

Contribution to the Study of the Mass Reduction of Stones by Some Medicinal Plants

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ABSTRACT: The cystine calculi's, consisting primarily of amino acids, is considered the rarest stones with a prevalence of 1% with adults and 10% with the gallstone child. Its treatment is difficult because of its resistance to extracorporeal lithotripsy and its frequent recurrence. Drug treatments used until now, based on citrate are effective, but often poorly tolerated. Morocco is one of the countries that has been using, for a long time, traditional medicine based on natural plants to treat many diseases including stones. The most medicinal plants used for this purpose are *Herniaria hirsuta* L. (HHL) , flowers of *Opuntia Ficus- Indica* (OFI) , *Zea Mays Styles* (ZMS) and seeds of *Ammi Visnaga* L. (AVL) . The objective of this work is to study experimentally the effectiveness of each plant on the dissolution of cystine stones. [1]

Material and methods: In 1L saline solution containing 9 g / L of NaCl and boiled, is introduced 5 g of plant extract powder. The powder is left soaked for 15 minutes and then filtered. A specific installation that resembles the urinary circuit was conducted in the laboratory. As a result, the cystine stone is placed in a tube then undergoes a steady flow through a dynamic circuit, the solution laden with extract for eight weeks; as an effective time to treat gallstones. [1] Every two weeks, the calculations are removed and dried for 16 hours at a temperature of 40 ° C. The same assembly was carried out for two other witnesses solutions to correct the loss mass (Dissolution Rate): The first is a solution of Potassium Citrate of 3 mmol / L and the second is NaCl of 9 g / L.

Results: After eight weeks, the loss mass is about $(61,43 \pm 11,12)$ % with. (HHL) , $(63,75 \pm 10,95)$ % to (OFI) , $(66,83 \pm 11,12)$ % to (AVL) and $(72,46 \pm 11,07)$ % to (SZM) , while the loss of mass in the presence of witnesses solutions is $(20,23 \pm 3,12)$ % for potassium citrate (C Pot) and $(18,38 \pm 5,32)$ % for the saline solutions.

KEYWORDS: Nephrolithiasis, medicinal plants, urinary stones, citrate, Dissolution Rate.

I. INTRODUCTION

Cystinuria is a hereditary tubulopathy, caused by a failed transtubular transport of cystine and other dibasic amino acid (ornithine, arginine, lysine). Cystine as less soluble amino acids, its excessive shedding leads to recurrent stones, which may begin in childhood. The latter is one of the rarest stones with an average rate of 1% in adults and 10% in the gallstone child compared to other calculations. Two morphological subtypes are listed: Va show a granular and embossed superficial morphology and correspond to recently formed or untreated stones. Consequently, these cystine stones have a modified morphology corresponding to the Vb subtype [2].

In developed countries, such as France, Cystinuria represents 1% of all urinary calculi among adults, whereas it is 8.75% among children. In the United Kingdom, the prevalence is 1.5% among adults. Remains substantially zero in Asia [3, 4].

In the Maghreb countries, for example Tunisia, the prevalence among children is 2.5% while in Algeria it has 0.7% among adults [5,6].

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Most of the researches carried out in Morocco do not mention to the presence of cystine stones. Furthermore, a lonely study done on a series of 42 children, mentions the presence of cystine lithiasis with a rate of 2% [7, 8, 9, 10]. Since a long time in Morocco, several medicinal plants have been used traditionally for the treatment of urinary stones such as *Herniaria Hirsuta* L. (HHL), the flowers of *Opuntia ficus-indica* (OFI), *Zea Mays* Styles (SZM) and seeds of *Ammi Visnaga* L. (AVL) gave often spectacular therapeutic results. This has been scientifically proven by F. Meiouet and al, according to a research done on many plants brought into contact with cystine. [1] The objective of this work is to show experimentally the effectiveness of some plants that are known by their results on the degradation of the calculations. The choice was focused on cystine because of its extracorporeal lithotripsy resistance and its recurrence.

