

Beneficial effect of the *Urtica dioica* aqueous extract on the crystallization of calcium oxalate in urine

Razika Laoufi¹, Karima Yahiaoui², Ouahiba Bouchenak², Narimen Benhabiles², Samia Lefkir², Karim Arab²

¹Department of Biology, Laboratory of Technologies Soft and Valorization of Biological Materials and Biodiversity, Faculty of Science, University M'Hamed Bougara of Boumerdes, Algeria, ²Department of Biology, Laboratory of Valorization and Conservation of Biological Resources, Faculty of Science, University M'Hamed Bougara of Boumerdes, Algeria

Abstract

Background: *Urtica dioica*, belongs to the family of *Urticaceae*, is widely spread in the world. It is known as one of the most famous medical plants and the more useful. The aim of this research is to quantify the polyphenolic aqueous extract of parts of the *U. dioica* in the region of Boumerdes and evaluate the antioxidant activity and antilithiasis activity. **Materials and Methods:** The antioxidant activity of the leaves extract was evaluated by 1,1-diphenyl-2-picrylhydrazyl's method. The antilithiasis activity followed by an observation on electronic microscope to be scanned. **Results and Discussion:** Phytochemical screening revealed worth parts in secondary metabolites (polyphenols). The extraction yield of polyphenols is from 2.8% for 20 g of vegetal powder, with a concentration of 126.28 mg EAG/g powders for all the parts. By coincidence, the obtained results show that the aqueous polyphenolic extract has an important antioxidant power, and of an antilithiasic effect, in particular, the crystallization of calcium oxalate in the urine. In fact, the observation in scanning electron microscope found that the augmentation of the extract concentration favored the reduction in the size of the crystals from 17 μm to 5 μm . **Conclusion:** These results indicate that aqueous extract from the leaves of *U. dioica* L. possesses significant antioxidant and antilithiasic potential, deserves to be valued by its integration into therapeutic applications.

Key words: Antilithiasic, antioxidant activity, polyphenols, scanning microscopy, *Urtica dioica*

INTRODUCTION

Urinary tract lithiasis becomes a major health-care problem in all countries in the world, it leads to prolonged immobilization and loss of renal function in some cases. Many drugs have been used to treat curb kidney stones, but the serious adverse side effects as well as the fact that they are not tolerated by all patients hinder their long-term use. This situation has given a place for other options about the treatment including herbal medicines. In fact, some of plants extract used for a long-term exert their antilithogenic effects, by changing the ionic composition of the urine such as calcium ions and magnesium ions. A lot of plants extract are rich in saponin that can disrupt mucoprotein suspensions favoring the crystallization.^[1] It is the case of *Urtica dioica* the family of *Urticacées*, well known by its therapeutic virtues, also indicated

as a cure for diabetes,^[2] rheumatoid arthritis, hypertension and allergic rhinitis,^[3,4] Cardio diseases,^[5] and healing.^[6] The known traditional indications of this plant are essentially diuretics, and the scientific knowledge of the use of this plant is still limited.^[7,8] Therefore, in the absence of extensive work and scientific studies on the medicinal potential and actual effect of nettle in the natural treatment of urinary lithiasis, particularly in the prevention of calcium oxalate stones, we proposed to determine the effect of this plant on oxalocalcic urinary supersaturation. The main objective of this study is to determine if a nettle based cure prevents the risk of

Address for correspondence:

Razika Laoufi, Laboratory of Technologies Soft and Valorization of Biological Materials and Biodiversity, Faculty of Science, University M'Hamed Bougara of Boumerdes, Algeria. E-mail: hibalaou@yahoo.fr

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crystallization of calcium oxalate in the urine, by testing the infusion of the plant to get as close as possible to the procedure used by the lithiasis patient.

MATERIALS AND METHODS

Collection and Authentication of the Medicinal Plant

U. dioica L. was collected in March 2014 in the Boumerdes region (Algeria) in the forest of Bouarbi and recognized by Dr. Abdekrim, taxonomist, Botany Department of the National Superior School of Agronomy (ENSA), Algeria.

