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SYMPOSIUM PAPER

Inhibition of crystallization of calcium oxalate by the extraction of *Tamarix gallica* L

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Abstract The main objective is to study the inhibitor effect of acid fraction of the extract of *Tamarix gallica* L on the crystallization of calcium oxalate. The extract of *Tamarix gallica* L is very rich by acid compounds that are used as an inhibitor of nephrolithiasis (calcium oxalate). Our study of the calcium oxalate crystallization is based on the model of turbidimetry by means of a spectrophotometer. The calcium oxalate formation is induced by the addition of oxalate solutions of sodium and of calcium chloride. The addition of inhibitor with various concentrations enabled us to give information on the percentage of inhibition. The comparison between the turbidimetric slopes with and without inhibitor gives the effectiveness of inhibitor for the acid fraction. By comparing the photographs of with and without inhibitor, we concluded that the extract of *Tamarix gallica* L acts at the stage of growth. The acid fraction of the extract of *Tamarix gallica* L gives an activity remarkable in the formation of urinary lithiasis (calcium oxalate); this effectiveness is due to the presence of functions of acid.

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Introduction

The genus *Tamarix* belongs to the Tamaricaceae family [1], and is also employed in the traditional medicine as astringent, aperitif, stimulus and diuretic [1]. The *Tamarix* is found to be rich in polyphenolic compounds such as flavonoids, phenolic acids, tannins and coumarins [2–4]. Nephrolithiasis term indicates that the disease is characterized by the formation of a stone in the kidneys or urinary tracts [5], and is a frequent disease that affects about 10% of people in the western countries [6]. The crystals of calcium oxalate (CaOx), are the primary constituent of more than 60% of the majority of human kidney stones [7]; they exist in the form of CaOx monohydrate (COM) and CaOx dehydrate (COD) [8]. The crystallization studies of calcium oxalate (CaOx) have been an interest to the researchers and urologists for many years [7]. The clinical use of inhibitors to prevent the formation of CaOx stones has been limited to some minerals (Selenium, Magnesium, etc.) and orthophosphates [9, 10].There are several methods for measuring the inhibitory activity such as photometry, turbidimetry and mixed suspension, mixed product removal (MSMPR) [11]. The medicinal plants contain chemical compounds which themselves possess an inhibitor effect in the crystallization of calcium oxalate [11]. The use of some natural substances as inhibitors in the formation of nephrolithiasis [12–15] allowed us to study the inhibitor effect of the acid fraction of the extract of *Tamarix gallica* L on the inhibition of COD crystal. In this work, we have used the model turbidimetric [16].

Materials and methods

Plant material

Leaves of *Tamarix gallica* L were collected from Djelfa in September. Identification was performed in the Department of Agronomic Sciences of University of Djelfa.

Preparation of the extract

Plant material was air-dried in shade at room temperature. A total of 200 g powder from plant was extracted with 400 ml diethyl ether at 40°C in Soxhlet extractor for 24 h. The extracts were then filtered through Whatman filter paper. After the evaporation of solvent, the residue is agitated with ether petroleum. We let it decanted. We collected the phase of ether petroleum. The operation is repeated until the ether petroleum is colorless. The extracts of the ether petroleum are gathered. After the evaporation of solvent, the residue is agitated with diethyl ether, and extracted five times with sodium carbonate (1 M). We collected the aqueous phase and acidified with 12N sulfuric acid, then extracted by diethyl ether. After washing with water up to neutrality, drying by sodium sulfate, and evaporation of the diethyl ether, we get the fraction acid.

Turbidimetric study

The Precipitation of calcium oxalate at 37°C and pH 6.5 has been studied by the measurement of turbidity at 620 nm. A spectrophotometer UV/Vis (Beckman Du series 520) was employed to measure the turbidity of the formation of calcium oxalate. Pure chemicals, including calcium chloride dehydrate $CaCl_2$, $2H_2O$ (Riedel–De Haën), sodium oxalate $Na_2C_2O_4$ (Riedel–De Haenag) and sodium chloride NaCl (Fluka), are used for this study.

Study without inhibitor

We prepared the solutions $CaCl_2$, $2H_2O$ (10 mM) and $Na_2C_2O_4$ (4 mM) using solvent such as sodium chloride solution (0.15 M). A volume of 1.5 ml of calcium chloride dehydrate is transferred into the cell and blank reading was taken. Add 1.5 ml of sodium oxalate, to the previous volume, and the measurement is immediately started for a period of 10 min. For each experiment, six replicates were taken.

