countries<sup>1-3</sup>. There are

Several types of urinary stones whose physicochemical analysis provides information that can effectively contribute to understanding the mechanisms involved in their formation<sup>4,5</sup>. We note there is high frequency of oxalocalcic stones which represent 70 to 85% of urinary stones<sup>6</sup>.

Several factors of an endogenous, metabolic<sup>7,8</sup>, infectious, anatomical and also nutritional and medicinal nature<sup>9</sup> are at the origin of urolithiasis. Oxalocalcium urinary stones are found in the urine in three distinct crystalline forms<sup>10,11</sup>, the predominant form of which is that of calcium oxalate monohydrate (COM), followed by the dihydrate (COD), then the trihydrate form (TOC) very rare.

The essential mechanism of urinary stone formation<sup>12</sup> is dependent on the urine concentrating too much of poorly soluble compounds, which can then precipitate as crystals and then clump together in the form of stones. The mechanism of stone formation passes, schematically, through seven stages: supersaturation, germination or nucleation, crystal growth, aggregation of crystals with formation of particles greater than 100 µm, retention of crystals in the epithelium, the accretion of new crystals and finally crystalline conversion leading to the most thermodynamically stable form<sup>13</sup>. These seven steps can be brought together in two

main phases. First, crystallogenesis which includes the first four stages, followed by calculogenesis. Crystallogenesis is not a pathological phenomenon in itself, it is observed in normal subjects as well as in lithiasis subjects, while calculogenesis is a phenomenon indicating urolithiasis.

The major lithogenic factor, in all chemical varieties of lithiasis, is the supersaturation of urine with solutes entering into the composition of stones. The latter leads to the precipitation of crystals, an obligatory initial step in the formation of stones<sup>16-18</sup>. Indeed, in the case of oxalocalcium lithiasis, the supersaturation results from the increase in the urine concentration of oxalate and / or calcium and possibly leads to the crystallization of calcium oxalate which has been studied<sup>14,15</sup>. The action of the promoter solutes is partially counterbalanced by inhibitors of nucleation and aggregation of crystals, such as citrate, magnesium, potassium ions and protein macromolecules<sup>19,20</sup>. In general, stone formation results from the disruption of the balance between promoter solutes and those acting as inhibitors, capable of opposing one or more steps in the lithogenesis of calcium salts, present in the urine. Some authors<sup>21-23</sup> have been able to show that certain juices with a high content of citrate, potassium and magnesium increase citraturia, in turn increasing the inhibitory power of urine<sup>24-26</sup>.

Thus, in Morocco as in many countries, a large number of patients use natural substances in the treatment of urolithiasis. In fact, studies have been carried out on the effect of medicinal plants on the crystallization of urinary calculi, either in cystine form or in oxalocalcic form<sup>27,28</sup>. Citrate inhibits crystallization of calcium oxalate and calcium phosphate by decreasing urinary saturation with calcium salts, hence the importance of hypocitraturia as a factor promoting stone formation. Potassium reduces the excretion of calcium by combining with oxalate, thereby reducing the risk of kidney stone formation. Magnesium increases the

solubility of calcium in urine; it seems to have a preventive effect on kidney stones. The potassium-magnesium combination in the form of citrate reduces the recurrence of kidney stones<sup>29</sup>.

As part of our investigation on medicinal plants growing in Moroccan flora, we reported here, *in vitro*, the effect on calcium oxalate crystallization of aqueous extract of four medicinal plants used in Moroccan traditional pharmacopoeia.

## Material and Methods

The model adopted to follow the crystallization of calcium oxalate in the supersaturated solution at 37°C and pH = 5.6, is followed by the turbidimetric method. The kinetic by turbidimetry was measured using a Model SP8-400UV-Visible spectrophotometer and the wavelength was set at 620 nm corresponding to the maximum absorption of calcium oxalate<sup>30</sup>. A quartz cell is used to perform the analyzes. Thermostatization at 37°C is maintained in the solution to be analyzed and stirring is ensured using a 5 mm magnetic bar.

The crystallization medium is obtained by mixing two solutions A and B, the constitution of which is as follows respectively: Na<sub>2</sub>SO<sub>4</sub>, 10H<sub>2</sub>O; MgSO<sub>4</sub>, 7H<sub>2</sub>O; NH<sub>4</sub>Cl; KCl; CaCl<sub>2</sub>; and NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O; Na<sub>2</sub>HPO<sub>4</sub>, 12H<sub>2</sub>O; NaCl; Na oxalate; The products were of higher analytical purity.