Chemicals

Folin–Ciocalteu reagent, 2% sodium carbonate, gallic acid, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and sodium oxalate were used and supplied from the chemical company Sigma-Aldrich (Germany).

Vegetal material

It consists of polyphenolic aqueous extract of the *U. dioica* leaves. They were dried in the shade and then crushed to give a powder from which the polyphenolic extract has been prepared.

Phytochemical characterization

The phytochemical test carried out on the leaves of *U. dioica* aims to find the bioactive substances synthesized by this plant. It is made either on the powder or on the infused at 10%. The characterization methods used derive from those described by Harborne.^[9,10]

Evaluation of *in vitro* antioxidant activity

The evaluation of the antioxidant effect is realized by the scavenging of the free radical DPPH test, adopting the method described by Sánchez-Moreno *et al.*^[11] This study is based on trapping the stable free radical DPPH by a scavenging molecule, causing its discoloration.^[12]

The method is quick and convenient to be implemented. It is carried out at an ambient temperature, to eliminate any risk of thermal degradation of the tested molecules. For this, a volume of 25 µl of each methanolic extract at various concentrations (0.0125–1 mg/ml) is added to 1.95 ml of the methanol solution of DPPH (0.0024 g/l). In parallel, a negative control is prepared by mixing 25 µl of methanol with 1.95 ml of the DPPH methanol solution. The reading of the absorbance is made against a blank prepared for each concentration at 517 nm after 30 min incubation in the dark and at room temperature. The positive control is represented by a standard solution of an antioxidant

(ascorbic acid), whose absorbance is measured under the same conditions as the samples. The test is repeated 3 times for each concentration.

The results are expressed as percentage inhibition (I%) according to the following formula:

$$I\% = \frac{(A \text{ reference} - A \text{ test})}{A \text{ reference}} \times 100$$

Where:

A reference: The absorbance of the control

A test: The absorbance of the extract

IC₅₀ values are determined graphically by linear regression.

Antilithiasis activity

The plant is washed several times with distilled water to rid it of dust. Then, a stock solution of the plant, at 40 g/l in boiling distilled water, was prepared. After infusion for 10 min, the solution is filtered under vacuum and on a 0.2 µm membrane and then lyophilized.

Solutions in distilled water at different concentrations (0.0625–1 g/l) were prepared. The evaluation of the antilithiasis effect is carried out by adopting the method described by Hamilton.^[13] The urine of 24 h of the male subject with no history of stones were collected and kept in a plastic bottle, without addition of antibacterial agent. An aliquot of 4 ml of urine and 100 µl of infusion of the plant at different concentrations (C₅ = 1 g/l, C₄ = 0.5 g/l, C₃ = 0.25 g/l, C₂ = 0.125 g/l, and C₁ = 0.0625 g/l) have been placed in several tubes. In parallel, a tube without plant extract is left as a reference. All tubes are incubated at 37°C. The calcium oxalate crystallization is induced by the addition of 100 µl of sodium oxalate solution to 0.1 mol/l, previously kept at 37°C. Then, all tubes were incubated again at 37°C for 30 min. Reading the optical density, each sample is at 620 nm. Finally, the samples were filtered under vacuum and 0.2 µm membrane. The filters were observed by scanning electron microscope (SEM).

SEM observation

Observation at the SEM required a preliminary preparation of samples to overcome the insulating nature of the latter. At first, the samples consisting of pieces about 1 cm in size (filter paper) were glued with silver lacquer on a sample holder adaptable to the slide of the SEM. A sputtering metallization was then carried out to deposit a gold layer of about 20 nm on the assembly, which allows the evacuation of the electric charges during the observation. The SEM used is an LEO Gemini 982 with a field-effect gun that can obtain highly resolved images at low voltage (<5 kV), which has the advantage of avoiding damaging the samples.

