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Zizyphus Lotus Anti-lithiasis activity in vitro of aqueous extracts of pulp fruit in human urine

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Abstract

The aim of this work was to evaluate the in vitro anti-lithiasic effectiveness of aqueous extract obtained from pulp of Zizyphus Lotus fruits in urine of lithiasic patients so as to valorize it within the framework of sustainable regional development and as local product. So, aqueous extracts of pulp of Z. Lotus fruits were prepared at different concentrations (1-10 mg/ml), submitted to a cold maceration during 48 hours. The crystallization of calcium oxalate was induced in human urine with extract. The study of the crystallization of calcium oxalate is carried out using optical microscope with polarized light (OMPL).The in vitro anti-lithiasic activity was evaluated against aggregation of calcium oxalate. The results show that adding the aqueous extract to the urine will decrease the size and the number of calcium oxalate also *Z. Lotus* showed in vitro anti-lithiasic activity by inhibiting the aggregation of calcium oxalate.

1. Introduction

Urolithiasis is a pathology characterized by the formation of crystalline concretions called urinary calculi that develop in the urinary tract, usually in the kidneys [1]. This pathology is frequent and recurrent; some forms are particularly severe and can lead to kidney failure [2]. Calcium oxalate ($CaC_2O_4 \times H_2O$) is the major constituent of stones formed in the urinary system of patients with urolithiasis [3]. Calcium oxalate can exist in three crystalline forms: whewellite, weddellite and calcium oxalate trihydrate [4]. In addition, urolithiasis will increase more and more and our need is to find a natural, preventive, and curative treatment, which has no side effect on health. The fruits of jujube (Zizyphus Lotus L), commonly called " nbag " are among the forgotten fruits of Morocco. Z. Lotus (L) are considered as medicinal plant [5]. The bush of jujube is thorny, belonging to the family of Rhamnaceae and is also called Sedra, Zarb, Azouggar or Tazougart [6]. In Morocco, this species is located in different areas, mainly in semi-arid ones; this is the case of the area of Beni Mellal- khenifra. After an hibernation period, between Octobre and March, Z. Lotus begins to bloom in May and June; then, it produces fruit in August [7]. These fruits are used in traditional medicine for treating various diseases such as Bronchitis; Diabetes, Diarrhea and abscesses [8]. Moreover, it has therapeutic activities: anti-ulcer activity [9]; anti-inflammatory and analgesic activity [10] and antispasmodic activity [11].

The aim of our work was to search in vitro the anti-lithiasis effect of the aqueous extracts of the of Z. Lotus fruits' pulp toward calcium oxalate in Urolithiasis patients' urine.

2. Material and Methods

2.1. Plant collection

Zizyphus Lotus fruits have been collected since September 2015-2016 from six areas of the Beni Mellal - khenifra region. After taking the representative sample, the fruits were first pitted and then the pulp was ground with a mortar to obtain a fine powder that will be used to prepare the aqueous extracts (Figure 1).

