International Journal of Minor Fruits, Medicinal and Aromatic Plants. Vol. 8 (2) : 36- 42, December 2022

Phytotherapy of urinary calculi: the mass reduction of calcium oxalate stones in vitro by the aqueous extract of *Urtica dioica* l.

Ahmed Reda Belmamoun[1], Abdelkader Ammam[2]*, Redouane Chadli[3], Chafik Mhamdia[4] and Afaf Bakdi[5]

[1]Laboratory of Process, Materials and Environmental Engineering, Djillali Liabes University, BP 22000, Sidi Bel-Abbes, Algeria.

[2] Laboratory of Pharmacognosy, Bio toxicology, and Biological Valorization of Plants, Moulay Tahar University, BP 20000, Saida, Algeria.

[3] Laboratory of Organic and macromolecular physical chemistry, Djillali Liabes University, BP 22000, Sidi-Bel-Abbes, Algeria.

[4]Laboratory of Nutrition, Pathology, Agro Biotechnology and Health, Djillali Liabes University, BP 22000, Sidi-Bel-Abbes, Algeria.

[5] Department of Environmental Sciences, Faculty of Nature and Life Sciences, Djillali Liabes University, BP 22000, Sidi- Bel-Abbes,Algeria.

**Email: vetokadi@yahoo.fr*

Received : 05.05.2022 ; Revised : 12.07.2022 ; Accepted : 16.07.2022

DOI : 10.53552/ijmfmap.8.2.2022.36-42

ABSTRACT

The purpose of this study is to evaluate in vitro the effect of an aqueous extract of Urtica dioica on the dissolution of oxalocalcic type kidney stones at the mesoscopic scale. The weight of the stones used in our experiment varied from: 0.0625g to 1.1049g. Type identification of kidney stone samples is performed by Infrared spectroscopic analysis. The presence of carboxylate ion of calcium oxalate is highlighted by absorption bands in the 1312.41 cm^{-1} and 1606.36 cm^{-1} areas. The aqueous extract of the aerial part of the plant Urtica dioica was prepared by infusion for 30 min of 5g of powder in 100 ml of saline solution (9 g/L of NaCl), previously brought to the boiling point, and there was then filtered. The stones were left in contact with the extract for 6 weeks, under constant magnetic stirring in 50 ml of aqueous extract. Kinetic evolution of the pH and the evaluation of the dissolution capacity of the extracts were carried out every week. The results obtained are very satisfactory where we observe a loss of mass which increases with time in order to reach a rate of 63%. This confirms the dissolution of stones and the increase of pH by the effect of the presence of the base of calcium oxalate in the aqueous medium. According to this study, we emphasize the need to suggest Urtica dioica as a means to reduce the occurrence of this urological disease and to establish less expensive tests and treatments.

***Keywords*:** Calcium oxalate, dissolution rate, kidney stones, urticadioica

INTRODUCTION

Renal lithiasis is a disease that affects from 4 to 20% of the population in different countries, characterized by the formation of crystals in the kidneys (El Habbani *et al.,* 2021). It is a multifactorial disease due to a sequence of physicochemical steps, in particular supersaturation, nucleation, growth, aggregation, and retention in the renal tubules (Ammork *et al.,* 2020).The recurrence rate of this pathology is 70-81% in men compared to 47-60% in women (Annand *et al.,* 2021). Extra corporeal shock wave lithotripsy (ESWL) is the treatment of choice for this disease, but this technique causes acute kidney damage and small fragments of unremoved crystals (Parveen *et al.,* 2021). In Algeria, alternative medicine is still widely used by the population with the use of medicinal plants based on recommendations made here and there (Dif *et al.,* 2022). Therefore, we proposed to evaluate in vitro the effect of an aqueous extract of *Urtica dioica* on the dissolution of oxalocalcic type kidney stones.