II. MATERIALS AND METHODS

II-1.The Urinary Lithiases

Cystine is an amino acid composed of two cystine units linked by a disulfide bridge. It can be responsible for the formation of kidney stones especially among people with cystinuria.

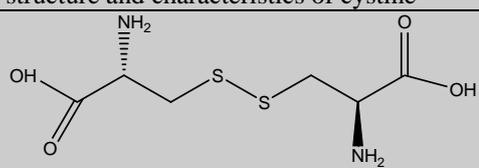
Table: 1	structure and characteristics of cystine
Chemical structure	
Molecular formula	$C_6H_{12}N_2O_4S_2$
Other name	2-amino-3-(2-amino-2-carboxy-ethyl)disulfanyl-propanoic acid
Molecular weight (g/mol)	240,3

Figure 1 shown several fragments of cystine; were removed by surgery in an elderly patient of 39 years old suffering from recurrent congenital cystinuria. These calculations whose mass is between 102-309 mg have morphology classified Va.

The chemical composition was analyzed by infrared spectroscopy (Figure 2). The different elongations presented in the IR spectrum are 845 cm^{-1} and 540 cm^{-1} features cystine depending on the laboratory of Tenon CRYSTAL Hospital, Paris- France.



Figure 1 : fragments of cystine.

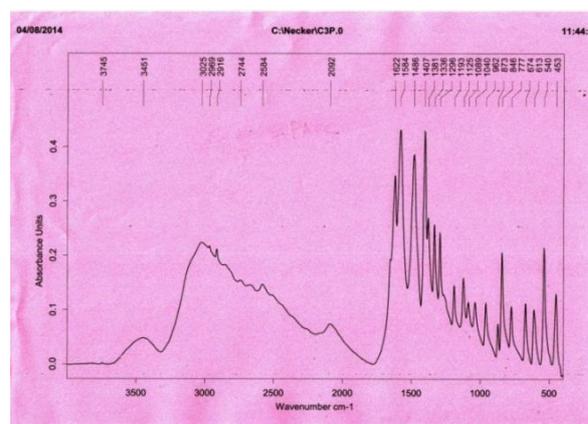


Figure 2 :Infra-Red spectrum of cystine

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

II-2.Medicinal plants

The first plant, HHL fully used (leaves and stems) shown in Figure 3; is a species of flowering plant in the pink family known by the common name hairy rupturewort. It is native to Eurasia and North Africa, and it is known on other continents, including North America, as an introduced species. This is an annual herb with stems up to 20 inches long and comes from the Taza region of North -eastern Morocco.

The second plant shown in Figure 4 is in the form of flowers of OFI, is a species of cactus that has long been a domesticated crop plant important in agricultural economies throughout arid and semiarid parts of the world. It is thought to possibly be native to Mexico.



Figure 3 : Herniaria Hirsuta L. (HHL)



Figure 4 :Florws of Opuntia Ficus-Indica (OFI)

The third plant shown in Figure 5 is AVL.it is a species of flowering plant in the carrot family known by many common names, including bisnaga,toothpickweed, and khella. It is native to Europe, Asia, and North Africa, but it can be found throughout the world as an introduced species. This is an annual or biennial herb growing from a taproot erect to a maximum height near 80 centimeters. Leaves are up to 20 centimeters long and generally oval to triangular in shape but dissected into many small linear to lance-shaped segments. The inflorescence is a compound umbel of white flowers similar to those of other Apiaceae species. The fruit is a compressed oval-shaped body less than 3 millimeters long. In our experiments we use the seeds removed from the end of the stems. They come from the Taounate region of northern Morocco.

The fourth plant illustrated in Figure 6 shows very fine filaments that come from the outer shell corn cobs SZM. Zea Maïs is an annual grass in the Poaceae (grass family) that originated in Central America and is one of the top three cereal crops grown in the world it is from the region of Fez in northern Morocco.

The four plants were dried at room temperature in a ventilated area and away from light and then ground.