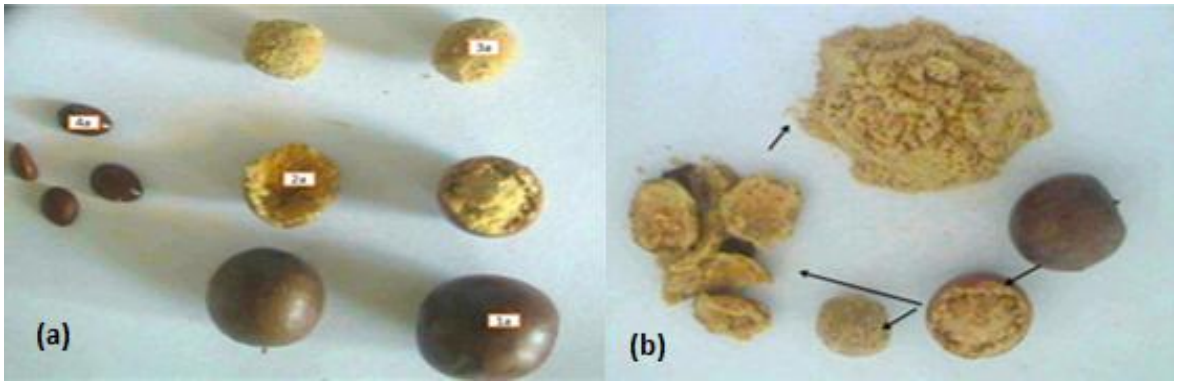

Figure 1: (a): Different parts of *Zizyphus Lotus*: 1a: the fruit; 2a: the pulp; 3a: the nucleus; 4a: the almond. (b): steps for obtaining the fine powder of Zizyphus Lotus pulp.

2.2. Extract preparation

The aqueous extracts of the Z. Lotus fruits' pulp were prepared in distilled water; this latter's concentration was ranging from 1mg / ml to 10mg / ml; then it was cold macerated at 4 ° C for 48 hours.

2.3. Preparation of urine samples

Urine samples come from JABRANE Multidisciplinary Clinic, in Béni Mellal. The urine was collected in a clean container of 250 ml capacity and stored at room temperature and analyzed within two hours of urination. Once the sample was received, the urine was homogenized, and its pH is measured with sufficient accuracy by a pH meter. Using a Pasteur pipette, a part of the sample was taken from the bottom of the container and up towards the middle in order to cover the larger crystals and aggregates, then the sample was transferred in a cell of Malassez for microscopic examination. The other part of the sample was destined to study the anti-crystallizing activity of calcium oxalate by adding the aqueous extracts of Zizyphus Lotus pulp's powder.

2.4. Anti crystallization of calcium oxalate in vitro in the presence of aqueous extracts in human urine

The aqueous extracts were prepared at different concentrations (1-10 mg / ml) of Z. Lotus fruit pulp powder.

2.5. Polarization microscope examination

Urines should be stored at room temperature or at 37 ° C (but not at 4 ° C) between the time they are emitted and the time they are examined.

3. Results and discussion

3.1. Microscopic observation results of urine without aqueous extracts

In the majority of cases, several ions, at least one of which in excessive concentration in the urine, will associate to form a chemical species such as calcium and may form calcium oxalate with the ions of oxalate [12]. This species is insoluble: the solubility is 3.10-9 (mmol/l)2 for whewellite in an aqueous medium [13,14]. The Hydrogen Potential pH is measured for each urine sample. Ideally, the urine should be emitted to the laboratory and examined without delay but these technical constraints make it difficult to practice this examination on a daily basis. Urine conservation studies at room temperature or at 37 ° C demonstrated that if the sample was kept less than 3 hours after emission above 20 ° C, the evolution of Crystalluria was very weak and the results were interpretable in the same way as those obtained on freshly expressed urine [15]. The crystals are identified by their morphological characteristics and their polarized aspect in lights. The crystalline species which are independent of the PH are few in number, this is the case of calcium oxalate which is partly dependent on PH but on the usual range of urine between 4.8 and 7.5 and we can consider that their sensitivity is low [12]. The crystalline facies is another important element in matters of crystalluria [12]. Indeed, all the crystals of a given species does not necessarily have the same shape [14; 16]. This depends essentially on two elements: the Biochemical composition of the urine and the presence of substance capable of interfering with the growth of certain faces of the crystals. This results in a change of shape of the observed crystals [12]. Dihydrated calcium

oxalate: the weddellite has an octahedral crystals form, composed of two flattened pyramids contiguous to the base and thus appears in the form of square envelopes (Figure 2 and 3).

