

# Study on the Inhibitory Effect of the Aqueous Extract from the Jujube Pulp against the Crystallization of the Calcium Oxalate

Younes AASSEM<sup>1</sup>, Mohamed BOUHA<sup>2</sup>, Rachid BOUHADI<sup>3</sup>, Mustapha EL BIR<sup>4</sup>, BATOUI SANAË<sup>5</sup>, Ahmed GAMOUH<sup>6</sup>, Mohamed MBARKI<sup>7</sup>

<sup>1,6</sup>Laboratory of Applied Spectro-Chemistry and Environment, Faculty of Science and Technology - Sultan Moulay Slimane University, Beni Mellal, Morocco

<sup>2,3,4,7</sup>Laboratory of Chemical Processes and Applied Materials, Faculty of Science and Technology - Sultan Moulay Slimane University, Beni Mellal, Morocco

<sup>5</sup>Laboratory of Environmental Engineering, Faculty of Science and Technology - Sultan Moulay Slimane University, Beni Mellal, Morocco.

**Abstract:** *The aim of this work is to study the effect of the aqueous extract of jujube pulp on the crystallization of calcium oxalate. This extract contains, among other chemical species, heterocyclic compounds that we are currently identifying. We prepared the aqueous extract of jujube pulp by boiling 5%, in terms of weight. Slices of 15 g of pulp were placed in 300 ml of distilled water and brought to the 60, 80 and 100 °C temperatures. A volume ranging from 0.5 to 2 ml of each sample was placed in 5 ml of the sodium oxalate (Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub>) presenting a concentration of 0.005 mol / l. Then, 5 ml of calcium chloride (CaCl<sub>2</sub>) at a concentration of 0.005 mol / l was added to the mixture. In order to observe the formed crystals, we performed polarizing light optical microscopy (PLOM). The first results show that the size and the number of calcium oxalate crystals is reduced by the presence of aqueous extract of jujube pulp. The monohydrate form of calcium oxalate is completely converted to the dihydrate form. Result that would tend to consider the state of the oxalocalcic solution close to that of calcium oxalate crystalluria. We conclude that the aqueous extract of jujube has an inhibitory effect on calcium oxalate crystals. This effect remains to be confirmed in vivo on the calcium oxalate crystallization in the formation of urine. Such a result is very important since it could contribute in order to avoid the development of urinary calculus.*

**Keywords:** Ziziphus jujube, decoction, extract, urinary lithiasis, Inhibitors, the crystallization.

## 1. Introduction

Plants are one of the main sources of discovery of new drugs [1]. One of these plants is Ziziphus jujube (Z. jujube), which is essential in medicine and is used traditionally in different countries to treat various diseases.

The selected plant belongs to the Rhamnaceae family [1]-[2]. The ziziphe is part of the jujubes species. There are about 40 species that are commonly found in the world [1]. Most species are used as drugs, particularly in India, China and other countries in Southeast Asia. It is found in almost all parts of Asia. The plant of this family contains fruits very close to the vitaceae family [3]-[4].

The urinary oxalocalcic lithiasis is the most common form of urolithiasis in the most kidney stones. The development of urolithiasis is a complex process that results from a succession of several physico-chemical events, including supersaturation, growth, aggregation and retention of crystals in the renal tubules [5]. Epidemiological data that has been collected over several decades have shown that the majority of urinary stones, up to 80%, are composed mainly of calcium oxalate (OxCa) [6]. It is important to underline that calcium lithiasis is characterized by the high rate of recurrence thus requiring a preventive approach.

Inhibitors are defined as molecules that increase the supersaturation threshold necessary for initiation of nucleation, which slow down crystal growth and secondarily

inhibit nucleation. Promoters reduce the formation product, like [Ox][Ca], of a supersaturated solution [7]. Citrate is considered to be one of the major low molecular weight inhibitors active against the crystallization of calcium oxalate. It has three acidities and effectively complexes urinary calcium. This has the effect of lowering the supersaturation of oxalates and calcium phosphates in the urine and thus increasing the formation product of these crystalline species [8]. In addition, citrate has inhibitory activity against growth and aggregation of calcium oxalate crystals and growth of calcium phosphate crystals in lithiasic patients [9]-[10].