MATERIALS AND METHODS

Urinary stones

Samples of kidney stones were eliminated spontaneously in patients suffering from urinary lithiasis, after shock wave treatment at the urology

department of the University Hospital of Sidi-Bel-Abbes. These stone samples were washed with distilled water, air-dried on filter paper, and stored in sterile containers. The weight of the stones used in our experiment varied from: 0.0625g to 1.1049g. Type identification of kidney stone samples is performed by Infrared spectroscopic analysis on a Mattson Genesis II FTIR instrument. The samples were processed as KBr pellets (Singh, 2008).

Plant material

The plant studied belongs to the Urticaceae family under the scientific name: *Urtica dioica*. We are interested in this study in the aerial part of the plant. The plant is harvested at the level of the forest of the Ex-ITMA, wilaya of Sidi-Bel-Abbes, during the flowering period: February-April 2019. The aerial part (leaves) was dried at room temperature and protected from light for a period of 4 months; then reduced to powder and sieved. The powder is stored in an airtight glass bottle protected from light. The identification of the plant *Urtica dioica* was done with the help of a specialist in Botany of the laboratory of Water Resources and Environment at the University of SAIDA.

Extraction process

Our experimental work was carried out at the laboratory of Agronomic Sciences, Faculty of Natural Sciences and Life, and laboratory organic physical chemistry and Macromolecular LCPOM of the University Djillali Liabès of Sidi-Bel-Abbes. The extract of *Urtica dioica* leaves was prepared by infusion for 30 min of 5g of powder in 100 ml of a physiological aqueous solution of sodium chloride (NaCl) at 9g /L, previously brought to boiling point (Hannache *et al.,* 2012). The extract was then filtered.

Experimental device

The aqueous extract of the plant (*Urtica dioica*) is used to demonstrate, in vitro, the dissolving power of urinary stones at the mesoscopic scale, by putting the stones in the presence of 50 ml of each sample at room temperature under constant magnetic stirring (130 rpm). The stones were left in contact with the extract for 6 weeks, which is the recommended treatment period in traditional medicine (Meiouet *et al.,* 2011). Each week, the stones are removed from the different solutions, washed with distilled water, dried at 40°C for 18 h in an oven, and then weighed with a 10^{-4} g precision balance to evaluate the mass loss. The sample is put back into the extract with the same conditions to count the second week, until the sixth week of the experiment. The study is performed on three samples of the same type of kidney stones. In parallel, the same experiment was performed, where physiological saline water (NaCl 9g/l) was used instead of *Urtica dioica* extracts. Furthermore, the dissolution of the urinary calculi in the extract is monitored by evaluating the pH values during six weeks of immersion with agitation and at room temperature. The pH is measured weekly using a pH meter with a 10^{-4}g precision scale. The Ca^{+2} and Mg^{+2} ions were determined according to the method described by Rodier *et al*. (2009), before and after the six weeks contact of calcium oxalate stones with the aqueous extract, by an EDTA solution containing ethylene diamine tetra acetate ion in the basic medium of ph=10.

RESULTS AND DISCUSSION

Analysis of kidney stones

The presence of oxalate is highlighted by absorption bands in the 1600 cm^{-1} and 1300 cm^{-1} areas (Fig. 1). The oxalocalcic dependent species is hyperoxaluria represents the major cause of urinary lithiasis, among its causes, is the excessive consumption of food rich in oxalate as tea. Urinary lithiasis ranks third among the most common urologic diseases (Wigner *et al*., 2022). With a wide range of sizes, kidney stones can have a diameter ranging from a few millimeters to several centimeters (Idm'hand *et al*., 2019). These require other treatments and techniques such as ESWL (Kim *et al*., 2020). On the other hand**,** Ahmed *et al.* (2018) stated that ESWL is less effective for calcium oxalate monohydrate (COM) stones.

The spectrum below shows two characteristic bounds, the first one around 1312.41 cm^{-1} of the C-O-C bond due to the presence of the carboxylate ions and the second one around 1606.36cm^{-1} of the carbonyl function C=O which confirms the presence of the carboxylate ion of calcium oxalate. The large bung presented between 3000 and 3500cm^{-1} is due to the presence of humidity from 50 to 75%. The IR spectrum data confirms that our urinary stone samples used in this study are of the oxalocalcic type.

