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Inhibition Effect of Some Algerian Sahara Medicinal Plants on Calcium Oxalate Crystallization

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The inhibition effect of six medicinal plants used in Algerian Sahara traditional pharmacopoeia on the calcium oxalate crystallization is studied. The inhibiting effect of two concentrations (0.5 and 1 mg/mL) of each extract and compared without extract. The turbidimetric parameters were given such as the time of induction (Δt), turbidimetric slopes of crystalline growth and aggregation, the coefficient of variation CV % and the rate of inhibition I % for each tests. The results showed that the aqueous extract of *Cynodon dactylon* L presents an inhibiting effect of a rate of 20 % on the crystalline growth and 60 % on the phase of aggregation, whereas the aqueous extract of *Quercus prinus* has an inhibiting effect of crystalline aggregation with a rate of 40 %. The aqueous extract of *Punica granatum* L under the concentration 1 mg/mL, exerts an inhibiting effect of crystalline aggregation but does not influence the crystalline growth. The other extracts did not show any effect on calcium oxalate crystallization.

Key Words: Urolithiasis, Calcium oxalate, Turbidimetry, Algeria, Sahara, Medicinal plant.

INTRODUCTION

Urolithiasis, multiform pathology, can lead in certain case to the loss of renal function and even to a significant morbidity. Recurrence problem make the prevention very important. Unfortunately, in spite of the considerable progress noted in medical therapy, there is not a satisfactory drug to treat this disease. It is a process that results from a combination of factors in which the main phenomenon is the supersaturation of some compounds in urine that might crystallize forming solid concretions[1]. The formation of such concretions involves several physicochemical events, *e.g.,* nucleation, growth and aggregation, but the mechanism of these processes remains incompletely understood[2].

Calcium oxalate is one of the main constituents of urinary stones found in more than 70 % cases[3]. Crystallization of calcium oxalate is of particular interest not only from the theoretical point of view but also because of its biological importance[4]. In safe subjects, crystallization process being opposed by inhibitors. Many studies

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have been carried to characterize these inhibitors[5-9]. Numerous inhibitors have characterized inorganic pyrophosphate, magnesium, citrate, metallic ions and protein from human kidney[9-13].

Local medicinal pharmacopoeia constitutes an important source of remedies for primary health care[14]. However, many plants have been studied for their beneficial effect in treating kidney stones among them *Agropyron repens*[15], *Herniaria hirsuta*[15,16], *Costus spiralis*[17], *Aerva lanata*[18], *Phyllantus niruri*[19], *Tribulus terrestris* and *Bergenia ligulata*[20].

As part of our investigation into medicinal plants growing in Algerian Sahara[14,21-24], we reported here, *in vitro*, of the effect on calcium oxalate crystallization of aqueous extract of six medicinal plants (*Punica granatum* L, *Cynodon dactylon* L, *Matricaria chamomilla* L, *Quercus prinus*, *Juniperus phoenicea* L and *Atriplex halimus* L) used in Algerian Sahara traditional pharmacopoeia.

EXPERIMENTAL

An ethnopharmacological survey of medicinal plants used by local population in South West of Algeria, for the treatment of the urinary affections were conducted by the intermediate of a questionnaire comprising following information: name of the plant, disease treated, part of plant used and the mode of preparation. This investigation was then refined and supplemented by interviews of herbalists in the Souks and old peoples who hold a significant knowledge. The analysis of 149 cards enabled us to identify and classify 11 plants belonging to 9 botanical families, six plants were selected by their frequency of local use for eleven plants listed by the ethnopharmacological survey[23](Table-1).

TABLE-1
MEDICINAL PLANTS USED IN SOUTH WEST OF ALGERIA FOR THE TREATMENT OF THE URINARY AFFECTIONS

Voucher No	Species	Botanical family	Local name	Parts used	Form use	Citation freq. (%)
CA 99/30	*Ammi visnaga* Lamk	Apiaceae	Nokha	Fruit	Decoction	04.70
CA 01/09	*Matricaria chamomilla* L	Asteraceae	Babounge	Aerial parts	Decoction	07.08
CA 99/70	*Centaurea benedicta* L	Asteraceae	Guernina	Aerial parts	Decoction	01.34
CA 99/68	*Scorzenera undulata* Vahl	Asteraceae	Guiz	Root	Infusion	01.34
CA 99/12	*Atriplex halimus* L	Chenopodiaceae	Guetaf	Leaf	Decoction	14.75
CA 99/08	*Juniperus phoenicea* L	Cupressaceae	Araâr	Leaf	Infusion	05.37
CA 05/01	*Mercurialis annua* L	Euphorbiaceae	Morkeba	Aerial parts	Decoction	0.67
CA 99/65	*Quercus prinus*	Fagaceae	Bellote	Fruit	Decoction	21.47
CA 05/00	*Hyparrhenia hirta* L	Poaceae	Saybous	Root	Decoction	03.35
CA 00/05	*Cynodon dactylon* L	Poaceae	Nedjem	Aerial parts	Decoction	24.46
CA 99/64	*Punica granatum* L	Punicaceae	Rommane	Fruit	Decoction	15.43