According to epidemiological surveys, among the main factor modulating the risk of stone formation, the frequency variations of urolithiasis inadequate nutritional behavior of lithiasic people that reports a urinary metabolic abnormality, and consequently the formation of urinary calculus [11]. The following table summarizes all direct and indirect effects of dietary habits in lithogenic mechanisms.

**Table 1:** Role of dietary habits in lithogenesis [11]

Eating habits	mechanisms
<b>Direct effects</b>	
High intakes of calcium	Hypercalciuria
High intakes of oxalate	Hyperoxaluria
High intakes of purines	Hyperuricosuria
Low intakes of vegetable fibers	Hypercalciuria and oxaluria
Low intake of drinks	Increased concentration of purines
<b>Indirect effects</b>	
High protein intakes	Hypercalciuria, acidic urinary PH,

	hypocitraturia
High intakes of fat	hyperoxaluria
High intake of refined sugars	hypercalciuria
High salt intake	hypercalciuria

Previous studies of some native species have shown that among all extracts based on polarity, leaves and fruits have important pharmacological and toxicological activities [3]-[4]-[12]-[13]-[14].

Jujube belongs to the family called rhamnaceous, ziziphus zizyphus species and it is the common jujube tree, that is a deciduous thorny fruit plant. It grows well in wild areas. It adapts to hot and dry climates, and even in arid regions. Several studies have been carried on jujubier. The aqueous ethanolic extract of its seeds appears to possess hypoglycemic activity [15]. Aqueous macerated from the leaves of *Ziziphus mauritiana* Lam caused an inhibition of blood glucose [16]. Other virtues have been detected for this plant: an anxiolytic effect of ziziphus jujuba seeds in vivo [17], an anti-inflammatory activity of the essential oil of ziziphus jujuba seeds [18], an antioxidant effect and antilisteria of essential oil and organic extracts of ziziphus jujuba seeds [19]. However, in Morocco the studies on the jujube remain relatively rare: Hence the interest of this work.

Through previous work the analysis of the data results carried out was collected according to ethnobotanical survey cards in other that regions of Morocco can identify the medicinal plants most used traditionally for the treatment of urolithiasis. *Herniaria hirsuta* has been used with a 21.6% frequency, followed by *Petroselinum crispum* (12.4%), *Zizyphus lotus* (10.5%), *Citrus limon* (9.7%), *Opuntia ficus-indica* (7%), *Coriandrum sativum* (6.6%), *Zea mays* (5.6%), *Apium graveolens* (4.8%) and *Crocus sativus* (3.6%) [20]-[21]-[22]-[23].

## 2. Vegetal material

The studied plant in our work is jujube that has been collected in the Moroccan Beni Mellal Khenifra area. The fruits were dried then shelled to obtain slices of the pulp.

### 2.1 Preparation of extracts

We performed three 5% aqueous decoctions: 15 g slices of the pulp were put in 300 ml of distilled water. The first decoction was heated to 100 °C, the second to 80 °C and the third to 60 °C. The three decoctions were performed for one hour. The cooled decocted were filtered on absorbent paper, the filtrates obtained are stored in at 4 °C temperature.

### 2.2 Preparation of calcium oxalate crystals.

To obtain synthetic crystals, 5 ml of sodium oxalate at a 0.005 mol / l concentration are mixed with 5 ml of calcium chloride at a 0.005 mol / l concentration.

### 2.3 Experimental protocol

For each filtrate we took three doses, 0.5 ml, 1 ml and 2ml, which we tested on the crystals as it follows:

Each dose was put in 5 ml of sodium oxalate at a 0.005 mol / l concentration. 5 ml of calcium chloride at a 0.005 mol / l concentration are added to the preceding mixture. The blank test is the mixture of sodium oxalate and calcium chloride without filtrate.

### 2.4 Observation and counting of crystals

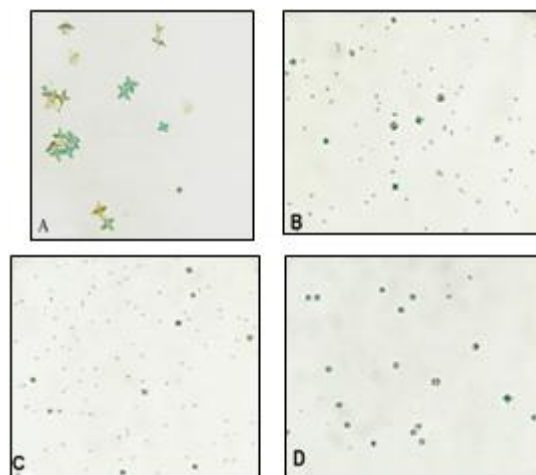
For the observation of crystals, we used the polarizing light optical microscope (PLOM). The micrographs were taken for each dose and for the blank test.

