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ROLE *GLOBULARIA* IN THE INHIBITION OF CALCIUM OXALATE CRYSTALLIZATION IN VITRO STUDY

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ABSTRACT

Plant extracts are known to modulate calcium oxalate (CaOx) crystallization. One of these is *Globularia*, the root and flower. Medicinals plants has been demonstrated to be an efficient inhibitor of CaOx crystal growth; however, its inhibitory activity against other events in CaOx crystallization has not been fully investigated. To assess the potential of *Globularia* as an effective inhibitor, its effects on CaOx crystal nucleation and aggregation were evaluated. Nucleation and aggregation of CaOx crystals were studied by artificial urine is prepared by mixing and stirring two equal volumes of 50 ml of solutions A and B at constant temperature (37°C). In the nucleation assay, crystallization was induced by mixing calcium chloride and sodium oxalate, The addition of extracts *Globularia* flower and root , acts on the phase of growth crystallisation ; the rate of inhibition capacity in concentration 100 % is 96.09% , and 96.77% for concentration 75% respectively, On note that these inhibitors developed an inhibition important on the low concentration. *Globularia* is an efficient inhibitor of crystal nucleation and aggregation. Its presence in the kidneys and urine may protect subjects against CaOx crystallization and kidney stone formation.

Key words: Crystallization, Inhibitors, Globularia, lithiasis, Artificial urine

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1. INTRODUCTION

Today, researchers have focused on the drug discovery from medicinal plants [1]. It has been estimated that at least one third of all medicinal product have plant origin [2]. Medicinal plants are regarded as an acceptable, cheap, easily available and safe source of ac-tive compounds for pharmaceutical [3]. The therapeutic effects of medicinal plants on kidney and urinary tract disorders have been variously studied and their efficacy has been demonstrated [4].

Globularia alypum L. is an aromatic and medicinal plant belonging to the Glob-ulariaceae family, found throughout the Mediterranean area and largely used and considered for its therapeu- tic virtues [5].The name "Turbith" in French *Globulariaalypum*, known locally under the name of "Tasselgha" or "AinLarneb" belongs to the family of Globulariaceae. It is a perennial wild shrub and one of the plant remedies most used in traditional Algerian medicine in the treatment of a large number of diseases (hypertension, cardiac disorders, renal colic and various cancerous lesions), stomach, urinary lithiasis, rheumatism, gout, typhoid fever, intermittent fever and diabetes colon, liver and esophagus[6]. Urine oxalate excretion is low (normal value <0.5 mmol/day in humans) but its affinity for calcium ions make it a major promoter of calcium oxalate crystals and kidney stone formation [7]. CaOx crystallization in the kidney tubules is caused by elevated urinary oxalate levels occurring in, for example, genetic disorders, such as primary hyperoxaluria or genetic forms of renal tubular acidosis [8]. Although patients with(IH) idiopathic hyperoxaluria are generally thought to have milder eleva-tions in UOx excretion compared to patients with (EH) enteric hyperoxaluria severe hyperoxaluria and recurrent nephrolithiasis may occur in either type of hyperoxaluria [9].*Garcinia cambogia* extract containing 60% hydroxycitric acid (HCA), is a popular weight-loss supplement and sold at most health supplement and drug stores. Chung *et al* showed that HCA induces dissolution of the calcium oxalate crystal *in vitro*, suggesting that *Garcinia cambogia* has the potential as a novel treatment for calcium oxalate kidney stone [10]. Plants provide food, raw materials for medicine and various other requirements for the very existence of life from the origin of human beings [11]. This work was carried out to investigate the inhibitory effects of plant extract medicinal on crystallization of calcium oxalate in order to clarify their therapeutic potential. Stone formation has different phases of calcium oxalate lithogenic: nucleation, crystal growth, aggregation and particle retention. So, the work can take stock of the steps therapeutic to adopt according to the type of injury to the urinary tract and the diversity of plants medicinal responded in kind. Many of the concentrations of plants are specialties human assets. Most of theme used in our work have proven effective in tests on the

inhibition of calcium oxalate major component in urinary stones. In this study extract *Globularia alypum L* is presented to inhibit the formation of crystals of calcium oxalate.

2. MATERIALS AND METHODS

Plant extract

Extracts plant of wild Algerian plant is studie (*Globularia alypum L*). The flowers and roots of the plant were harvested from natural resources in 2006 and 2007, mainly when plants were at flowering stage. Voucher specimens were deposited in the laboratory of Plant Physiology, Oran University. In some cases, a species was sampled at different times. Infusions were prepared daily just before handling by suspending a weighed amount of dry plant material in boiling tap water at room temperature. The suspension was stored at room temperature for 15 min and then filtered through filter paper.