Statistical Analysis

The results are statistically analyzed using Student's *t*-test. All values are expressed as mean \pm standard error at the mean. The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Analysis of *U. dioica* Leaf Extracts

Screening carried out on the leaves of *U. dioica* is summarized in Table 1. It should be noted that the leaves are very rich in total tannins, gallic tannins, flavonoids, mucilage saponins, and alkaloids. However, they are moderately rich in anthocyanins, coumarins, starch, and glucosides. In other side, there is a total absence of catechin tannins, iridoids, free quinones, and sennosides. Studies carried out on this species in different regions have shown molecular biodiversity as secondary metabolites directly related to an evolutionary adaptation to biotic (the species itself) and abiotic (middle) conditions. Indeed, in Kenya, Moses *et al.*^[14] noted a richness in saponins and the absence of free quinones; in Iraq, Ghaima *et al.*^[15] observed the presence of alkaloids, glycosides, and coumarins; in Russia, Krystofova *et al.*^[16] revealed the presence of flavonoids and tannins; and in Iraq, Ahmed *et al.*^[17] showed the richness of this species in tannins, saponosides, flavonoids, and alkaloids. Finally, Andersen and Wold^[18] mentioned the presence of mucilage in the aerial part of the nettle harvested in Norway. This richness in bioactive substances explains the use of *U. dioica* in folk medicine, as a diuretic and astringent and in the treatment of several diseases.^[19,20]

Table 1: Results of phytochemical tests carried out on the powder of *Urtica dioica* leaves

Bioactive substances	Results	Leaves
Tannins total	Color blue-black	(+++)
Tannins gallic	Color blue dark	(+++)
Tannins catechic	Color red	(-)
Anthocyanes	Red color	(++)
Flavonoids	Color red-orange	(++++)
Saponins	Precipitate white	(+++)
Mucilages	Precipitate flaky	(+++)
Coumarins	Formation of a disorder	(++)
Alkaloids	Red coloring	(+++)
Quinones	Red coloring	(-)
Sennosides	Purple coloring	(-)
Iridoïdes	Coloring blue	(-)
Glucosides	Coloring red brick	(++)

(-): Absence of substance, (+): Low presence of substance, (++) : Average presence of substance, (+++): Strong presence of substance

Determination of Total Polyphenols in *U. dioica* Leaves

The total polyphenol content obtained is 126.28 ± 0.04 mg EAG/g of leaf powder. A larger value, 208.37 mg EAG/g of *U. dioica* leaf powder harvested from the Banja Luka region (Turkey), is obtained by Zoran Kukrić *et al.*^[21] This difference is due to the method and the extraction solvent according to Lee *et al.*^[22]

Antioxidant Activity

The results show that the percentage of free radical inhibition for *U. dioica* phenolic extracts (0.124 ± 0.005 mg/ml) is slightly lower than that of ascorbic acid (0.110 ± 0.003 mg/ml) for all concentrations used. The phenolic extract of *U. dioica*, therefore, has an antiradical power close to ascorbic acid [Figure 1].

Zoran Kukrić *et al.*^[21] have reported an antiradical power 5 times lower than that of ascorbic acid. Lee *et al.*^[22] reported that the extraction method affects antioxidant capacities. This may partly explain the difference in results.

Antilithiasis Activity

The observations made by the SEM revealed, according to the samples, the following results: For sample 1, the reference (not treated with the phenolic extract), an absorbance of 0.404 ± 0.001 is noted. The SEM observation of the crystals retained in the filter of this sample revealed the presence of two kinds of crystals: Calcium oxalate monohydrate rounded with a slightly darker center, or whewellite, and some crystals of oxalate of calcium dihydrate, or weddellite of octahedral form (in square envelope), size between $11 \mu\text{m}$ and $17 \mu\text{m}$ [Figure 2].

The value recorded during the reading of the absorbance of sample 2 in the presence of *U. dioica* extract at a concentration of $C_1 = 0.0625$ g/l is 0.236 ± 0.002 . The crystals observed were larger than those observed for the rest of the samples, $13\text{--}15 \mu\text{m}$ [Figures 3 and 4].