The counting of the monohydrate and dihydrate crystals is carried out on the grid of the Malassez cell.

## 3. Results and discussion

### 3.1 Comparison of the micrographs of the various extracts

The figure 1 illustrates some examples of PLOM micrographs obtained in presence of the aqueous extract from jujube pulp that are prepared at different temperatures:



**Figure 1:** PLOM micrographs of crystals formed before and after the addition of extracts:

A: blank test, B: 2 ml of extract at 60 °C. C: 2 ml of extract at 80 °C., D: 2 ml of extract at 100 °C.

It can be seen that in the presence of the aqueous extract from jujube pulp the number of calcium oxalate crystals does not seem to decrease until after the 80°C temperature. We also observe that the size of the crystals seems to decrease beyond the 60°C temperature. These results explain that the inhibitory effect of the aqueous extract from the jujube fruit pulp would appear at around 60°C in terms of crystal size. However, the factor of the number of crystals only seems to play a role above 80°C. Thus, extracts at 80°C could ensure an inhibitory in vitro effect against the crystallization of calcium oxalate.

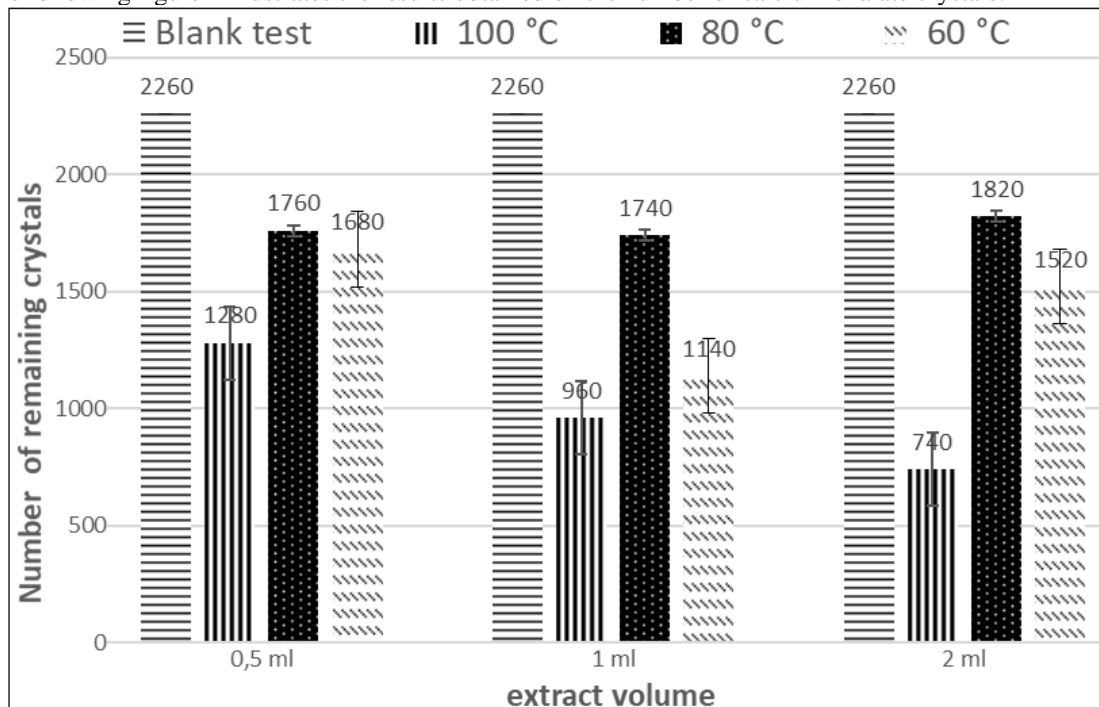
### 3.2 Counting of monohydrated and dehydrated crystals:

The following table presents the results of the counting of the crystals in the conditions of temperatures and extracts concentrations that have just been mentioned.

**Table II:** Number of calcium oxalate crystals according to different volumes, of aqueous extracts from jujube pulp after decoction at 100 ° C, 80 ° C and 60 ° C.