Simulation of the sedimentary crystal formation and synthetic urines

The model of crystallization calcium oxalate adopted in our work is a simple model that is used to prepare artificial urine, mixing two solutions: A solution of anhydrous sodium oxalate ($Na_2C_2O_4$) MERCK brandand type for analysis. B: solution of calcium chloride dehydrate (Ca $Cl_{22}H_2O$) MERCK brand and type for analysis.equal volume, thermo stated at 37 °C using a water bath. Stirring is provided by means of a magnetic stirrer whose speed of rotation is constant in all experiments. Sodium chloride (NaCl) MERCK brand and type extra pure, which reduces the initial solutions at an ionic strength of 0.15 is the concentration of 9g/l of solution. To be able to determine the effect of the inhibitors at each phase of crystallization (nucleation, crystal growth and aggregation) we have performed a study of urinary sediment using a Zeiss microscope with polarized light equipped with a Winder M 476079 camera. Pictures (shots) were taken first in the control setting, before the addition of the inhibitor, in the three phases of crystallization. The other shots were taken after the intake of the inhibitors in order to compare the number and size of the crystals. Percentage of inhibition of crystallization (I%) was calculated as previously described and based on the formula, I% = [(TSI-TAI) / TSI] x 100 [12] in which TSI and TAI represent numbers of calcium oxalate monohydrate crystals in absence and presence of inhibitors (plant extracts) Nucleation, growth and aggregation of crystals were visually assessed under the microscope. The effects of inhibition were followed using scanning electron microscopy Hitachi TM1000, and optical microscopy. Supersaturated solutions induce crystallization by nucleation and subsequent crystal growth

The growth of crystals without and with extracts plants was monitored at regular time intervals. The mechanisms for the formation of calcium phosphate urinary stones are still not understood.

3. RESULTS AND DISCUSSION

The number of crystals and the percent inhibition (I%) in presence extract of flowers *Globuaria* are shown in table 1 and figure 1,

Table 1. Summary of inhibition results with *Globularia alypum* (flowers)

Time (mn)	5	10	15	20	25	30
% Inhibition [25%]	21.20	54.40	47.01	58.31	22.13	75.53
% Inhibition [50%]	76.57	86.12	88.37	86.43	85.71	90.45
% Inhibition [75%]	89.00	88.29	88.37	89.85	92.66	89.73
% Inhibition [100%]	93.58	93.96	94.03	93.03	93.69	96.09

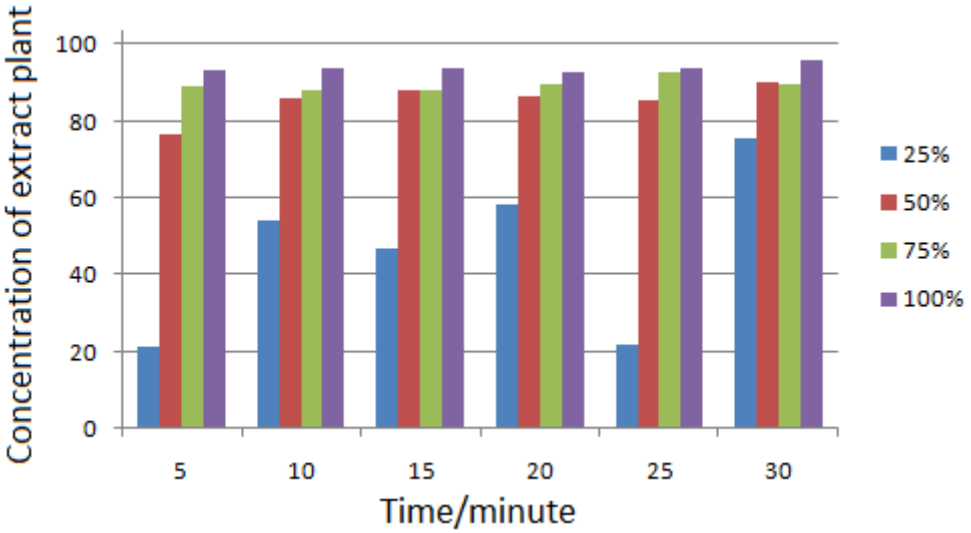

Fig.1. Inhibition of calcium oxalate crystals in Presence *Globularia alypum* (flowers)

The number of crystals and the percent inhibition (I%) in presence extract of root Globuaria are shown in table 2 and figure 2,