Ultra Violet - Visible molecular absorption spectrophotometry is used to monitor oxalocalcic crystallization in a synthetic urinary medium.

The experimental tests in fact consisted of mixing a volume of a few milliliters of a calcium solution A with the same volume of an oxalic solution B (Table-1), the stirring, the temperature and the pH being kept constant.

Table-1 Chemical composition of the two solutions forming synthetic urine

Solution	Compounds	Concentration (mol/l)
A	$\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$	$1.93 \cdot 10^{-3}$
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	$5.92 \cdot 10^{-3}$
	$\text{NH}_4 \text{Cl}$	$8.67 \cdot 10^{-2}$
	KCl	0.163
	$\text{CaCl}_2$	$7 \cdot 10^{-3}$
B	$\text{NaH}_2 \text{PO}_4 \cdot 2\text{H}_2 \text{O}$	$1.54 \cdot 10^{-2}$
	$\text{Na}_2 \text{HPO}_4 \cdot 12\text{H}_2 \text{O}$	$1.56 \cdot 10^{-2}$
	NaCl	0.223
	Na oxalate	$2 \cdot 10^{-3}$

The model for the study of inhibitors on oxalocalcium crystallization which we adopted was based on the principle of turbidimetry referring to crystal growth which involves nucleation, growth and aggregation<sup>31,32</sup>.

Turbidimetry is an optical method that measures the haze (absorbance) that exists in a solution. The value of absorbance A is determined with respect to a reference value and is expressed by the formula (1)

$$A = \log\left(\frac{I_0}{I}\right) \text{-----(1)}$$

Where I. represents the light intensity of the incident ray and the intensity transmitted to the photoelectric cell. We define from relation (1) the turbidity of a solution by the formula (2)

$$T = \left(\frac{1}{l}\right) \times A = \left(\frac{1}{l}\right) \times \log\left(\frac{I_0}{I}\right) \text{-----(2)}$$

Where  $l$  designates the length of the optical path traveled by the light ray in the cell. The general shape of the turbidimetric curves obtained (absorbance as a function of time) is

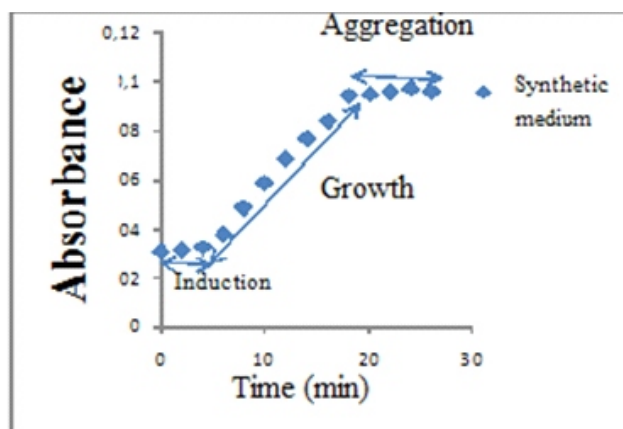


Figure-1 Evolution of calcium oxalate crystallization in the urinary synthetic urinary environment in the absence of plants.

This curve can be divided into three parts:

(i) A latency time during which the absorbance is practically zero thus corresponding to the crystalline nucleation phase and which is characterized by an induction time.

(ii) An ascending phase of strong growth, linear over a large part of time corresponding to crystal growth. It is characterized by a second kinetic parameter which is the turbidimetric slope.

(iii) Finally, a slow decrease passing through a maximum and reflecting the aggregation phase which is of great interest in the study of crystallization.

This model also has the advantage of quantifying the inhibitory power expressed in % relative to a control. It is given by the formula (3)

$$\%I = \left(1 - \frac{P_{ai}}{P_{si}}\right) \times 100 \quad \text{-----}(3)$$

Where  $P_{ai}$  and  $P_{si}$  represent respectively the values of the turbidimetric slopes with and without inhibitor.

#### Plant characterization

The plants studied are: parsley (*Petroselinum sativum*), nettle (*Urtica dioica*), oregano (*Origanum vulgare*) and corn beard (*Zea mays*). The plants material were identified by Professor Leila EL GHAZI from the department of biology, Faculty of Sciences, University Hassan II of Casablanca, Morocco, according to the flora of Morocco.