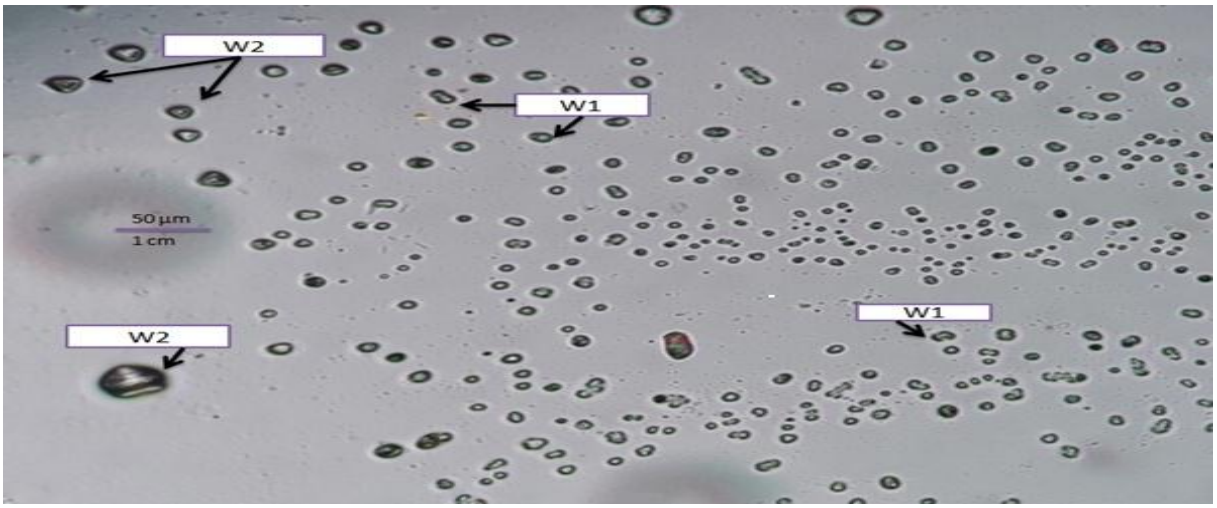

Figure 2: Micrograph of crystals of calcium oxalate monohydrate: whewellite (w1) and calcium oxalate dihydrate: weddellite (w2) of human urine to PH = 5.3 by Polarized light microscopy (Gross = 200)

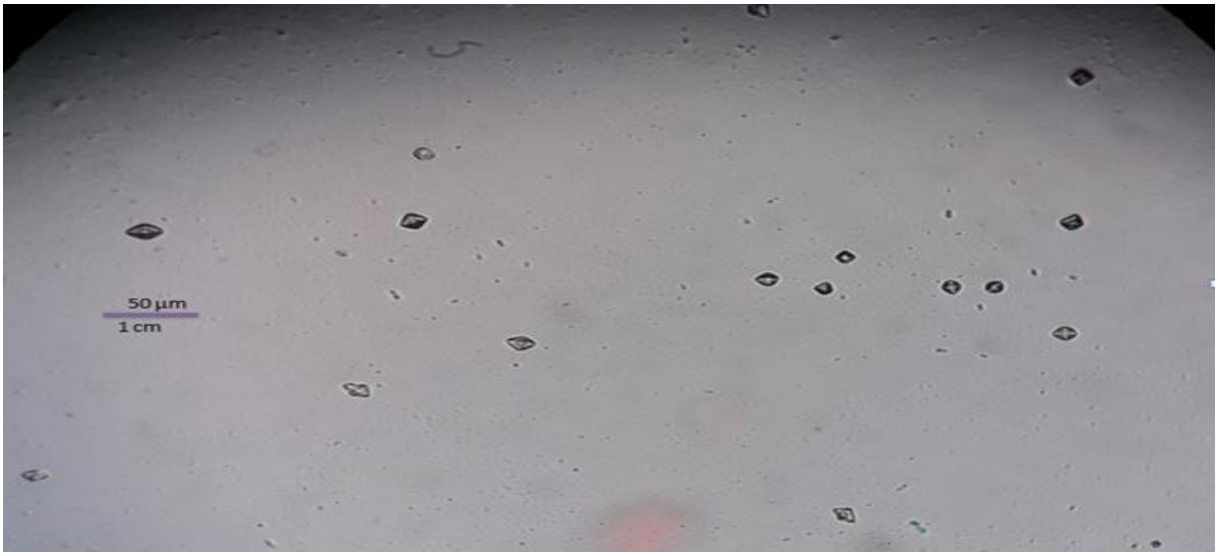

Figure 3: Micrograph of crystals of calcium oxalate dihydrate of human urine to PH = 5.3 by Polarized light microscopy (Gross=200)

When calciuria rises, a growing number of weddellite crystals shows a thickening of the separation edge between the two pyramids. In Figure 6, the pH of the urine is 5.7 and the only crystallographic form observed is the weddellite and the crystals form between them an aggregation. A study done by M. Daudon shows that in a highly hypercalciuric urine, the only observable facies is weddellite [12]. The characteristic crystals of calcium oxalate monohydrate: whewellite are oval to depressed center and swollen at the ends (Figure 4). It is essentially associated with hyperoxalluries [14, 16, 17 and 18].