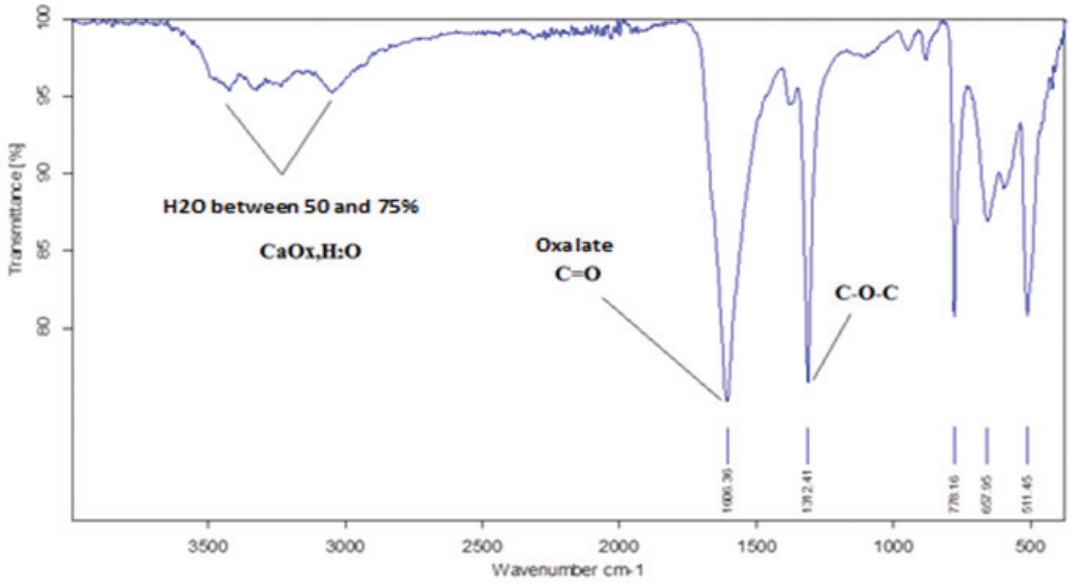


Fig. 1: FT-IR analysis of urinary stones

Kinetic evolution of ph and evaluation of the dissolution capacity of extracts

Herbal medicine is an integral part of the cultural practices of our local communities, which constitute an important heritage for the management of urological diseases (Bencheikh *et al.,* 2021; Guerrouj *et al.,* 2021). In recent years, various plants and traditional medicines have been proposed to reduce calcium oxalate kidney stones, which can be useful in its prevention and treatment (Afkari *et al.,* 2019). According to recent ethnobotanical studies in Algeria, the infusion of leaves is the most used practice (Belhouala *et al*., 2021; Zatout *et al.,* 2021). As well, pharmacological research on medicinal plants used in anti-lithiasis therapy has revealed their therapeutic potential in vitro models (Manasa Reddy *et al.,* 2018). The complete mechanisms of actions of medicinal plants are not well known, but herbal medicine is more effective in the treatment of urolithiasis (Chandel *et al.,* 2019).

The pH values are obtained at the beginning and at the end of the experiment for each week during the whole duration of our study (Figure 2).The urinary stone samples used in this study are of the calcium oxalate type.