Figure 5 :Seeds of Ammi Visnaga L. (AVL)



Figure 6 : Zea Mays Styles (SZM)

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

II-3. Main molecules in the studied plants

A bibliographical study on the four plants shows that the plant HHL contains saponosides as derivative médicagéniques acids and bidesmosidiques saponosides and flavonoids. The Styles of SZM consist of polyphénols of tanine type are rich in potassium, while the seeds of AVL are mainly composed of furanochromes as khelline, visnagine. As for the flowers of OFI reveal primarily consists of flavonoids: pendulétine, rutine, quercétine, luteoline [11, 12, 13, 14]. Tables 2 and 3 show the main molecules studied in plants and the citrate molecule

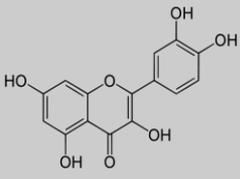
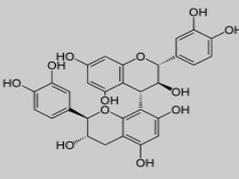
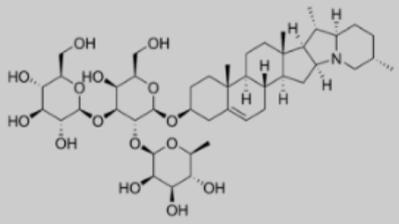
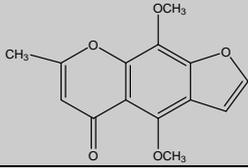
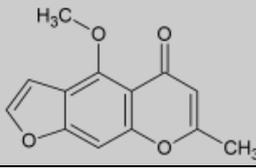
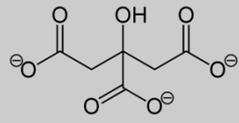
Table 2: Main molecules studied in plants.			
Name of the molecule	Quercétine	Tanins	Saponine
chemical Formula			

Table 2: Main molecules studied in plants and citrate' molecule.			
Name of the molecule	Khelline	Vesnigin	Citrate
chemical Formula			

II-4. Extraction process

In 1 L of physiological saline solution containing 9 g / L NaCl boiled; is introduced 5 g of plant extract powder. The whole is left to soak for 15 minutes, then undergo three filtration stages. The first through a sieve of 125 µm followed by filtration port hardened quantitative filter paper of 20 to 25 µm port then through a hardened quantitative filter paper from 7 to 10 µm port set on a Buchner Funnel porcelain.

The result of the plant extracts was compared to:

An aqueous solution of sodium citrate to 3 mmol / L, which corresponds to the average concentration of urinary citrate obtained during the treatment of cystinuria.

An aqueous NaCl solution at 9 g / L used as a witnesses solutions.

II-5. Experimental device

A specific installation that resembles the urinary circuit was conducted in the laboratory (Figure 7). Indeed, the solution laden with plant extract is placed in a large tank and three cystine stones are placed in an enclosure, using the sample holder, attached at both ends by two tubular. The first to pump the solution from the large tank and the second attached to a second collecting vessel. The stones undergo regular flow from the tank through two valves placed upstream and downstream of the chamber for controlling the flow rate of the solution at 1,5mL / min (2L / day): average flow of urine in the human body. The pH value of the recovered solution is monitored daily using a pH meter (sens Ion2). The

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(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

latter is filtered on a pore diameter of 125µm sieve, and given to the initial reservoir. The operation is repeated, daily, for eight weeks, period recommended in traditional medicine.

Every two weeks, the calculations are removed, dried for 16 hours at a temperature of 40 ° C and weighed with a precision balance to measure the mass loss and then delivered to the compound.

The same assembly was carried out for two other witnesses' solutions to correct the mass loss (Dissolution Rate): The first is a solution of Potassium Citrate 3 mmol / L and the second is a solution of NaCl 9 g / L.