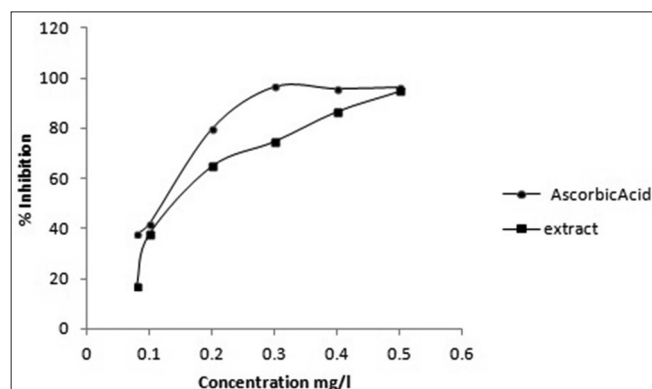


Figure 1: Percentage of radical inhibition of 1,1-diphenyl-2-picrylhydrazyl by the different extracts

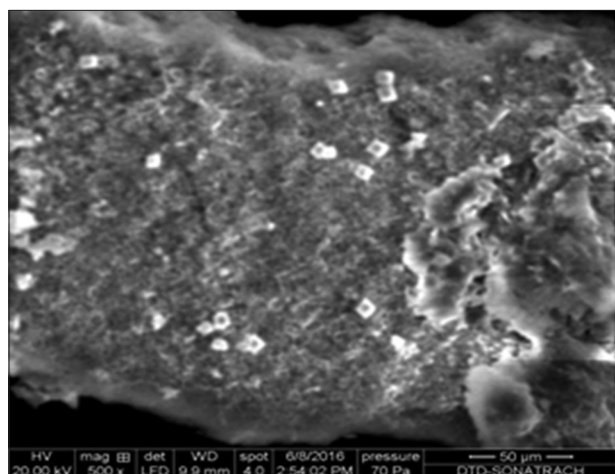


Figure 2: Reference sample picture without *Urtica dioica* extract taken by scanning electron microscope (overall view)

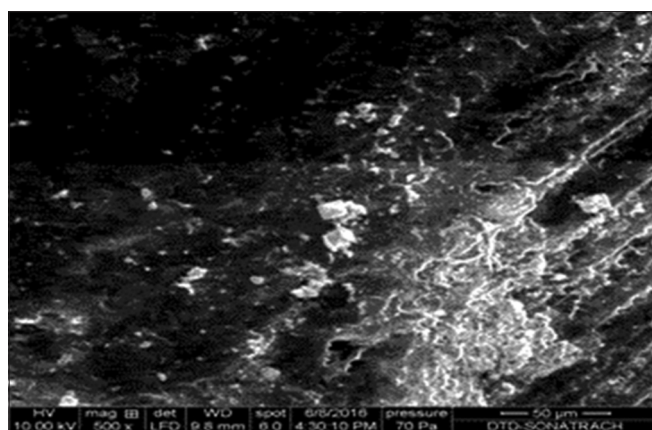


Figure 3: Picture of the sample in the presence of *Urtica dioica* extract at a concentration of $C_1 = 0.06$ g/l taken by scanning electron microscope (overall view)

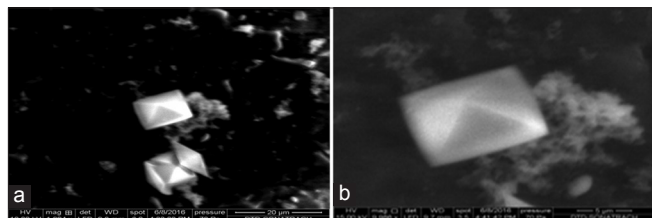


Figure 4: Pictures of sample 2 in the presence of *Urtica dioica* extract at a concentration of $C_1 = 0.06$ g/l taken by scanning electron microscope. (a) Agrandissement $\times 1000$. (b) Agrandissement $\times 5000$

The value of the absorbance recorded on sample 3 treated with *U. dioica* extract at a concentration of $C_2 = 0.12$ g/l is 0.363 ± 0.001 . The observation in SEM of the crystals retained in the filter of this sample showed the presence of crystals of size ranging from $9 \mu\text{m}$ to $11 \mu\text{m}$ [Figures 5 and 6a,b].

In the presence of *U. dioica* extract at a concentration of $C_3 = 0.25$ g/l (sample 4), the absorbance retained is 0.392 ± 0.001 . The crystals and aggregates observed are illustrated in Figure 7a and b. The size of the crystals varies between $9 \mu\text{m}$ and $11 \mu\text{m}$.