The plants were dried in the open air for 48 hours in the shade. The extracts of the plants studied were prepared by the infusion method with a concentration of 1mg / 1ml (plant / water). The same amounts of extract were added to the mixture of solutions A and B. Four plant extracts (parsley, nettle, oregano and corn barb) were investigated in the synthetic urinary medium.

#### Mineral content determination

The determination of potassium (K) and magnesium (Mg) contents was carried out using the standard methods<sup>33</sup>. Dried samples powder (0.5g) was ashed in a porcelain crucible at 550°C, in a muffle furnace for 24

hours. Then, 25 ml of aqua regia ( $\text{HNO}_3$ ,  $\text{HCl}$ , 2/5: 3/5 v) were added and the mixture is heated at reflux for 2 hours at a temperature of 150 ° C. After cooling, 20 ml of distilled water were added, then we filter, we collect the filtrate in a 50 ml flask and make up to the mark with distilled water. The mineral constituents present in samples were analyzed separately using an atomic absorption spectrophotometer (Shimadzu, AA., 7000, Japan). A calibration curve for each of the mineral to be determined, was prepared using the standard solutions, before the readings were obtained.

## Results and Discussion

### Kinetics of crystallization in aqueous medium, synthetic medium and hypercalcic medium.

The experimental device that we have developed has the advantage of maintaining constant agitation, a fixed temperature at 37 ° C and introduction of the solutions at the same temperature. A volume of 70 ml of solution A is poured into a 250 ml beaker placed in a water bath thermostatically controlled at working temperature with continuous stirring. The same volume of solution B is also poured into a 250 ml beaker and placed in the same thermostated bath with continuous stirring.

The two solutions A and B are mixed under the same experimental conditions and the stopwatch is started by taking a sample in the quartz cuvette with a volume of 1.5 ml and an optical path of 1 cm.

The aqueous medium has the same concentration of calcium and of oxalate as the synthetic medium except that the other constituents are not added; for the hypercalcic medium; it has the same constituents as the synthetic medium except that the concentration of calcium has been doubled.

Absorbance is measured every 2 minutes for an average time of 30 minutes. Thus, we obtained the absorbance variation curve as a function of time, the shape of which is given in Figure-2.

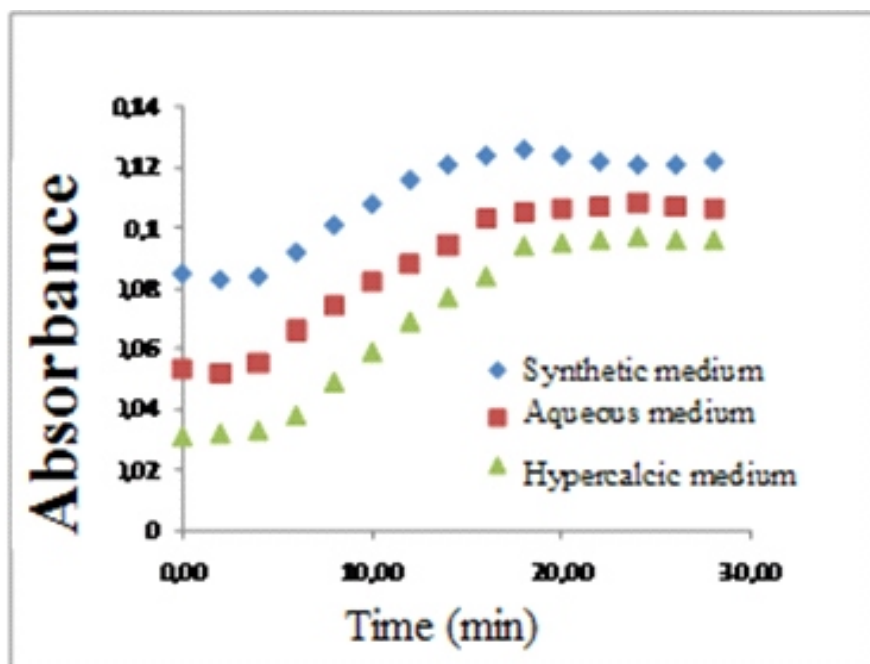


Figure-2 Evolution of calcium oxalate crystallization in the urinary synthetic urinary, aqueous and hypercalcium.