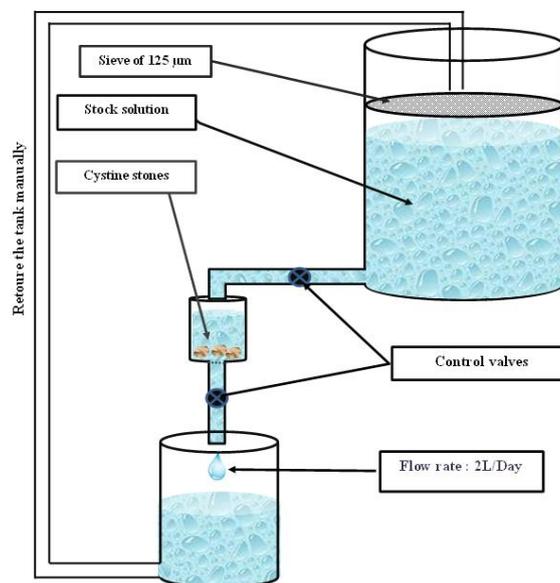


Figure 7: Diagram of experiment installation.

III. RESULTS

III-1. Effect of plant extracts on calculations

The results given express the dissolution rate (DR) calculated based on the final average weight calculations, using the following formula, with their standard deviations.

$$(DR\%) = \frac{W_i - W_f}{W_i} * 100$$

With W_i : Initial average weight of three calculations
 W_f : Final average weight of three calculations

The initial average weight " W_i " (\pm standard deviation) of stone fragments in solution loaded with plant extract is:

- ✓ W_i (OFI) = 102,29 \pm 3,27 mg
- ✓ W_i (HHL) = 106,38 \pm 3,07mg
- ✓ W_i (AVL) = 220,63 \pm 4,12 mg
- ✓ W_i (SZM) = 309,10 \pm 5,07 mg

The average initial weight (\pm standard deviation) of stone fragments in the control solutions is:

- ✓ W_i (solution of NaCl) = 203,42 \pm 5,97 mg
- ✓ W_i (solution of citrate) = 240,45 \pm 4,93 mg

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

Figure 8 is given the kinetic evolution of the dissolution rate DR of the calculations of cystine, for the four plants for eight weeks.

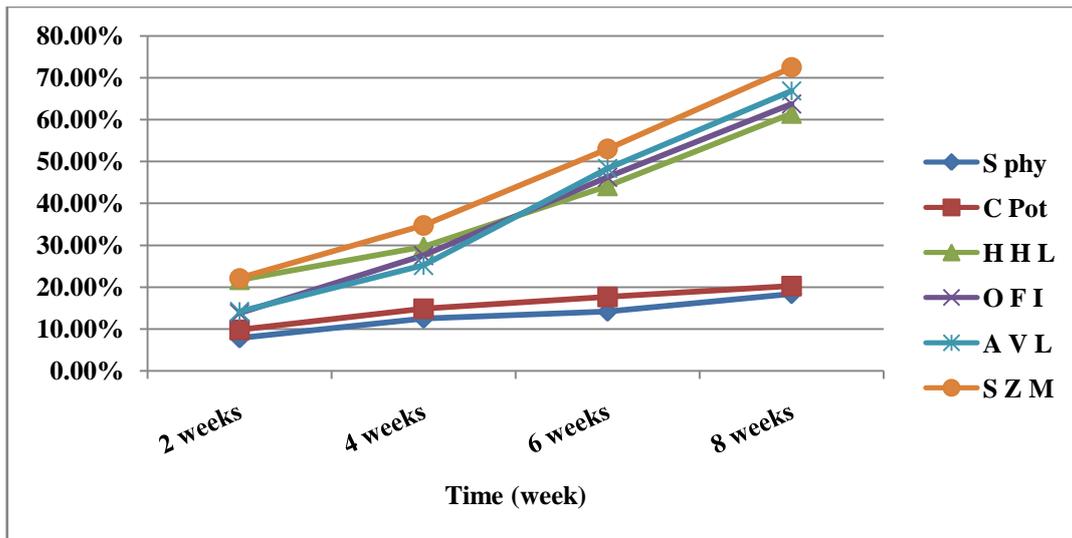


Figure 8 :kinetic evolution of the dissolution rate of the calculations.