Preparation of extracts: The whole plants were collected in March 2005 from Bechar and El Bayadh region (Western South of Algeria). The botanical identification and voucher specimens are conserved at the phytochemical herbarium of Phyto-

chemical and Organic Synthesis Laboratory at Bechar University[24]. Plant materials were air-dried and then powdered. A phytochemical screening was performed for identification of composition in various types of natural substances. Samples of plant materials were extracted with Soxhlet extractor and hydrous methanol and lyophilized. The powders obtained were used to prepare solution at different concentrations (0.5 and 1 mg/mL).

Crystallization study: The completed works made on the study of crystallization kinetics of calcium oxalate *in vitro* made it possible to specify the thermodynamic and kinetic conditions of this process[25]. The model selected in this study followed up by turbidimetry of crystallization kinetics. We followed the kinetics of crystallization by a standard UV/visible spectrophotometer H λLIOS γ thermostated using a Marie bath maintaining a circulation of the solution to constant temperature (37 °C). The prepared solutions are maintained with 37°C under agitation constant (500 rpm) controlled by a magnetic stirrer Mini-Mr type.

Study in absence of plant extract: Two solutions $CaCl_2.2H_2O$ (40 mM) and $Na_2C_2O_4$ (4 mM) were prepared using sodium chloride (0.15 M) and made all necessary dilutions. The two solutions of calcium chloride and sodium oxalate of equal volume (70 mL) are maintained at a temperature of 37 °C. After 0.5 min, a sample of the calcium chloride solution is taken in a cell of 1.5 mL and 1 cm of optical way installed in the spectrophotometer. The absorbance is read against distilled water at 620 nm. As soon as the absorbance is stable, the volume of sodium oxalate is added and measurements were noted immediately[26]. The absorbance is measured every 30 s for a period of 20 min. The curve of variation of the optical density with time (t) is obtained.

Study in presence of plants extract: In the presence of plants extracts, the methodology of studying the kinetics of calcium oxalate crystallization is the same. Equal volumes of calcium chloride, sodium oxalate and plant extract were mixed and the measurement is immediately started. The rate of inhibition was calculated by the relation:

$$I(\%) = \left(1 - \frac{S_{pe}}{S_{ae}}\right) \times 100$$

where S_{pe} and S_{ae} are the turbidimetric slope respectively in presence and in absence of plant extract.

RESULTS AND DISCUSSION

In Algeria, most patients use medicinal plants as an alternative remedy to treat various types of illness including urolithiasis. In this regard, six medicinal plants are selected from eleven listed by the ethnopharmacological investigation to studying there inhibiting effect on calcium oxalate lithiasis. *Cynodon dactylon* L, *Quercus prinus* L and *Punica granatum* L represent the most popular plant used by local population (Table-1).

As indicated in Table-2, the phytochemical screening shows that *Punica granatum* L has an average presence of each type of natural products. *Cynodon dactylon* L show a strong presence of alkaloids and medium to weak presence of the other metabolite types. *Matricaria chamomilla* L and *Atriplex halimus* L has a medium to weak presence of all types of natural products. *Quercus Prinus* and *Juniperus phoenicea* L have a strong presence of tannins but average to weak presence of the other constituents.

TABLE-2
PHYTOCHEMICAL SCREENING RESULTS OF SELECTED PLANTS

Plant species	Phytochemical constituents of plants			
	Alkaloids	Saponins	Tannins	Flavonoids
Punica granatum L	++	++	++	++
Cynodon dactylon L	+++	++	++	+
Matricaria chamomilla L	–	–	++	+
Quercus prinus	+	++	+++	+
Juniperus phoenicea L	+	+	+++	+
Atriplex halimus L	–	++	+	–

(–): Absence (negative test), (+): Weak presence, (++): Average presence, (+++): Strong presence.

In present crystallization study, the turbidimetric model is chosen which has sensitivity and a rather significant reliability[25,26]. To test the reliability and the reproductiveness of present model, the coefficient of correlation (R) and the coefficient of variation (CV) is calculated.