The growth lines for each medium, as well as their slopes are shown in Figure -3.

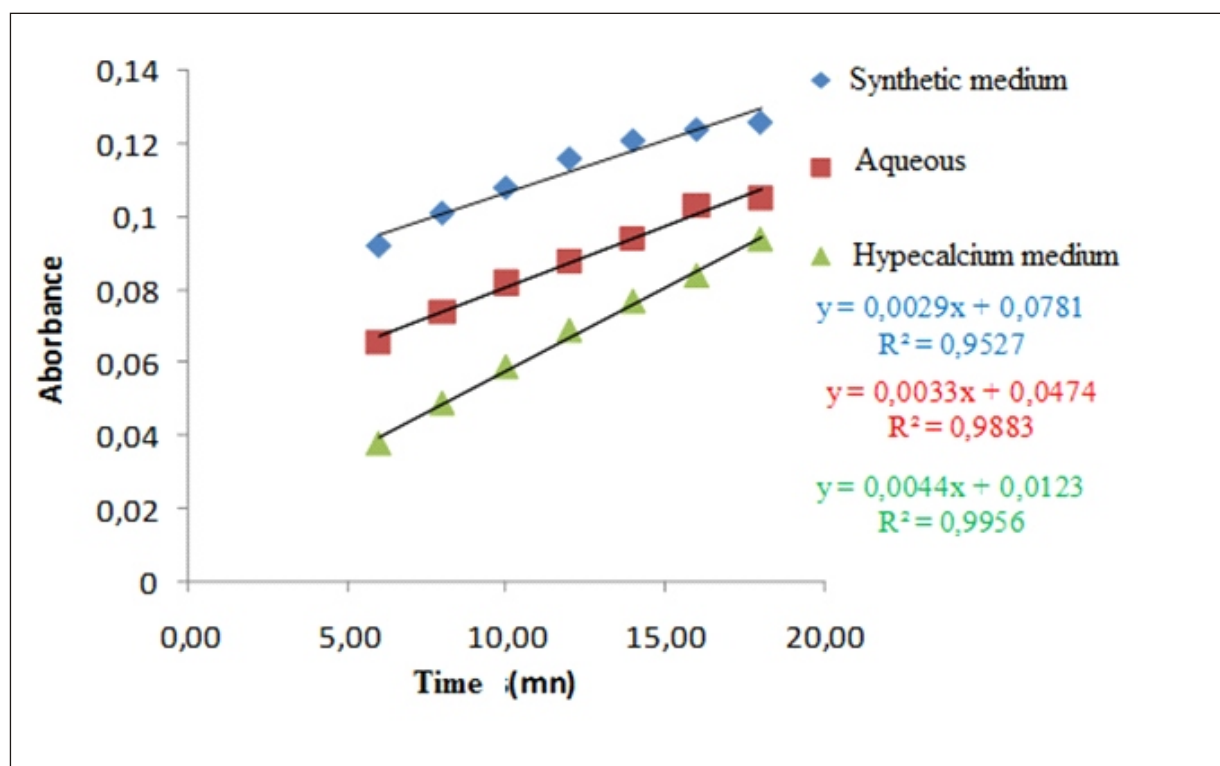


Figure-3 Determination of the crystalline parameters from the crystalline growth rights for the synthetic, aqueous and hypercalcium medium.



We then determined the induction time  $T_i$  which corresponds to the time elapsed between the addition of sodium oxalate and the moment when the growth is experimentally measurable.

The curves admit linear ranges characterized by turbidimetric slopes whose correlation coefficients  $R$  are greater than 95%.

In Table-2 below, the different crystalline parameters of the three media studied are shown.

Table - 2 crystalline parameters for the synthetic, aqueous and hypercalcic medium

Medium	$T_i$ : Induction time	$T_c$ : Growth time	$T_a$ : Aggregation time	Slope	$R^2$
Synthetic	4	14	From 18	0,0044	99,56
Aqueous	4	14	From 18	0,0033	98,83
Hypercalcic	4	14	From 18	0,0029	95,27

Based on the results obtained it was observed that hypercalcic medium promotes more crystallization of calcium oxalate followed by the aqueous medium and finally the synthetic medium.