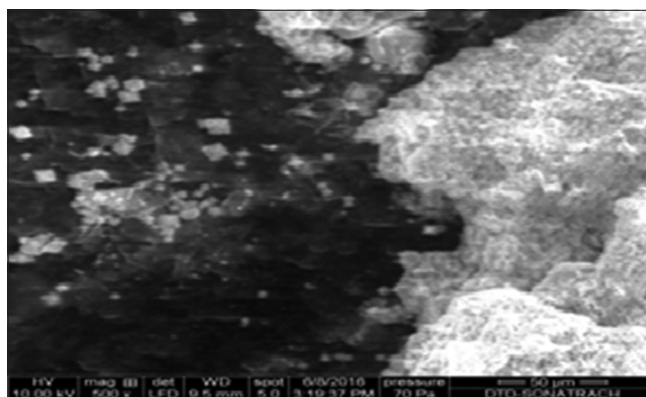


Figure 5: Picture of sample 3 treated with *Urtica dioica* extract at a concentration of $C_2 = 0.12$ g/l taken by scanning electron microscope (overall view)

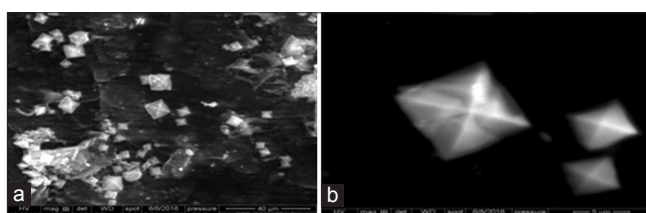


Figure 6: Pictures of sample treated with *Urtica dioica* extract at a concentration of $C_2 = 0.125$ g/l taken by scanning electron microscope. (a) Agrandissement $\times 1000$. (b) Agrandissement $\times 5000$

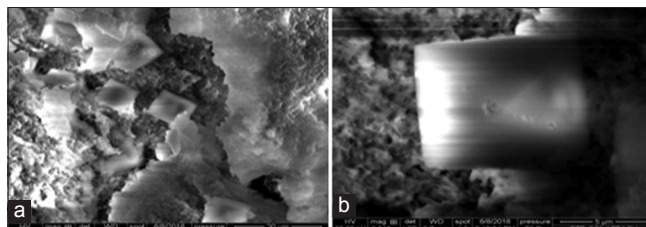


Figure 7: Pictures of sample 4 in the presence of *Urtica dioica* extract at a concentration of $C_3 = 0.25$ g/l taken by scanning electron microscope. (a) Agrandissement $\times 2000$. (b) Agrandissement $\times 8000$

In the presence of *U. dioica* extract at a concentration of $C_4 = 0.5$ g/l, the absorbance recorded is 0.393 ± 0.002 . Weddellite crystals are observed in Figure 8, the size of which is between $7 \mu\text{m}$ and $10 \mu\text{m}$. Other crystals are observed in Figure 9a and b.

The reading of the absorbance of sample 6, in the presence of *U. dioica* extract at the concentration of $C_5 = 1$ g/l, is 0.404 ± 0.002 . Several weddellite crystals have been observed in Figures 10 and 11a,b with a size $< 5 \mu\text{m}$.

At the end of these observations, a strong and interesting evolution emerges from the reference sample to the samples in the presence of different concentrations of *U. dioica* extract. This is the complete disappearance of calcium oxalate monohydrate whewellite following the addition of the

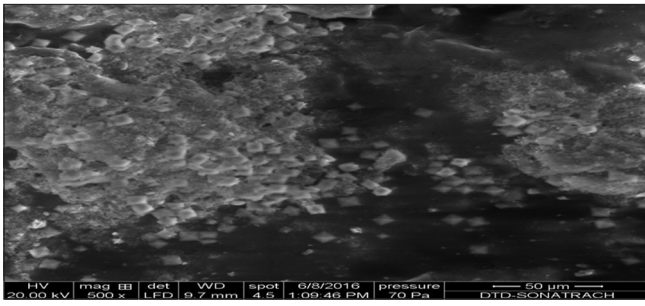


Figure 8: Picture of sample 5 in the presence of *Urtica dioica* extract at a concentration of $C_4 = 0.5$ g/l taken by scanning electron microscope (overall view)