Study with inhibitor

The inhibitor (100%) is prepared taking 0.9 g of the fraction acid with 60 ml of sodium chloride (0.15 M). From this inhibitor, we prepared diluted inhibitory solutions (10%, 50%) using solvent such as sodium chloride solution (0.15 M). A mixture of 1 ml of calcium chloride dehydrates (10 mM) and 1 ml of inhibiting solution is versed in the cell. Take a blank reading, and then add a volume of 1 ml of sodium oxalate (4 mM) and the measurement is immediately started for a period of 10 min. The percentage of inhibition was calculated using the following formula:

$$I(\%) = \left[1 - \left(\frac{T_i}{T_c}\right)\right] \times 100$$

where T_i is turbidemetric slope with inhibitor, T_c turbidemetric slope without inhibitor.

Microscopic study

The photographs were taken using a microscope optic equipped with a digital camera and connected with a micro-computer. At time corresponds at the stage of growth and aggregation (t_1, t_2) a drop of the mixture of crystallizable solution, or inhibiting solution is placed in the cell of Malassez, which is immediately placed under the objective of the microscope.

Results

Turbidimetric study

The maximum values of the variation of absorbance, and the turbidimetric slopes relating to the curves of crystallization without and with inhibitors (10, 50, and 100%) are gathered in Table 1.

The variation of absorbance according to the time for tries without inhibitor is shown in Fig. 1.

The Figs. 2, 3 and 4 represent the variation of absorbance according to time for the tries with inhibitors 10, 50 and 100%.

The Fig. 5 shows the difference between the curve without and with inhibitor.

Table 1 The maximum values of the variation of absorbance, and the turbidimetric slopes relating to the curves of crystallization without and with inhibitors

CI (%)	TS	*I* (%)	Δ*D*	R^2	Cv (%)
0	0.517	00.00	0.399	0.856	4.79
10	0.148	71.37	0.206	0.924	4.00
50	0.101	80.46	0.181	0.986	4.19
100	0.079	84.71	0.140	0.940	6.84

CI concentration of inhibitor, *TS* turbidimetric slope, R^2 linear regression of the quadruplicate data, *Cv (%)* coefficient of variation, *ΔD* variation of absorbance, *I* percentage of inhibition

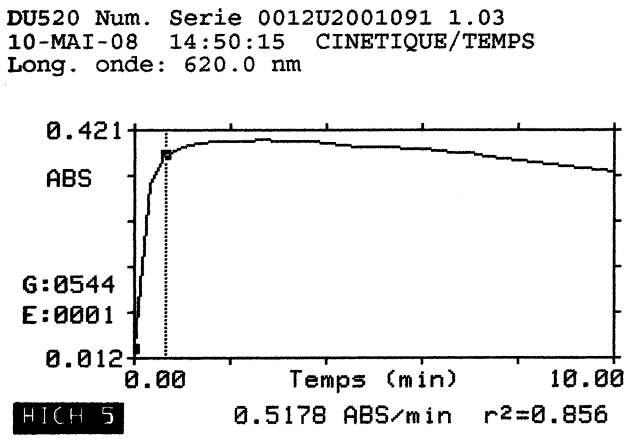

Fig. 1 Variation of absorbance according to time for tries without inhibitor

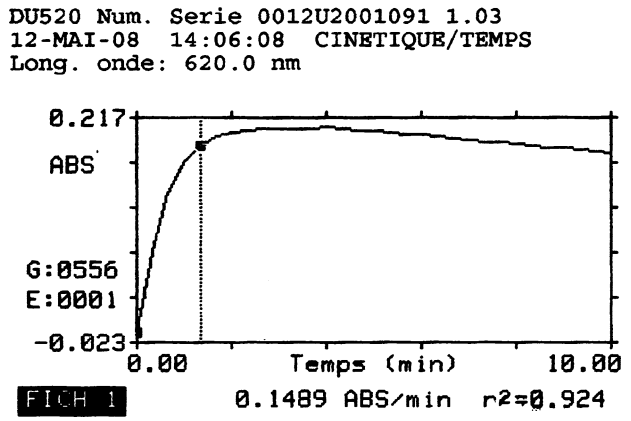

Fig. 2 Variation of absorbance according to time for tries with inhibitor (10%)