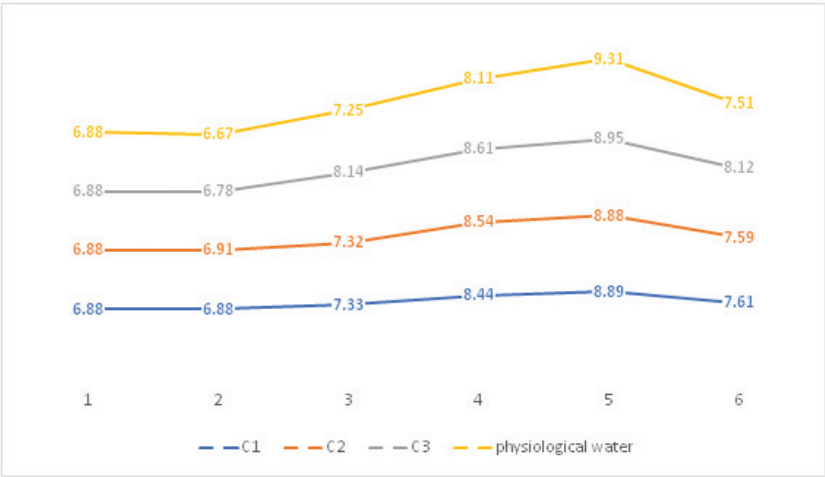


Fig. 2: pH evaluation

We observed a very important increase in the pH values and the highest value of 9, 31 where we find the greatest efficiency. The pH in physiological water remains almost constant where we can say that the properties do not change during the experiment (no dissolution of kidney stones in physiological water).

The dissolution capacity of the extracts (A%) was evaluated by the mass lost from the samples of the kidney stones by the effect of the tested extracts in a period of one week. The formula used to calculate the dissolution capacity in our extract (A%) is:

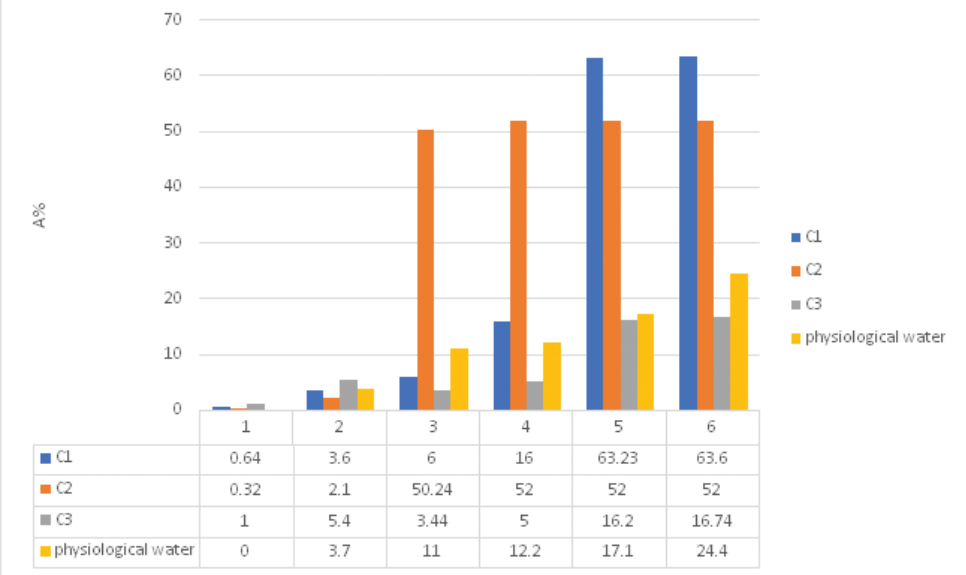
$$A\% = \frac{\Delta m}{m_{initial}} \times 100$$

A%: The dissolution rate of the kidney stones

Δm : The lost in a week

$m_{initial}$:The mass of the initial sample0

The different results of the dissolution rates in the experimental medium with the sample masses of the kidney stonesare shown in the Fig. 3.



	1	2	3	4	5	6
C1	0.64	3.6	6	16	63.23	63.6
C2	0.32	2.1	50.24	52	52	52
C3	1	5.4	3.44	5	16.2	16.74
physiological water	0	3.7	11	12.2	17.1	24.4

Fig. 3: Dissolution capacity of extracts

The results show a rapid evaluation for the C2 stone sample, a rate of 50% is obtained in the third week and the maximum in the fourth week. The sample C1 has a good dissociation but the dissolution takes five weeks with a rate of 63%. Sample C3 does not give good results because it is very bulky.