3.2. Results of crystallization of calcium oxalate in urine by adding aqueous extract of Zizyphus Lotus pulp

The inhibitory effect of the aqueous extract of Z. lotus fruit on the crystallization of calcium oxalate is evaluated in vitro in human urine at concentrations ranging from 1 to 10 mg / ml of its pulp's powder. Results of previous work made by our team [19] showed that the aqueous extract of Z. Lotus fruit pulp's powder inhibits the aggregation of calcium oxalate in the aqueous solution of calcium oxalate. S. Deepti et al [20] approached the anti-lithiasic effect of an aqueous extract of Chenopodium album leaf. According to the study by Montealegre et al [21], the Results of previous work made by our team [19] showed that the aqueous extract of Z. Lotus fruit

pulp's powder inhibits the aggregation of calcium oxalate in the aqueous solution of calcium oxalate. S. Deepti et al [20] approached the anti-lithiasic effect of an aqueous extract of Chenopodium album leaf. According to the study by Montealegre et al [21], the dose of 0.5 and 1 mg / ml of the extract of Blumea Balsamifera will decrease the size of the crystal of calcium oxalate, also it will tend to displace the phase of the crystals towards the phase of calcium oxalate dihydrate (COD) and will inhibit the aggregation of crystals as well. S. Sarmistha et al [22] worked on aqueous and alcoholic extracts of Bergenia Ciliata rhizome by challenging their inhibitory effect on nucleation and aggregation of calcium oxalate crystals.

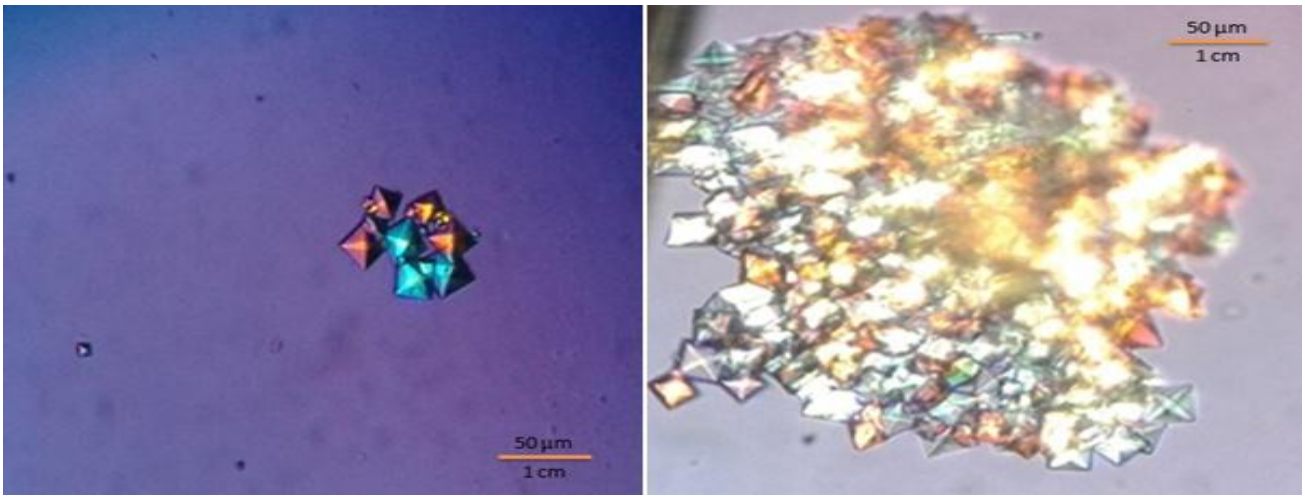

Figure 4: Micrograph of crystals aggregation of dihydrated calcium oxalate in human urine at PH = 5.7 by polarized light microscopy (Gross = 200)