In all present tests, the values of the coefficient of correlation (R) are found to be higher than 0.99 and the values of the coefficient of variation are lower has 10 %, which reassures the reliability and the reproductiveness of present model (Figs. 1-7).

The time of induction Δt is almost the same one in all the tests, except that with *Quercus prinus* extract which is of 1.5 min, suggesting that nucleation lasts more in the presence of this extract than without *Quercus prinus* extract.

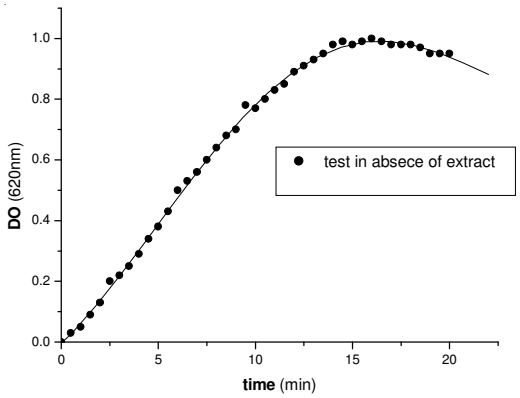

Fig. 1. Evolution of calcium oxalate crystallization in absence of plant extract

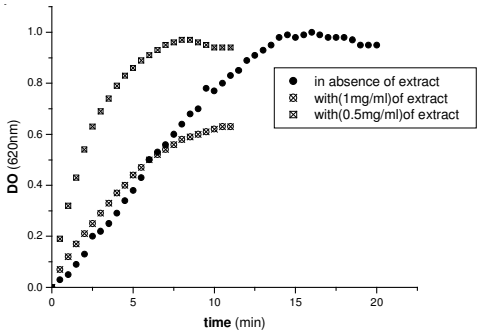

Fig. 2. Evolution of calcium oxalate crystallization in the presence of *Punica granatum* L extract

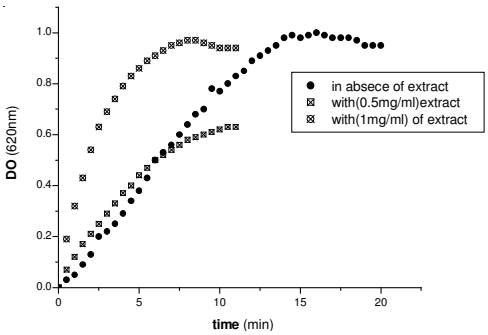

Fig. 3. Evolution of calcium oxalate crystallization in the presence of *Cynodon dactylon* L extract

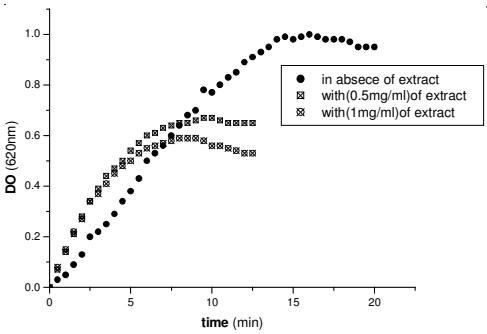

Fig. 4. Evolution of calcium oxalate crystallization in the presence *Matricaria chamomilla* L extract

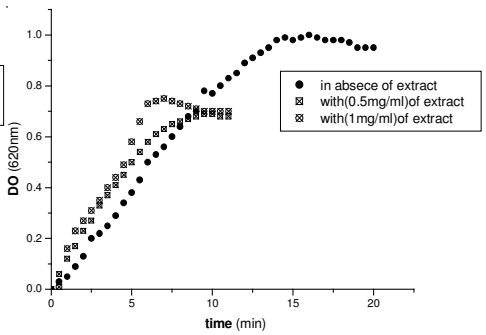

Fig. 5. Evolution of calcium oxalate crystallization in the presence of *Quercus prinus* extract

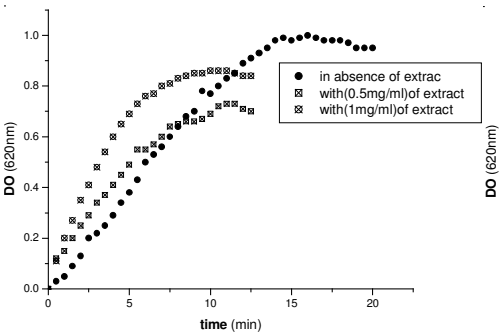

Fig. 6. Evolution of calcium oxalate crystallization in the presence of *Juniperus phoenicea* L extract