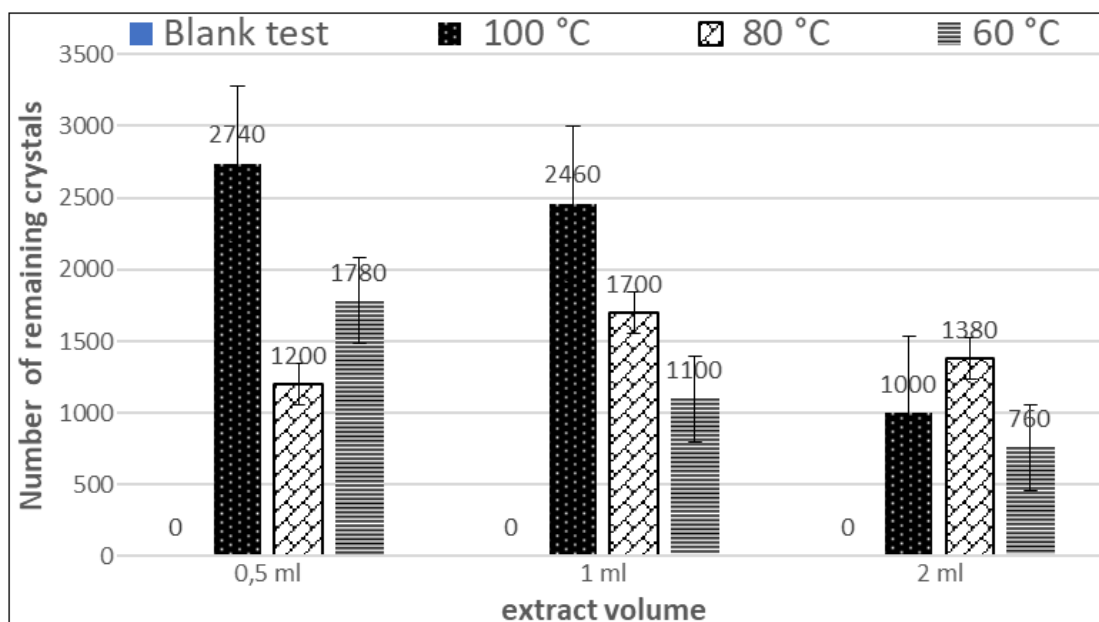
	blank test	0.5 ml			1 ml			2 ml		
		60 ° C	80 ° C	100 ° C	60 ° C	80 ° C	100 ° C	60 ° C	80 ° C	100 ° C
dehydrated crystals	2260	1680	1760	1280	1140	1740	960	1520	1820	740
monohydrated crystals	0	1780	1200	2740	1100	1700	2460	760	1380	1000

The following figure 2 illustrates the results obtained on the number of calcium oxalate crystals.



**Figure 2:** Evolution of the number of crystals of the dihydrate calcium oxalate crystals in the presence of different volumes of aqueous extract from jujube pulp.

The figure 3 shows the evolution of the number of crystals of the monohydrate calcium oxalate crystals in presence of different volumes of aqueous extract of the jujube pulp.



**Figure 3:** Evolution of the number of crystals of the monohydrate calcium oxalate crystals in presence of different volumes of aqueous extract of the jujube pulp.

So, one could observe that the number of dihydrate crystals formed decreases according to increasing contents of the extract at 100 ° C, whereas the other two decoctions do not represent a sharp decrease.

Regarding the number of monohydrated crystals, after their appearance, they decreased according to the increase of the contents of the extract at 100 ° C and at 80 ° C.

We have found, also, that the extract at 100 ° C of jujube has a better effect of decreasing the number of the monohydrate and dihydrate calcium oxalate crystals according to increasing contents compared to other extracts.

#### 4. Conclusion

Nature would be a rich source of future vegetal drugs treating various diseases since ancient times. The present work would contribute to the valorization of the so-called forgotten fruits. The work has optimized the material of analyses and characterization of lithogenic species like calcium oxalate crystals thanks to Polarizing Light Optical Microscopy.

According to the present work, the aqueous extract of jujube has an in vitro inhibitory effect on calcium oxalate crystals. Such an effect remains to be confirmed, in vivo, on the oxalocalcic crystallization during the formation of urinary calculi.

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