Previous work has described a method of isolating soluble and insoluble fractions in methanol from Humulus lupulus L that is rich in compounds that inhibit the formation of calcium oxalate. In fact, both fractions could be effective in treating kidney stone disease [23]. Similarly, the anti-crystallizing effect against calcium oxalate by extract of the dolichos biflorus seed in vitro has been confirmed [24]. In addition, magnesium, citrate and phytate are inhibitors of crystallization of calcium oxalate, preventing the formation of monohydrate and calcium oxalate trihydrate and avoiding the crystallization of calcium oxalate by decreasing its supersaturation [25]. Bellakhdar et al [26] studied the effect of Zyziphus Lotus leaves in the treatment of urinary tract infections. Through the present work, 4mg/l of the aqueous extract of Z. Lotus powder inhibits the aggregation of dihydrated calcium oxalate (widdellite) in human urine of PH = 5.7 there is remarkable degradation of the wedellitte aggregate (figure 5).

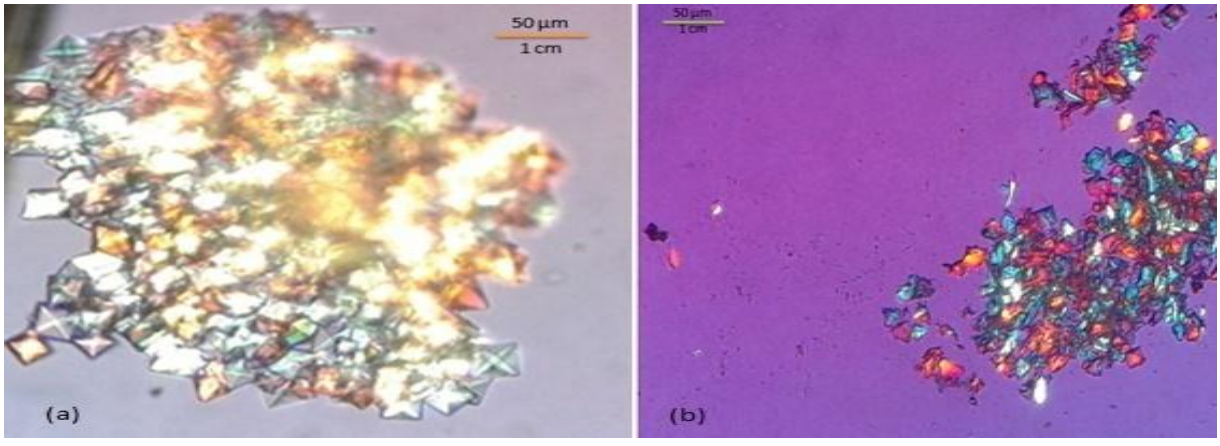

Figure 5: Micrograph of crystals of dihydrated calcium oxalate in human urine at pH = 5.7 by polarized light microscopy (Gross =200).(a) : aggregation of dihydrated calcium oxalate without aqueous extract;(b) : degradation of aggregation of dihydrated calcium oxalate with 4mg / ml of aqueous extract of Z. Lotus pulp

The dose 10mg/ml of the aqueous extract of Z. Lotus pulp's powder has an anti-crystallizing effect towards the calcium oxalate by decreasing the size and number of crystals of calcium oxalate dehydrated in human urine of lithiasic patients of pH = 5.3 (figure 6).