The analysis of the curve shows a continuous increasing kinetic evolution of DR for the different solutions over the eight weeks. This trend is more important for loaded solutions than the control solutions.

The Comparison of the response calculation processing by the plants is given in Figure 9.

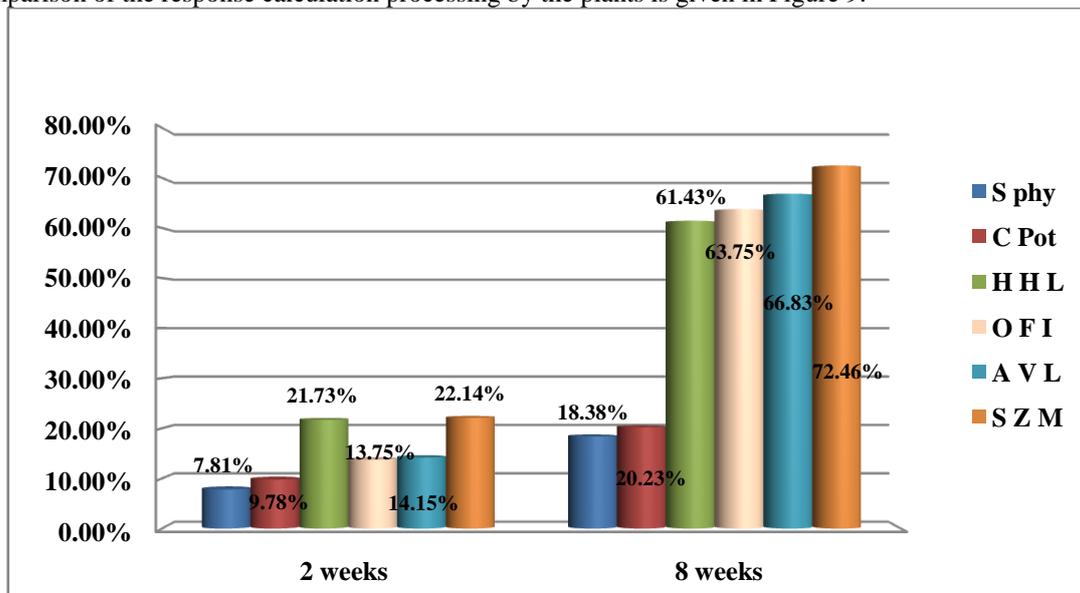


Figure 9:The Comparison of the response calculation processing by the plants.

The Comparison of DR for the four plants shows that SZM have a slightly greater impact than others because after two weeks, the DR of this latter is $(22,14 \pm 10,35) \%$, compared to $(13,75 \pm 8,11) \%$ for flower extracts of OFI, $(14,15 \pm 7,15) \%$ for seeds AVL and $(21,73 \pm 6,41) \%$ for the whole plant of HHL. Citrate and physiological saline solution gave a TD $(7,81 \pm 2,45) \%$ and $(9,78 \pm 1,22) \%$ respectively.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

The same result is observed after eight weeks of contact calculations with the plant extracts, since the DR is $(72,46 \pm 11,07)$ % in extracts of SZM, it is $(66,83 \pm 11,12)$ % , $(63,75 \pm 10,95)$ % and $(61,43 \pm 11,12)$ % with the seeds of AVL, with the flowers of OFI and with the whole plant of HHL respectively. Then with citrate, and the physiological solution, the dissolution rate after eight weeks was $(20,23 \pm 3,12)$ % and $(18,38 \pm 5,32)$ % respectively

III-2. Kinetic Evolution of pH for the four plants

The change in pH as a function of time is given in Figure 10.