The results of the measurements of the concentration of calcium Ca^{2+} and magnesium Mg^{2+} ions, in the extract solution before and after the emersion of the stones obtained are presented in the Table 1:

Table 1: Concentration of Ca^{2+} and Mg^{2+} ions

	$[Ca^{2+}] + [Mg^{2+}]$ mmol/l	
Aqueous extract of Urtica dioica	Before	After
	15	900

The possible mechanism by which *Urtica dioica* affects calcium oxalate could be the result of several valuable chemical compounds, such as phenolic compounds (such as chlorogenic acid, ferulic acid, ellagic acid, naringin, myricetin, rutin), flavonoids (such as kaempferol), tannins, coumarins (e.g., scopoletin), lignans (secoisolariciresinol, 9,9-bisacetyl-neo-olivil and their glucosides), phytosterols (e.g., β-sitosterol), fatty acids, polysaccharides, isolectins, triterpenic acids and

monoterpendiols Taibi *et al.,* 2021). Studies have shown that the main bioactive of *Urtica dioica* are flavonoids, anthocyanins, and saponins (Nirumand *et al.,* 2018), which could inhibit calcium and oxalate deposition and crystal growth by disintegrating mucoproteins (Al-Assaf *et al*., 2020). In our study, the dissolution or degradation of the stones in the medium leads to the release of calcium oxalate ions of basic character. The presence of oxalate will increase the pH of the medium. The experimental data show a positive evaluation of pH with the loss of mass of the studied urinary stones. Tannins and polyphenols inhibit CaO_x crystal formation and dissolve preformed CaO_x crystals by promoting calcium complexation (Bawari *et al.,* 2018). The results obtained justify well the evaluation of the dissolution of the stones in the extract of our plant. We observe a very important increase in the concentration of Ca^{2+} and Mg^{2+} ions in the solution of the extract after emersion of the stones during the six weeks. Flavonoids could effectively inhibit CaO_x stone formation in vitro, correlating with their diuretic, antioxidant, and anti-inflammatory properties (Zeng *et al.,* 2018). According to the study of Taïbi *et al.* (2021) in the Tiaret region of Algeria, the medicinal benefits of *Urtica dioica* are related to its nutritive, depurative, diuretic, antioxidant, anti-inflammatory, and stimulating effects. Antioxidant therapy could be one of the effective methods to prevent the nucleation and fixation and growth of calcium oxalate crystals (Keles *et al.,* 2020). The process of calcium oxalate crystallization is of great interest in medicine, it is the main component of 75-80% of kidney stones (Khan *et al.,* 2021). A more negative crystallite surface inhibits the growth and agglomeration of urinary crystals (Sun *et al.,* 2017). The study of Polat (2019) shows that *Urtica dioica* extracts can reduce the size of calcium oxalate crystals leading to the formation of COD (calcium oxalate dihydrate: $Ca\text{-}C_2O_4\text{-}2H_2O$, weddellite) and inhibiting the growth and aggregation of COM (calcium oxalate monohydrate: $CaC_2O_4\text{-}H_2O$, whewellite) crystals. Furthermore, Polat, (2019) indicates that the surface of the crystals becomes negative due to the coverage of the crystal surface by the negatively charged *Urtica dioica* ions, which induces the increase of the zeta potential over time.

CONCLUSION

The present study shows the efficacy of the aqueous extract of *Urtica dioica* in dissolving oxalocalcic type urinary stones in vitro. Therefore, we emphasize the need to suggest *Urtica dioica* as a means to reduce the occurrence of this disease and to establish less expensive tests and treatments. However, further studies are needed to determine the mechanism of action of this plant in preventing kidney stone formation in vivo.

REFERENCES :

Afkari, R., Feizabadi, M. M., Ansari-Moghadam, A., Safari, T. and Bokaeian, M. 2019. Simultaneous use of oxalate-degrading bacteria and herbal extract to reduce the urinary oxalate in a rat model: A new strategy. *International Braz j Urol*, **45**(6): 1249–1259.