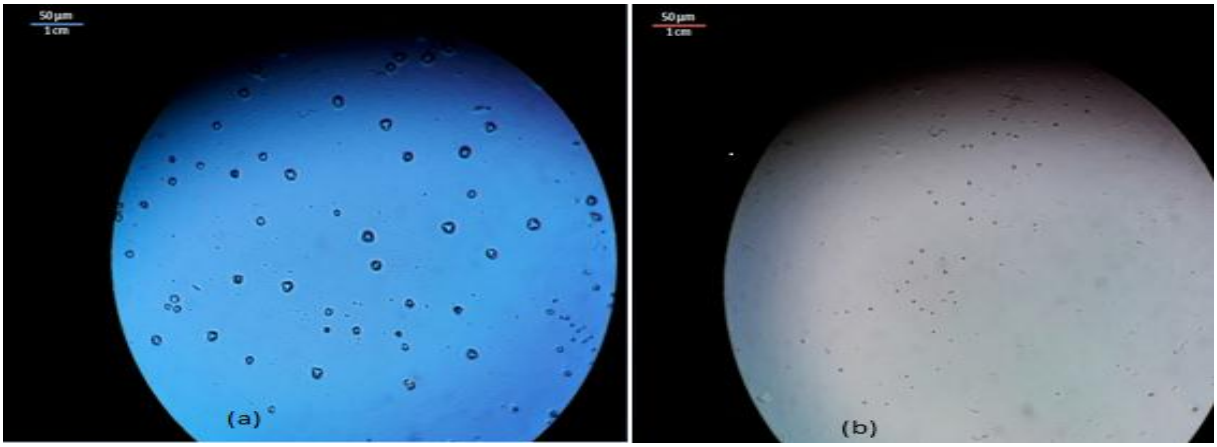

Figure 6: Micrograph of crystals of dihydrated calcium oxalate in human urine at PH = 5.3 by polarized light microscopy (Gross =200). (a): without aqueous extract; (b) : with 4mg/ml of aqueous extract of Z. Lotus pulp

Conclusion

The aqueous extract of pulp of Z. Lotus can inhibit the nucleation and aggregation of calcium oxalate crystallization in vitro. The activity of de fruits extract might be due to phytochemicals present in it, further characterization and isolation of the major active component from the Z. lotus fruits. In addition, thanks to the present work, the optical microscope with polarized light (MOLP), which is a priori routine technique in terms of analysis in laboratories, could be optimized and valued for the study of urinary lithogenesis. The in vivo study and the clinical trials remain decisive and sharp in the question of the therapeutic and medicinal valorization of the bioactive elements extracted from the medicinal plant Zizyphus Lotus.

References

1. P. Dalibon, La lithiase urinaire, une affection sous surveillance, *Actualités Pharmaceutiques*. 54 (2015) 23-29.
2. K. El Hmidi, S. Aloui, S. Akremi, K. Soltane, M. Ben Salem, H. Skhiri, N. Ben Dhia, A. Letaief, M. Hammouda, M. El May, Épidémiologie de la lithiase urinaire : expérience de service de néphrologie de CHU de Monastir, *Néphrologie et thérapeutique*, 9(2013) 357.
3. A. Trinchierie, Epidemiology of urolithiasis: an update, *Clin Cases Miner Bone Metab.* 5 (2008) 101-106.
4. C. Conti, M. Casati, C. Colombo, E. Possenti, M. Realini, G. Diego Gatta, M. Merlini, L. Brambilla, G. Zerbi, Synthesis of calcium oxalate trihydrate: New data by vibrational spectroscopy and synchrotron X-ray diffraction, *Spectrochimica Acta Part, Molecular and BiomolecularSpectroscopy*. 150 (2015) 721–730.
5. A. Zyyat, A. Legssyer, H. Mekhfi, A. Dassouli, M. Serhrouchni, Phytotherapy of hypertension and diabetes in oriental Morocco, *Journal of Ethnopharmacology*. 58 (1997) 45-54.
6. N. Rsaissi, M. Bouhache, La lutte chimique contre le jujubier, *Programme National de transfert de technologie en agriculture. (PNTTA)*. *DERD*. 94 (2002) 1-4.
7. M. Maraghni, M. Gorai, M. Neffati, Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of Ziziphus lotus, *South African Journal of Botany*. 76 (2010) 453–459.
8. Edouard Le Floc'h, Contribution à une étude ethnobotanique de la flore tunisienne, *Publication Scientifique Tunisiennes*. (1983) 129**.**
9. W. Borgi, N. Chouchane, Anti-spasmodic effects of Zizyphus lotus (L.) Desf. extracts on isolated ratduodenum, *Journal of Ethnopharmacology*. 126 (2009) 571–573.