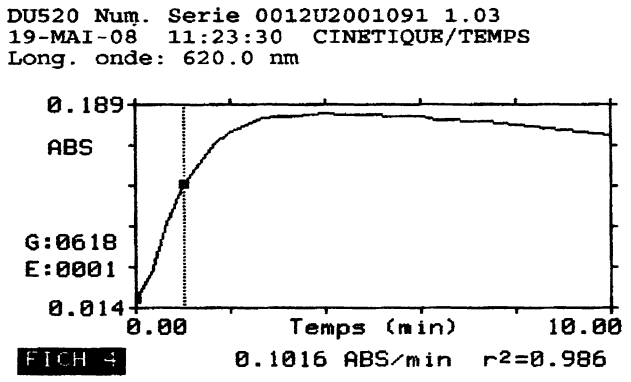

Fig. 3 Variation of absorbance according to time for tries with inhibitor (50%)

Microscopic study

The times of photographs 1, 2, 3, and 4 correspond, respectively, to the stage of growth and aggregation for tries without and with inhibitor, and are gathered in Table 2 and Fig. 6.

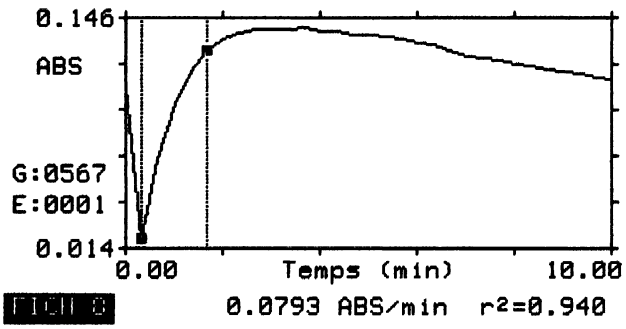

Fig. 4 Variation of absorbance according to time for tries with inhibitor (100%)

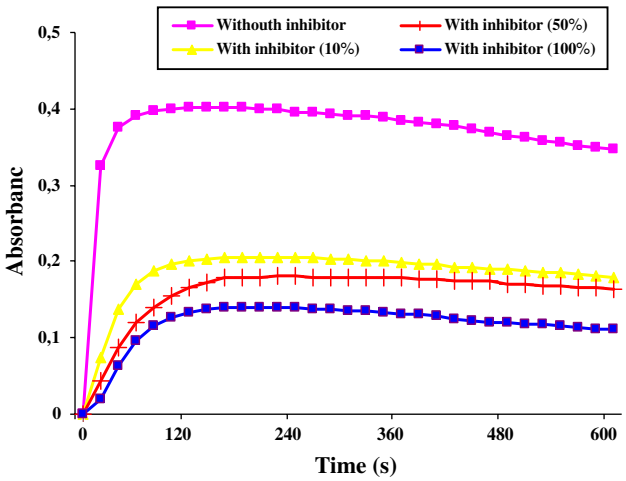

Fig. 5 Variation of absorbance according to time for tries with inhibitor

Table 2 times corresponding to the stage of crystallization for tries without and with inhibitor

NP	SC	CI (%)	TC (S)
1	Growth	00	20
2	Aggregation	00	60
3	Growth	10	50
4	Aggregation	10	120

NP number of photographs, *SC* stages of the crystallization, *CI* concentration of inhibitor, *TC* time corresponding to the stage of crystallization

Discussion

The richness of Mediterranean area by the medicinal plants enabled us to give the access to study the effect of these natural substances on the crystallization of calcium oxalate (CaOx) in vitro [14, 15]. Finally, majority of the work is directed toward studying the inhibiting effect of the extracts of medicinal plants on the lithiasis urinary [17, 18]. The values in Table 1 and the curves in Fig. 5 show that the variation of the absorbance decreases gradually with the