Table 2. Summary of Inhibition Results with *Globularia alypum* (Roots)

Time (mn)	5	10	15	20	25	30
% Inhibition [25%]	81.41	85.52	88.94	85.94	84.81	88.78
% Inhibition [50%]	89.52	88.66	93.29	93.39	89.31	95.58
% Inhibition [75%]	89.79	86.36	92.67	91.68	92.14	96.77
% Inhibition [100%]	93.71	95.77	96.12	96.21	95.88	95.22

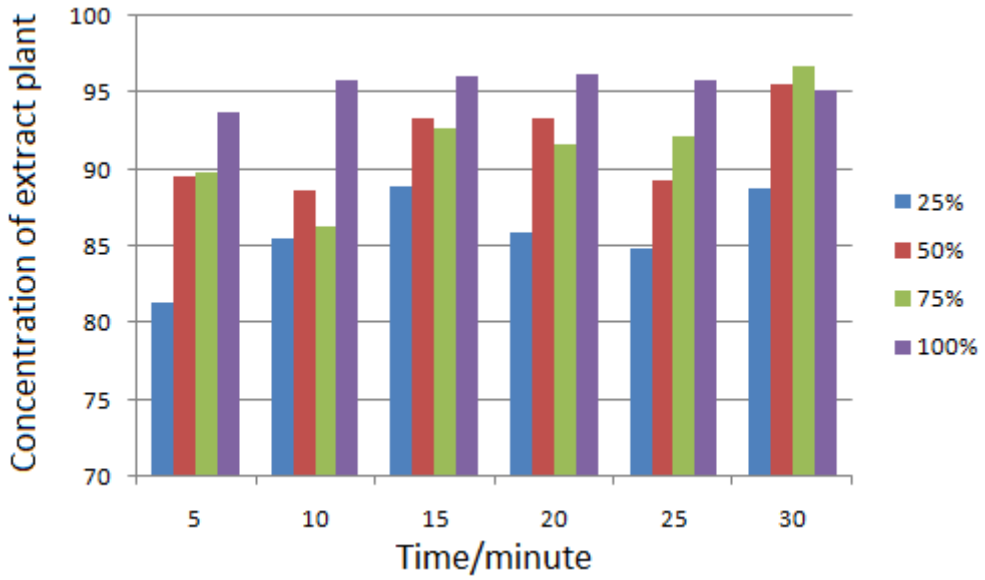

Fig.2. Inhibition of calcium oxalate crystals in Presence *Globularia alypum* (Root)

The present work was carried out to study inhibitory effects of medicinal plant extracts on crystallization of calcium oxalate in order to clarify their therapeutic potential. The formation of the calculations involves different phases of calcium oxalate lithogen: nucleation, crystal growth, aggregation and particle retention. So, the work has made it possible to take stock of the therapeutic approaches to be adopted depending on the type of involvement of the urinary tract and on the diversity of medicinal plants that are very much responded to in nature. Many of these extract plants are active in human specialties. Most of the plant extracts used in our work have proved their effectiveness in tests carried out on the inhibition of calcium oxalate major component in urinary stones. It should be noted that, in general, crystallization occurs rapidly at all the different concentrations, with a gradual decrease in the number of crystals as a function of the doses and the time of the extract plant. The leaf extract effect of *Globularia* flower on calcium oxalate crystals is important. After 30 minutes the inhibition rate reached a maximum value of 96.09% for a pure extract. The majority of concentrations reaches significant inhibition rates and it is co-confirmed with published research work:

The anti-hypercalciuria and anti-urolithiasis effects of this plant attracted considerable attention toward pomegranate for use in the prevention of renal calculus formation. Its therapeutically beneficial phytochemicals are responsible for muscle relaxation in the urinary and biliary tract; consequently, stones can be easily removed from the kidney [13]. In a synthetic urine system for calcium oxalate crystallization, the hydro-alcoholic extract of seeds showed inhibitory activity on nucleation and aggregation of calcium oxalate monohydrate crystals[14].Showed

that phenolic compounds of ethanol extract from Bergenia ciliata had suitable ability to dissolve calcium oxalate and calcium phosphate stones in vitro (67.74% and 36.95%, respectively) compared to Cystone that commonly used (67.74% and 48.48%). [15]. who tested the effect of the same plants on the dissolution of cystine and oxalo-calcic stones (without agitation). After eight weeks of contact, the aqueous extract of the stigma of Z. mays shows a dissolution rate of 72 % for cystine stones and 55% for calcium oxalate stones. The extracts of A. visnaga solubilize 67 % of cystine stones and 47 % of calcium oxalate stones. O. ficus-indica extract can reach a DR of 64 % and 45 % of the total mass of cystine stones and calcium oxalate stones, respectively. The DR of H. hirsute extracts gave 61 % for cystine stones, whereas it is 50 % when using calcium oxalate stones [16].