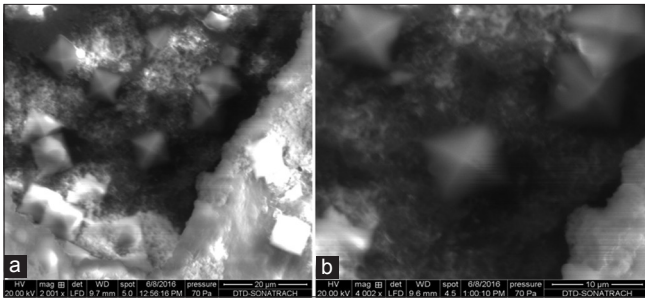


Figure 9: Pictures of sample 5 in the presence of *Urtica dioica* extract at a concentration of $C_4 = 0.5$ g/l taken by scanning electron microscope. (a) Agrandissement $\times 2000$. (b) Agrandissement $\times 4000$

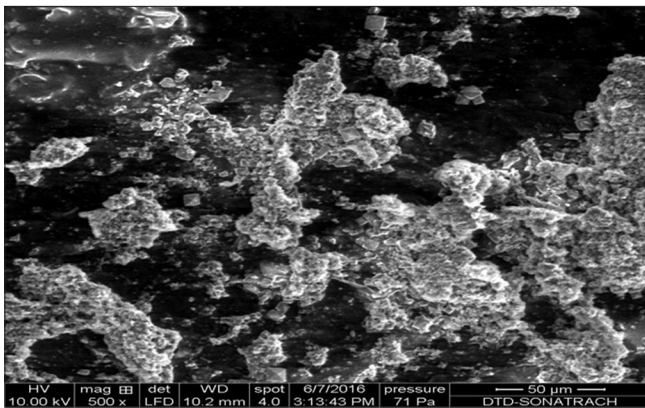


Figure 10: Picture of sample 6 in the presence of *Urtica dioica* extract at a concentration of $C_5 = 1$ g/l taken by scanning electron microscope (global view)

polyphenolic extract of *U. dioica*, and these at all concentrations taken. The presence of whewellite in urine is a lithogenic risk parameter.^[23,24] This change in urine composition is in favor of corroborating the antilithiasis effect of *U. dioica* at different concentrations in this study. The plant extract may contain substances that inhibit the growth of sodium oxalate monohydrate. This property of *U. dioica* could be very interesting in the prevention of oxalocalcic lithiasis whose frequency is high.^[25,26] In fact, the higher the plant infusion concentration is the more, the absorbance increases, due to the increase in the number of crystals [Figures 12 and 13], and consequently, the supersaturation decreases. Thus, increasing

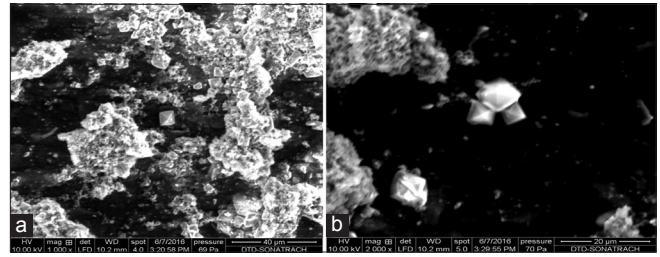


Figure 11: Pictures of sample 6 in the presence of *Urtica dioica* extract at a concentration of $C_5 = 1$ g/l taken by scanning electron microscope. (a) Agrandissement $\times 1000$. (b) Agrandissement $\times 2000$