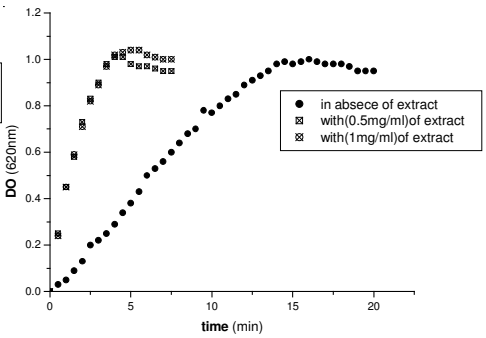

Fig. 7. Evolution of calcium oxalate crystallization in the presence of *Atriplex halimus* L extract

The results showed that the *Cynodon dactylon* L extract presents an inhibiting effect of a rate of 19.3 % on the crystalline growth and 60 % on the phase of aggregation (Tables 3 and 4). This beneficial effect confirms the results of the ethno-pharmacological survey. An inhibiting effect of the crystalline aggregation of 40 %

was marked by *Quercus prinus* extract. The extract of *Punica granatum* L 1 mg/mL, exert an inhibiting effect of the crystalline aggregation of 20 % but do not influence the crystalline growth (Table-4).

TABLE-3
GROWTH PARAMETERS IN ABSENCE AND IN PRESENCE OF PLANTS EXTRACTS

	Extract concentration (mg/mL)	Δt (mn)	Growth slope (A mn^{-1})	R	CV (%)	I (%)
In absence of plant extract	–	0.5-5.0	0.082	0.995	4.3	–
Punica granatum L	1.0	1.0-5.0	0.132	0.998	1.3	–
	0.5	0.3-5.0	0.084	0.998	4.1	–
Cynodon dactylon L	1.0	1.0-4.0	0.066	0.996	7.2	19.3
	0.5	1.0-4.0	0.068	0.997	8.3	17
Matricaria chamomilla L	1.0	0.5-3.0	0.116	0.994	7.8	–
	0.5	0.5-4.0	0.114	0.993	5.4	–
Quercus prinus L	1.0	1.5-4.5	0.086	0.999	9.2	–
	0.5	0.5-3.5	0.103	0.998	8.6	–
Juniperus phoenicea L	1.0	1.0-5.5	0.117	0.994	3.4	–
	0.5	1.0-5.0	0.085	0.995	2.6	–
Atriplex halimus L	1.0	1.0-3.5	0.208	0.992	2.7	–
	0.5	1.0-3.0	0.225	0.991	2.9	–

TABLE-4
AGGREGATION PARAMETERS IN ABSENCE AND IN PRESENCE OF PLANTS EXTRACTS

	Extract concentration (mg/mL)	Δt (mn)	Aggregation slope (A mn^{-1})	R	CV (%)	I (%)
In absence of plant extract	–	16.5-17.5	0.010	0.922	–	–
Punica granatum L	1.0	8.0-9.5	0.008	0.954	4.2	20
	0.5	10-10.5	00	–	–	–
Cynodon dactylon L	1.0	9.5-12.5	0.004	0.960	4.2	60
	0.5	10.5-12.5	0.004	0.974	4.8	60
Matricaria chamomilla L	1.0	8.5-10.5	0.012	0.977	4.2	–
	0.5	9.5-10.5	0.010	0.955	5.1	–
Quercus prinus L	1.0	8.0-8.5	0.006	0.940	5.2	40
	0.5	9.0-12.0	0.020	0.942	5.2	–
Juniperus phoenicea L	1.0	11.0-13.0	0.010	0.924	6.1	–
	0.5	11.5-13.0	0.016	0.982	4.2	–
Atriplex halimus L	1.0	5.5-7.0	0.060	0.951	5.2	–
	0.5	5.5-7.0	0.080	0.970	4.6	–

Other extracts did not show any effect on calcium oxalate crystallization. These plants of traditional medicine may have another effect on the urolithiasis (diuretic effect or biochemical change of the urines parameters).

According to present result, it is noted that the extracts of the plants having alkaloids or tannins, reveal an outstanding effect on calcium oxalate crystallization, which makes the importance of a phytochemical study of these extract to separate and identify the substance responsible of the inhibiting effect.

Conclusion

Calcium oxalate urolithiasis can be dangerous for the renal function and the use of inhibitors to prevent, slow down or reduce crystallization or aggregation phases might be very helpful. The, *in vitro*, inhibition of calcium oxalate crystallization by some plants used in Algerian Sahara traditional was studied. The *Cynodon dactylon* L extract produced maximum inhibition effect of calcium oxalate crystals growth, followed by *Quercus prinus* primes and *Punica granatum* L extract.

Finally, additional work will be required in order to study the phytochemical composition of the active extract and the effect of pure natural compounds.

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