10. W. Borgi, K. Ghedira, N. Chouchane, Antiinflammatory and analgesic activities of Zizyphus lotus root barks, *Fitoterapia*. 78 (2007) 16–19.
11. W. Borgi, N. Chouchane, Anti-spasmodic effects of Zizyphus lotus (L.) Desf. extracts on isolated rat duodenum, *Journal of Ethnopharmacology*. 126 (2009) 571–573.
12. M. Daudan, Cristallurie, *néphrologie et thérapeutique.* 11 (2015) 174-190.
13. B. Tomazik, G.H. Nancollas, The kinetics of dissolution of calcium oxalate hydrates, The kinetics of dissolution of calcium oxalate hydrates, *J Crystal Growth*. 46(1979) 355-361.
14. P. Jungers, M. Daudan, A. Le Duc, Lithiase urinaire, F*lammarion Médecine-Sciences, Paris.* 158 (1989) 95.
15. JS. Elliot, IN. Rabinwitz, Calcium oxalate crystalluria: Crystal size in urine, *Journal Urol.* 123(1980) 324-327.
16. M. Daudon, P. Kamoun, JP. Fréjaville, Cristallurie, F*lammarion Médecine-Sciences, Paris*. 303(2002) 1274.
17. M. Daudon, E. Letavernier, V. Frochot, JP. Hzymann, D. Bazin, P. Jungers, Respective influence of calcium and oxalate urine concentration on the formation of calcium oxalate monohydrate or dihydrate crystals, *C.R Chimie*. (2016) 1-10**.**
18. M. Daudon, La cristallurie : un marqueur diagnostique et pronostique des pathologies cristallogènes et des lithiases rénales, *Revue francophone des laboratories.* 445 (2013) 67-73.
19. L. Baddade, M. Elbir, M. Oubenali, M. Echajia, S. Rabi, M. Berkani, M. Mbarki, Inhibition de la cristallisation in vitro de l'oxalate de calcium par extrait aqueux de Zizyphus Lotus, *International Journal of Innovation and Applied Studies.* 23 (2018) 583–589.
20. S. Deepti, YN. Dey, I. Sikarwar, R. Sijoria, MM. Wanjari, AD. Jadhav, In vitro study of aqueous leaf extract of Chenopodium album for inhibition of calcium oxalate and brushite crystallization *Egyptian journal of basic and applied sciences.* 3 (2016) 164–171.
21. CM. Montealegre, RL. De Leon, Effect of Blumea balsamifera extract on the phase and morphology of calcium oxalate crystals, *Asian Journal of Urology.* 4 (2017) 201–207.
22. S. Sarmistha, RJ. Verma, Inhibition of calcium oxalate crystallisation in vitro by an extract of Bergenia ciliate, *Arabe Journal of Urology*. 11 (2013) 187–192.
23. A. Frackwiak, T. Kozlecki, P. Skibinski, W. Gawel, E. Zaczynska, A. Czarny, K. Piekarrska, R. Gancarz, Solubility, inhibition of crystallization and microscopic analysis of calcium oxalate crystals in the presence of fractions from Humulus lupulus L., *Journal of Crystal Growth.* 312 (2010) 3525–3532.
24. S. Sarmistha, RJ. Verma, Evaluation of hydro-alcoholic extract of Dolichos biflorus seeds on inhibition of calcium oxalate crystallization *Journal of Herbal Medicine.* 5 (2015) 41–47.
25. A. Rodriguez, A. Costa-Bauza, RM. Prieto, F. Berga, F. Grases, Magnesium, citrate and phytate: Effect of their binary mixtures as calcium oxalate crystallization inhibitors in urine, *European urology supplements*. 14 (2015) 29-78.
26. J. Bellakhdar, J. Claisse, R. Fleurantin, J. Younos, Repertory of standard herbal drugs in the Moroccan pharmacopoeia, *Journal of Ethnopharmacology*. 35 (1991) 123-146.

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