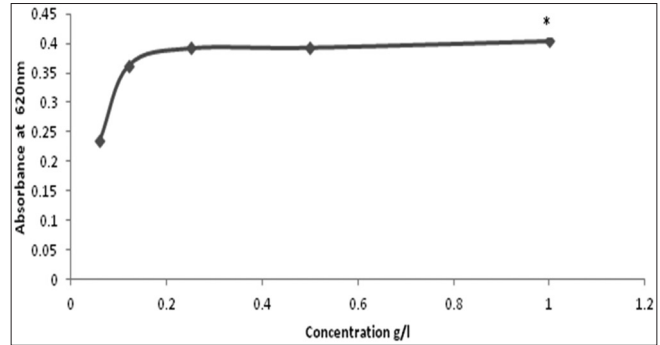


Figure 12: The crystallization of sodium oxalate in the urine of healthy subjects in the presence of aqueous extracts of *Urtica dioica*

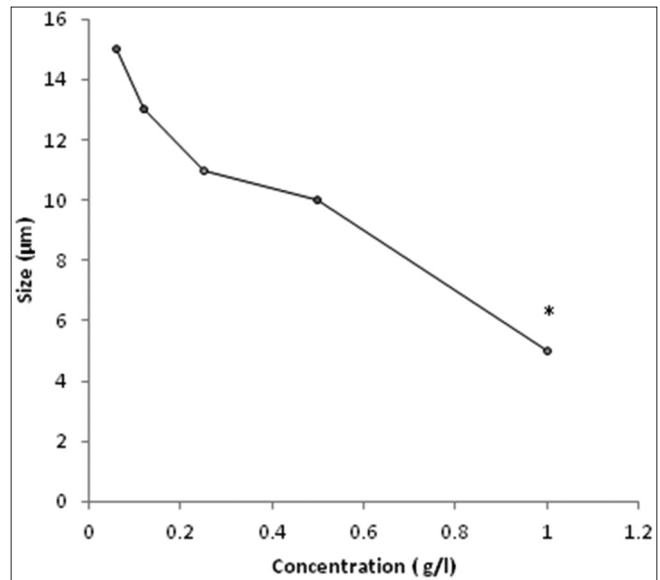


Figure 13: Evaluation of the crystal size according to the concentration

the concentration of the tested extract promotes a reduction in the size of the crystals in the urine.

Although the infusion of leaves of *U. dioica* induces the crystallization of calcium oxalate dihydrate (weddelite) in the urine of healthy subjects, it has an advantageous property in the preventive treatment of urolithiasis by significantly decreasing the size of the crystals, thus inducing

the excretion of small crystals, which makes it possible to avoid their retention in the collecting tubes, on the renal papilla or at the level of a calyx fold, first-step lithiasis process. It is also important to note that the presence of crystals in the urine is an insufficient condition to develop a urinary calculus since all the urine can contain it. The interpretation of a crystal factory must take into account the chemical nature of the crystals, the crystalline facies, the crystalline aggregation, the frequency, and the abundance of the crystal factory.^[23] The size of the weddellite crystals is usually between 5 µm and 12 µm. In the case where it exceeds 25 µm, it becomes indicative of hypercalciuria associated with hyperoxaluria, reflecting a high risk of formation of urinary calculus in lithiasis subjects. The presence of a single octahedral weddellite crystal larger than 35 µm is often indicative of an active lithogenous process even in subjects with no history of lithiasis. In addition, the aqueous extract of *U. dioica* allowed the formation of crystals of sodium oxalate dihydrate (weddellite) rather than those of whewellite (significant crystals by their simple presence in the urine). In addition, the presence of weddellite crystalline aggregates, in the presence of the aqueous extract of the plant irrespective of the concentration, may be an indication of a deficit of crystallization inhibitors which add to the supersaturation of the medium. Certain crystalline species, such as uric acid or brushite, have great ease of aggregation, unlike weddellite bipyramidal crystals which aggregate moderately, except in the case of a significant decrease in inhibitory power or significant oversaturation.^[24,27,28] In this biological model that is the total urine, *U. dioica* has shown controversial properties, it is, on the one hand, inhibiting the crystallization of calcium oxalate monohydrate, and, on the other hand, a diminished promoter effect, to diluted concentrations, on the crystallization of weddellite.

CONCLUSION

The richness of *U. dioica* polyphenolic extract in molecules with a reducing power capable of neutralizing cell damage caused by free radicals and antilithiasis activity deserves to be valued by its integration into therapeutic applications.

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