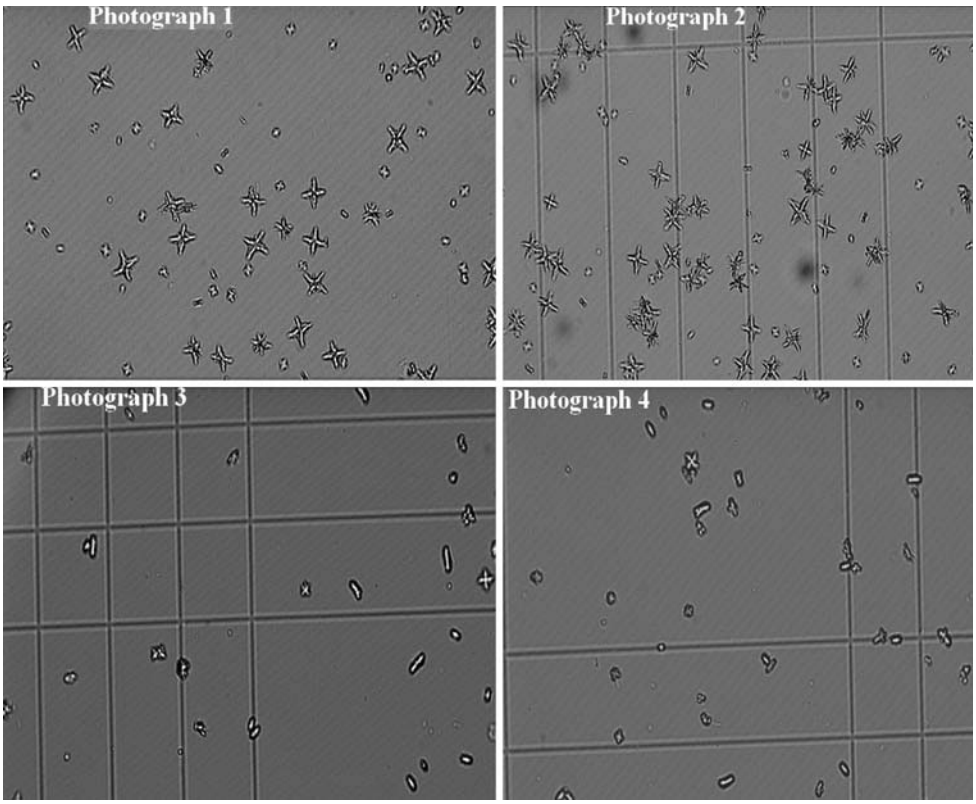

Fig. 6 Photographs of crystallization for tries without and with inhibitor

increase in the concentration of the inhibitor. We explain these results owing to the fact that the fraction acid of *Tamarix gallica* L has an inhibiting activity. The fraction acid of *Tamarix gallica* L rich in the acid compounds, which has acid functions, and is directly implied in the complexation of the calcium ions. Several studies are carried out using microscope to validate the results obtained by the turbidimetric model [16]. Then we observe that the number and the size of the crystals are important for photographs 1 and 2 corresponding to the stages of growth and aggregation for the crystallization without inhibitor of calcium oxalate. The comparison of photographs of the tries with inhibitor (10%) enabled us to give the number and size of the crystals for the inhibitor are not important, which explains why inhibition increases according to the concentration of inhibitor. The number and size of the crystals existing in the photograph 3 correspond to the stage of growth for the tries with inhibitor are reduced when compared with the photograph 1 that corresponds at the stage of growth for the tries without inhibitor, which explains that the acid fraction of *Tamarix gallica* L produces a significant quantity of inhibition on the growth of the crystals. Many works are shown that the extracts of the medicinal plants are reacted at the stage of growth [12, 19]. In conclusion, the various results obtained in our work (Turbidimetric slope, absorbance and percentage of inhibition) show that the acid fraction of *Tamarix gallica* L has an important inhibiting effect in vitro due to the effect of the functions of acid. On the other hand, the mechanism of action of the inhibitors being related to its chemical composition. The photographs obtained by microscope, show clearly that the acid compounds manifests at the stage of growth.