4. CONCLUSION

The result of our work does not allow us to confirm the use of these plants in the field of urolithiasis. However, all our extracts gave an inhibitory activity with the aqueous decoction. These activities may help to strengthen the body in depressed situations. In addition, a detailed study of the toxicity would be necessary to achieve determine doses. The plant extracts are generally used in the rough. This is why we utilize high doses for our tests. To overcome this, it is best to isolate the active principles of different plant extracts and present them in an acceptable dosage form. Awareness is still needed on the appropriate use of traditional medicine and the importance of the environment. Through this work, we hope to bring our modest contribution to the promotion of traditional medicine to reach place at the disposal of the population-based drugs effective medicinal plants and accessible.

5. REFERENCES

1-Saki K, Bahmani M, Rafieian-Kopaei M. The effect of most important medicinal plants on two important psychiatric disorders (anxiety and depression)-a review. Asian Pac J Trop Med. **2014**; 7:34-42. doi: 10.1016/s1995-7645(14)60201-7.

2-. Asadbeigi M, Mohammadi T, Rafieian-Kopaei M, Saki K, Bahmani M, Delfan B. Traditional effects of medicinal plants in the treatment of respiratory diseases and disorders: an ethnobotanical study in the Urmia. Asian Pac J Trop Med. **2014**;7: S364-8. doi: 10.1016/s1995-7645(14)60259-5.

3. Gupta A, Chaphalkar SR. Anti-inflammatory and immunosuppressive activities of some flavonoids from medicinal plants. J HerbMed Pharmacol. **2016**; 5:120-4.

4- Sarrafchi A, Rafieian-Kopaei M. The role of community in discovery of new drugs from herbal medicines. J Herbmed Pharmacol. **2014**; 3:69-70

5-B. Fehri, J.M. Aiache, KK.M. Ahmed, Pharmacogn. Rev. 4, 138 (**2012**).

6- Boussoualim,N., Trabsa,H., Krache,I.,Arrar,L.,Baghiani,A.J. App. Pharm. Sci. **2016**, 6, 159

7- Khan SR, et al. Kidney stones. *Nat Rev Dis Primers*. **2016**; 2:16008.

View this article via: PubMed Google Scholar

8- S. R. Mulay, H.-J. Anders, *N. Engl. J. Med.* 2016, 374, 2465.

9- Khan A (**2018** May) Prevalence, pathophysiological mechanisms and factors affecting urolithiasis. Int Urol Nephrol 50(5):799–806

10-*Chung, J., Granja, I., Taylor, M. G., Mpourmpakis, G., Asplin, J. R. & Rimer, J. D. (**2016**), "Molecular modifiers reveal a mechanism of pathological crystal growth inhibition", Nature, Vol. 536 No. 7617, pp. 446–50.*Google Scholar

11- Sumayya sikandari and Prathima Mathad(**2015**).In vitro antiurolithiatic activity of Butea monosperma Lam. and Nigella sativa Linn.seeds.Ukaaz-Annals of Phytomedicine,4(1):105-107.

12- Hennquine C, Lalame V, Daudon M, Lacour B, et Drueket T **1993**. Anew approche to studing inhibitors of calcim oxalate crystal grawth. Urol Res,21:101-108.

13- Rathod, N.; Biswas, D.; Chitme, H.; Ratna, S.; Muchandi, I.; Chandra, R. Anti-urolithiatic effects of Punica granatum in male rats. J. Ethnopharmacol. **2012**, 140, 234–238.

14- Saha, S.; Verma, R.J. Evaluation of hydro-alcoholic extract of Dolichos biflorus seeds on inhibition of calcium oxalate crystallization. J. Herb. Med. **2015**, 5, 41–47.

15-Byahatti VV, Pai KV, D'Souza MG. Effect of phenolic compounds from *Bergenia ciliata* (Haw.) sternb. leaves on experimental kidney stones. Anc Sci Life **2010**; 30:14-7.

16- Kenza Ammor, Fatima Mahjoubi, Dalila Bousta, Radouane Elhabbani and Abdellah Chaqroun. In vitro litholytic activity of extracts and phenolic fractions of some medicinal plants on urinary stones. Mediterranean Journal of Chemistry **2020,** 9(6), 468-477

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