This can be explained by the fact that the hypercalcic medium contains more calcium which is a promoter of calcium oxalate crystallization than the other two media.

For the same concentrations of calcium and of oxalate, the synthetic medium exhibits slightly greater inhibition than that of aqueous medium, this is due to the presence of the inhibitory ions potassium and magnesium in the synthetic medium.

#### Crystallization kinetics in the presence of medicinal plants in a synthetic urinary medium

We tested the plants mentioned above using the turbidimetric model which has a fairly high sensitivity and reliability. The results obtained for this study are presented in Figure-4 and likewise the growth lines corresponding to each plant.

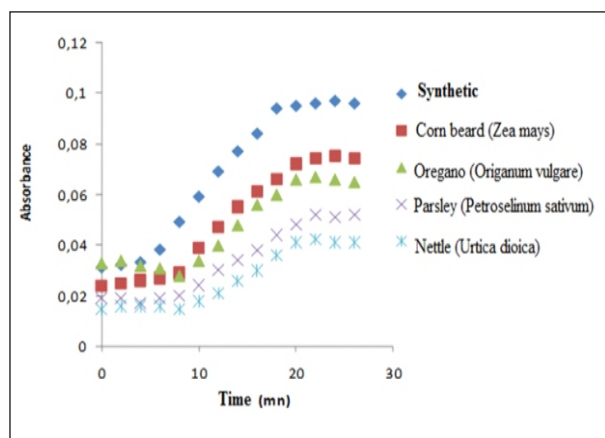


Figure-4 Evolution of the crystallization of calcium oxalate in the synthetic urinary environment in the presence of medicinal plants.

In Table-3 below, the different crystalline parameters of the synthetic medium are shown in the presence of the different plants studied with different concentrations as well as the inhibition rates corresponding to each inhibitor in the synthetic medium.

Table-3

	Concentration mg/ml	Ti : Induction time	Tc : Growth time	Ta: Aggregation time	Slope	R <sup>2</sup>	I%
Synthetic	0	4	14	From 18	0,0044	99,38	--
Corn Beard	1	6	14	From 18	0,0040	99,26	9,09
Oregano	1	8	12	From 18	0,0035	99,43	20,45
Parsley	1	8	14	From 18	0,0023	99,44	47,73
Nettle	1	8	14	From 18	0,0019	98,90	56,82

From the results in Table-4 it can be seen that the rate of inhibition increases in the following order: Corn bar (9.09%), Oregano (20.45%), Parsley (47.73%), and finally the nettle (56.82%). To explain this difference in inhibition rates, we thought to find a

Chemical composition of inhibitory elements in these plants.

Table-4 below shows the plants used and their chemical composition. The table shows that inhibition rate increases with potassium and magnesium content in each plant.

Table-4 Chemical composition of plants in minerals

	Potassium (mg/100g)	Magnesium mg/100g	Inhibition rate
Corn Beard	287	127	9,09
Oregano	1000	270	20,45
Parsley	1272	372	47,73
Nettle	2196	473	56,82
Solubilities of the oxalates	K <sub>2</sub> C <sub>2</sub> O <sub>4</sub> 39.9 g / 100g water	MgC <sub>2</sub> O <sub>4</sub> 10.4g/100g water	CaC <sub>2</sub> O <sub>4</sub> 6.7-10.4 g / 100g water

By comparing the solubilities of the oxalates of these elements with that of calcium oxalate, we notice that these solubilities are classified in decreasing order; K<sub>2</sub>C<sub>2</sub>O<sub>4</sub> (39.9 g / 100g of water) followed by MgC<sub>2</sub>O<sub>4</sub> (10.4 g / 100g of

water) and finally CaC<sub>2</sub>O<sub>4</sub> (6.7-10.4 g / 100g of water); therefore the oxalate combines first with potassium, then with magnesium and finally with calcium, which decreases the amount of calcium oxalate formed, which happens in majority of urinary stones.

## Conclusion

The results demonstrated the efficacy of the plants studied on the crystallization of oxalocalcium urinary stones, particularly *Urtica dioica* which exhibit a higher inhibitory effect in views of its high potassium and magnesium content. We can conclude that the natural extracts could, therefore be used in the treatment of urolithiasis of patients in traditional pharmacopoeia.

## Conflict of interest

The authors declare that they have no competing interests.

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