References

1. Saïdana D, Mahjoub MA, Boussaada O, Chriaa J, Chéraif I, Daami M et al (2008) Chemical composition and antimicrobial activity of volatile compounds of *Tamarix boveana* (Tamaricaceae). Microbiol Res 163(4):445–455. doi:10.1016/j.micres.2006.07.009
2. Sultanova N, Makhmoor T, Abilov ZA, Parween Z, Omurkamzinova VB, Rahman A et al (2001) Antioxidant and antimicrobial activities of *Tamarix ramosissima*. J Ethnopharmacol 78:201–205. doi:10.1016/S0378-8741(01)00354-3
3. Mahmoud A, Nawwar M, Sahar A, Hussein M (1994) Gall polyphenolics of *Tamarix aphylla*. Phytochemistry 36(4):1035–1037. doi:10.1016/S0031-9422(00)90486-2
4. Djurdjević L, Mitrović M, Avlović P, Gajić G, Ostić O (2006) Phenolic acids as bioindicators of fly ash deposit revegetation. Arch Environ Contam Toxicol 50(4):488–495. doi:10.1007/s00244-005-0071-2
5. Rieu P (2005) Lithiases d'infection. Ann Urol (Paris) 39:16–29. doi:10.1016/j.anuro.2005.01.001
6. Daudon M (2005) Épidémiologie actuelle de la lithiase rénale en France. Ann Urol (Paris) 39:209–231. doi:10.1016/j.anuro.2005.09.007

7. Emel A, Mualla O (2007) Inhibition of calcium oxalate monohydrate crystal growth using polyelectrolytes. J Cryst Growth 307:137–144. doi:10.1016/j.jcrysgro.2007.06.014
8. Doddametikurke RB, Chandra SB, Browning AJ, Cartledge JJ (2007) The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. Eau Ebu Update 5:126–136
9. Sakly R, Chaouch A, Elhani A, Najjar MF (2003) Effects of intraperitoneally administered vitamin E and selenium on calcium oxalate renal stone formation: experimental study in rat. Ann Urol (Paris) 37:47–50. doi:10.1016/S0003-4401(03)00007-X
10. Hess B, Jordi S, Zipperle L, Ettinger E, Giovanoli R (2000) Citrate determines calcium oxalate crystallization kinetics and crystal morphology—studies in the presence of Tamm-Horsfall protein of a healthy subject and a severely recurrent calcium stone former. Nephrol Dial Transplant 15:366–374. doi:10.1093/ndt/15.3.366
11. Saravanan DM, Amzad H, Salman Z, Gam LH, Zhari I (2006) The use of principal component analysis and self-organizing map to monitor inhibition of calcium oxalate crystal growth by *Orthosiphon stamineus* extract. Chemom Intell Lab Syst 81:21–28. doi:10.1016/j.chemolab.2005.09.007
12. Joshi VS, Parekh BB, Joshi MJ, Vaidyaa B (2005) Herbal extracts of *Tribulus terrestris* and *Bergenia ligulata* inhibit growth of calcium oxalate monohydrate crystals in vitro. J Cryst Growth 275:1403–1408. doi:10.1016/j.jcrysgro.2004.11.240
13. Ishwar D, Gupta SK, Pandey VN, Shoeb AA (2004) Inhibition and dissolution of calcium oxalate crystals *Berberis Vulgaris-Q* and other metabolites. J Cryst Growth 267:654–461. doi:10.1016/j.jcrysgro.2004.04.022
14. Marcio EB, Roberta L, Lucildes PM, Jivaldo RM, Nestor S, Mirian AB (2006) Effect of extract of *Phyllanthus niruri* on crystal deposition in experimental urolithiasis. Urol Res 34:351–357. doi:10.1007/s00240-006-0065-1
15. Atmani F, Khan SR (2000) Effects of an extract from *Herniaria hirsuta* on calcium oxalate crystallization in vitro. BJU Int 85:621–625. doi:10.1046/j.1464-410x.2000.00485.x
16. Hennequin C, Lalanne V, Estepa L, Drueke T, Daudon M, Lacour B (1997) Validation by image analysis of a turbidimetric method to study calcium oxalate crystallization. Clin Nephrol 48(5):292–299
17. Mayur DG, Siu PW (2006) Chinese herbal medicines and their efficacy in treating renal stones. Urol Res 34:365–372. doi:10.1007/s00240-006-0068-y
18. Abdelkhalek O, Touhami M, Mbarki M (2005) In vitro and in vivo study of effect of lemon juice on urinary lithogenesis. Arch Esp Urol 58:1087–1092
19. Suzuki K, Kawamura K, Tsugawa R (1999) Formation and growth inhibition of calcium oxalate by Takusha (*Alisma Rhizoma*). Scanning Microsc 13:183–189