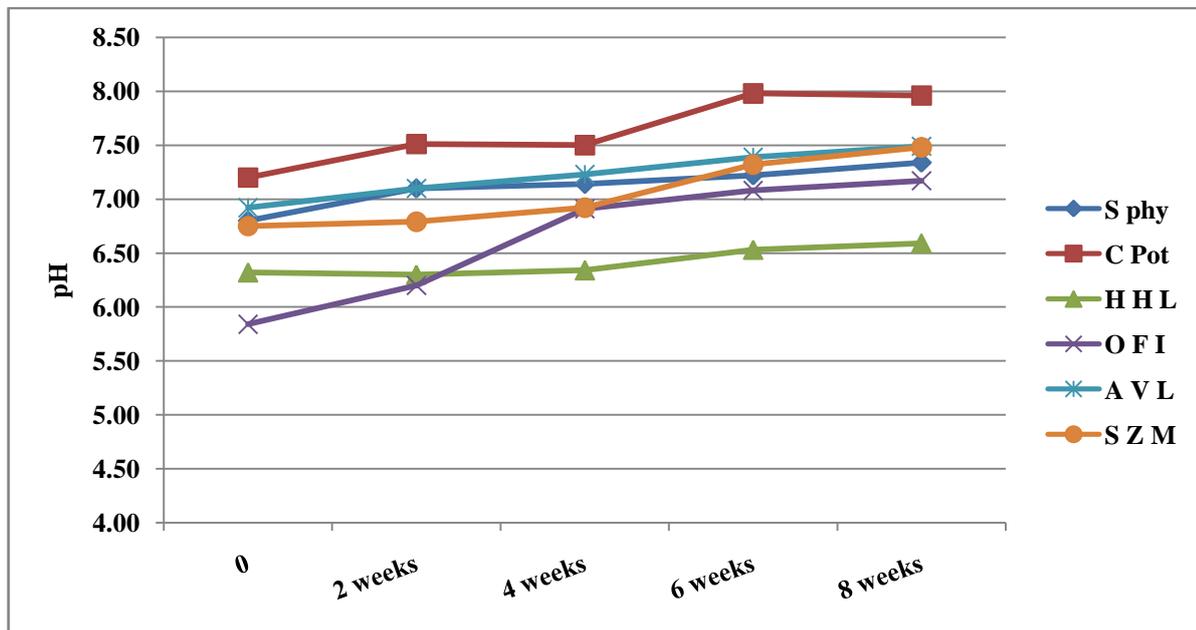


Figure 10: Evolution of pH of solution with time.

The initial pH of all solutions is slightly acid ranging from 5.8 to flower extracts of OFI, 7.2 to the citrate solution . These pH values undergo a slight linear increase during the eight week period from 6.6 for *Hirnia Hirusta*, and to 7.9 potassium citrate.

III-3. Proposed mechanisms of action.