Ahmed, S., Hasan, M. M., Khan, H., Mahmood, Z. A. and Patel, S. 2018. The mechanistic insight of polyphenols in calcium oxalate urolithiasis mitigation. *Biomedicine & Pharmacotherapy*, **106**:1292–1299.

Al-assaf, I. N., Al-Dabbagh, A. G., Al-Abachi, R. Y. and Al-Bajari, S. A. 2020. Protective role of *Urtiea dicica* on the pathological alteration induced by ethylene glycol in male rabbits. *Systematic Reviews in Pharmacy*, **11**(12): 1180-1183.

Ammor, K., Mahjoubi, F., Bousta, D., Elhabbani, R. and Chaqroune, A. 2020. In vitro litholytic activity of extracts and phenolic fractions of some medicinal plants on urinary stones. *Mediterranean Journal of Chemistry*, **9**(6): 468–477.

Anand, D., Chandrasekar, R. and Sivagami, B. 2021. A critical review on antiurolithiatic activity of bioactive phytoconstituents. *Research Journal of Pharmacognosy and Phytochemistry*, **13**(2): 95-100.

Bawari, S., Negi Sah, A. and Tewari, D. 2018. Antiurolithiatic Activity of Daucus carota: An in vitro Study. *Pharmacognosy Journal,* **10**(5): 880–884.

Belhouala, K. and Benarba, B. 2021. Medicinal plants used by traditional healers in Algeria: A multiregional ethnobotanical study. *Frontiers in Pharmacology*, **12**: 760492.

Bencheikh, N., Elbouzidi, A., Kharchoufa, L., Ouassou, H., Alami Merrouni, I., Mechchate, H., Es-safi, I., Hano, C., Addi, M., Bouhril, M., Eto, B. and Elachouri, M. 2021. Inventory of medicinal plants used traditionally to manage kidney diseases in North-Eastern Morocco: Ethnobotanical Fieldwork and Pharmacological Evidence. *Plants,* **10**(9): 1966.

Chandel, V. K., Jain, S. and Choubey, A. 2019. An overview on phyto-molecules and screening method of antiurolithiatic activity. *Journal of Drug Delivery and Therapeutics,* **9**(4-A): 848-857.

Dif, M.M., Benchohra, H.A., Dellal, A., Feddal,W. and Chebab, N. 2022. Ethnobotanical study of medicinal plants in Telagh region (North-western Algeria). *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **8** (1): 65-69.

DWaghmare, S. 2020. Ethnobotanical survey on antiurolithiatic activity of some medicinal plants. *International Research Journal on Advanced Science Hub*, **2**(Special Issue ICARD): 268–275.

El Habbani, R., Lahrichi, A., Sqalli Houssaini, T., Kachkoul, R., Mohim, M., Chouhani, B. A. and Chaqroune, A. 2021. In vitro mass reduction of calcium oxalate urinary calculi by some medicinal plants. *African Journal of Urology,* **27**(1): 28.

Guerrouj, B. E., Bencheikh, N., Bouhrim, M., Kharchoufa, L., Ouassou, H. and Imtara, H. 2021. Attitude and awareness of medicinal plants in treatment of kidney lithiasis in eastern Morocco: a retrospective study. *Herba Polonica*, **67**(2): 17–28.

Hannache, B., Bazin, D., Boutefnouchet, A. and Daudon, M. 2012. Effet des extraits de plantes médicinales sur la dissolution des calculs rénaux de cystine in vitro : étude à l'échelle mésoscopique. *Progrès En Urologie,* **22**(10) : 577–582.

Idm'hand, E., Msanda, F. and Cherifi, K. 2019. Étude ethnobotanique des plantes médicinales utilisées dans le traitement de la lithiase urinaire dans la province de Tarfaya (Maroc). *International Journal of Innovation and Applied Studies*, **26**(3): 711-719.