some proposed mechanisms of action

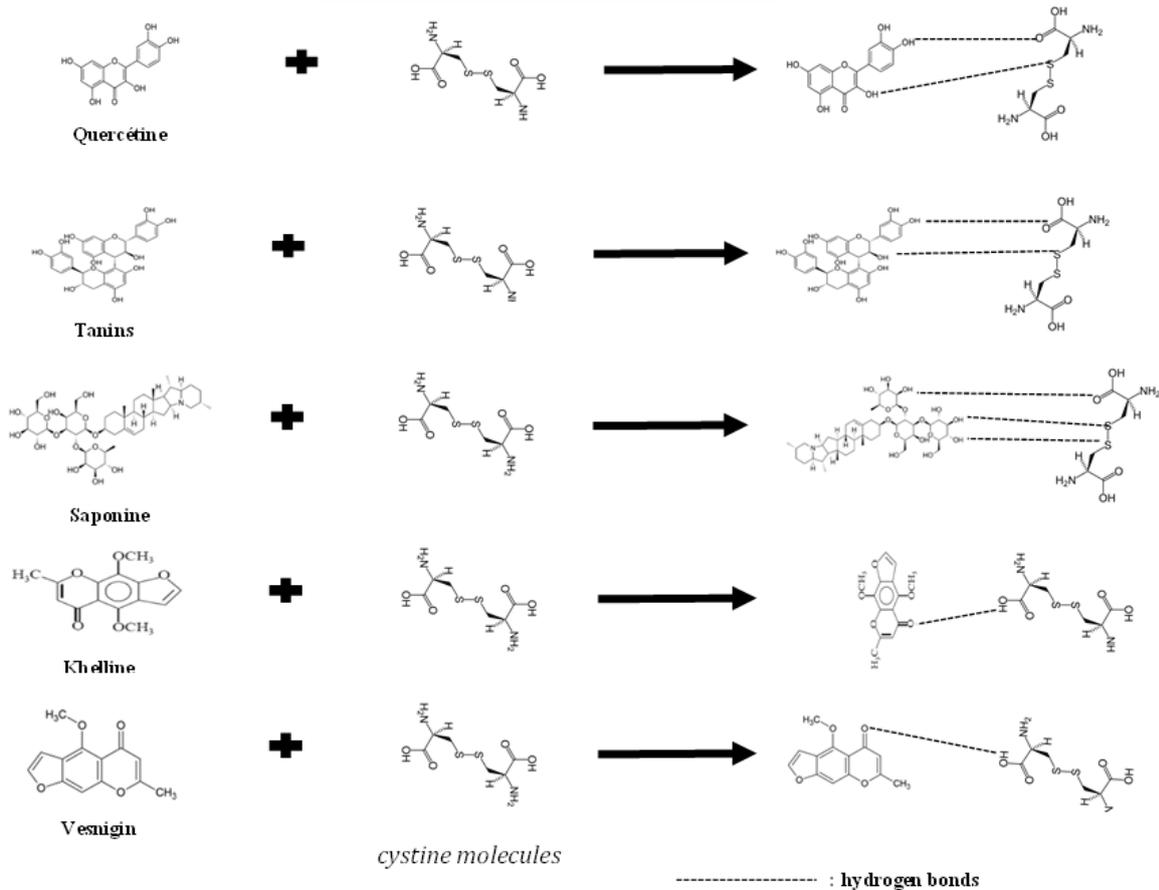


Figure 11: Proposed mechanism of action for the dissolution of cystine stones by the main constituents of medicinal plants.

VI. DISCUSSION

The Urinary solubility of cystine is closely related to the change of the pH since it does not exceed 250 mg / L at a pH below 7 but reached 500 mg / L from a pH equal 7.5 [15] . The first treatment of cystinuria is proposed hyperdiuresis based on a limiting dietary intake of methionine and salt. The objective is to maintain the soluble cystine by acting mainly on the concentration of the urine and the pH value. In case of persistence of cystine, an additional treatment of pharmacological agents based on sulfhydryl is proposed. However, this treatment has many side effects that limit its long term use. [16]

The use of medicinal plants to dissolve stones of cystine remains an interesting alternative. The tests performed in vitro in the presence of different extracts showed kinetics of solubilization sound much higher than that of saline and sodium citrate up to 72% for corn styles. The dissolving power of the extracts of maize styles, flowers *Opuntia ficus- indica*, *H. Hirsuta L.* and seeds of *A. visnaga* on cystine stones results from an interaction between the cystine and the molecules present in the plant extracts.

Examination of all chemical constituents present in the various plants used suggests that an independent mechanism of action of pH may be responsible for the dissolution of cystine stones. This effect could be related to the formation of complex cystine-molecule, such as cystine-flavonoïdes, tanins-cystine or cystine-sapnines whose stability would be

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

ensured by hydrogen bonds and hydrophilic bonds between the functional groups of active molecules carboxylic functions or amines of cystine molecule.

The complexes formed are much more soluble than cystine itself, resulting in the dissolution of the calculations while maintaining solution sometimes high amounts of dissolved cystine.

V. CONCLUSION

The results of our experiments show the effectiveness of extracts of four plants. These extracts may therefore be a curative and / or prophylactic interesting for cystinuriques patients.

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