Keleº, R., ªen, A., Ertaº, B., Kayali, D. and Eker, P. 2020. The effects of *Urtica dioica* L. ethanolic extract against urinary calculi in rats. *Journal of Research in Pharmacy*, **24**(2): 205–217.

Khan, A., Bashir, S. and Khan, S. R. 2021. Antiurolithic effects of medicinal plants: results of in vivo studies in rat models of calcium oxalate nephrolithiasis—a systematic review. *Urolithiasis*, **49**(2): 95–122.

Kim, C. H., Chung, D. Y., Rha, K. H., Lee, J. Y. and Lee, S. H. 2020. Effectiveness of percutaneous nephrolithotomy, retrograde intrarenal surgery, and extracorporeal shock wave lithotripsy for treatment of renal stones: A Systematic Review and Meta-Analysis. *Medicina*, **57**(1): 26.

Manasa, R.J., Prathyusha, K., Himabindhu, J. and Ramanjaneyulu, K. 2018. Evaluation of in vitro antiurolithiatic activity of *Mentha piperita*. *Journal of Pharmaceutical Sciences and Medicine*, **3**(8): 22-28.

Meiouet, F., El Kabbaj, S. and Daudon, M. 2011. Étude in vitro de l'activité litholytique de quatre plantes médicinales vis-à-vis des calculs urinaires de cystine. *Progrès en urologie*, **21**(1): 40-47.

Nirumand, M., Hajialyani, M., Rahimi, R., Farzaei, M., Zingue, S., Nabavi, S. and Bishayee, A. 2018. Dietary Plants for the prevention and management of kidney stones: preclinical and clinical evidence and molecular mechanisms. *International Journal of Molecular Sciences*, **19**(3): 765.

Parveen, R., Wadud, A., Shamsi, S. and Parveen, S. 2021. Antilithiatic activity of a non-pharmacopoeial Unani formulation in chemically induced urolithiasis in rats. *Journal of Complementary and Integrative Medicine*, **18**(4): 685–694.

Polat, S. 2019. An in vitro evaluation of the effects of Urtica dioica and Fructus Urtica Piluliferae extracts on the crystallization of calcium oxalate. *Journal of Crystal Growth*, **522**: 92–102.

Rodier, J., Legube, B. and Merlet, N. 2009: L'analyse De L'eau.Dunod (9th ed). Paris. pp. 249.

Singh, I. 2008. Renal geology (quantitative renal stone analysis) by "Fourier transform infrared spectroscopy." *International Urology and Nephrology*, **40**(3): 595–602.

Sun, X.-Y., Xu, M. and Ouyang, J. M. 2017. Effect of crystal shape and aggregation of calcium oxalate monohydrate on cellular toxicity in renal epithelial cells. *ACS Omega*, **2**(9): 6039–6052.

Taïbi, K., Aït Abderrahim, L., Boussaid, M., Taibi, F., Achir, M., Souana, K., Benaissa, T., Farhi, K.H., Naamani, F.Z and Nait Said, K. 2021. Unraveling the ethnopharmacological potential of medicinal plants used in Algerian traditional medicine for urinary diseases. *European Journal of Integrative Medicine*, **44**: 101339.

Wigner, P., Bijak, M. and Saluk-Bijak, J. 2022. Probiotics in the Prevention of the Calcium Oxalate Urolithiasis. *Cells*, **11**(2): 284.

Zatout, F., Benarba, B., Bouazza, A., Babali, B., Nacer Bey, N and Morsli, A. 2021. Ethnobotanical investigation on medicinal plants used by local populations in Tlemcen National Park (extreme North West Algeria). *Mediterranean Botany*, **42**: e69396.

Zeng, X., Xi, Y. and Jiang, W. 2018. Protective roles of flavonoids and flavonoid-rich plant extracts against urolithiasis: A review. *Critical Reviews in Food Science and Nutrition*, **59**(13): 2125–2135.