



Antioxidant effects and *ex vivo/in vitro* mass reduction of calcium oxalate urinary stones of several extracts from *Paronychia argentea* L. Efeitos antioxidantes e redução de massa *ex vivo/in vitro* de cálculos urinários de oxalato de cálcio de vários extratos de *Paronychia argentea* L.

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Abstract

Our study focused on the evaluation of the antioxidant and antilithiasis activity of *Paronychia argentea* L. extracts used in traditional medicine to treat or prevent urolithiasis, particularly their ability to dissolve natural oxalocalcic stones from a human. Aerial parts of the *Paronychia argentea* L. plant were extracted by two distinct methods, using five solvents of varying polarities. They were identified by assays of total polyphenols and flavonoids. Antioxidant activity was assessed using five different methods, while evaluation antilithiasis activity was carried out through three distinct approaches. The herb *Paronichia argentea* L possesses antilithiasis and antioxidant properties that could be beneficial in treating urinary stone disease by reducing their size, achieving a rate of 52%.

Keywords: Medicinal plant. Anti-lithiasis activity. Radical scavenging. Natural human urinary stone. Dissolution.

Resumo

Nosso estudo focou na avaliação da atividade antioxidante e antilítase de extratos de *Paronychia argentea* L. usados na medicina tradicional para tratar ou prevenir urolitíase, particularmente sua capacidade de dissolver cálculos oxalocalcic naturais de um humano. Partes aéreas da planta *Paronychia argentea* L. foram extraídas por dois métodos distintos, usando cinco solventes de polaridades variadas. Elas foram identificadas por ensaios de polifenóis totais e flavonoides. A atividade antioxidante foi avaliada usando cinco métodos diferentes, enquanto a avaliação da atividade antilítase foi realizada por meio de três abordagens distintas. A erva *Paronichia argentea* L possui propriedades antilítase e antioxidantes que podem ser benéficas no tratamento de cálculos urinários, reduzindo seu tamanho, atingindo uma taxa de 52%.

Palavras-chave: Planta medicinal. Atividade antilítase. Eliminação de radicais. Cálculo urinário humano natural. Dissolução.

1. Introduction

Stone formation in the kidney is one of the oldest and most widespread diseases known to man (AGARWAL; VARMA, 2014). They affect 5 to 15% of adults (ANAND et al., 2021) while urinary stone disease is a common disorder estimated to occur in approximately 12% of the population (VIEL et al., 1999). A stone is an aggregation of solute materials from urine, such as calcium, oxalate, phosphate and uric acid (AGARWAL; VARMA, 2014). Nowadays, kidneys and their problems have gained increasing interest concomitant with life changes, industrialization and malnutrition (BENCHEIKH et al., 2021).

Calcium oxalate stones represent up to 80% of analyzed stones. Calcium phosphate account for 15-25%, while 10- 15% is mixed stones. The others are struvite 15-30%, cystine 6-10%, and uric acid stones 2-10% (AGARWAL; VARMA, 2014; ANAND et al., 2021) followed by minute amount of calcium carbonate (PHATAK et al., 2015). Struvite stones are classified as infectious lithiasis because their presence indicates the presence of a ureolytic germ that can cause a sufficiently high alkalinity of the urine to cause the simultaneous precipitation of ammonium and magnesium phosphates (MAMMATE et al., 2023).

Calcium oxalate (CaOx) crystals are usually present in different forms: calcium oxalate monohydrate (whewellite), dihydrate (weddelite) and the rarer trihydrate (AGARWAL; VARMA, 2014). High concentrations of CaOx crystals, or of oxalate itself, lead to toxic effects on renal cells which induce cell surface alterations, thus unmasking attachment sites for adhesion and/or internalization of crystals by renal epithelial cells (DE BELLIS et al., 2019).

The pathogenesis of calcium oxalate stone formation is involved nucleation, crystal growth, crystal aggregation, and crystal retention in multistep process (PHATAK et al., 2015).

Lithiasis is the term used to refer to kidney stones; this could be triggered by low activity, diet, or genetics (TIENDA-VAZQUEZ et al., 2022).

Intake of oxalate-rich foods should be limited. These include spinach, beets, nuts, chocolate, tea, and strawberries which have been shown raising and significant urinary oxalate excretion (PHATAK et al., 2015). Excess fluid intake, salt restriction, and protein consumption are all recommended. The use of calcium during mealtime is advised to avoid calcium oxalate formation (PHATAK et al., 2015).

Drug prescribed for the treatment of renal stone dependent on the type of the stone and the patient characteristics. Thiazide diuretics are frequently used to reduce the risk of calcium stone recurrence (AL-SNAFI, 2023).

Several medicinal plant extracts have been reported for *in vitro* anti-crystallization activities till date such as: *Punica granatum* L. (KACHKOUL et al., 2023) *Zizyphus Lotus* (BADDADDE et al., 2019), *Peperomia tetraphylla* (NISHANTHI et al., 2016), *Melastoma malabathricum* Linn (SULEIMAN et al., 2018) and *Juncus acutus* (ALIAT et al., 2020). In ayurveda many plants having the property of disintegrating and dissolving the stone are referred to as “pashanbhedha” (AGARWAL; VARMA, 2014; PHATAK et al., 2015).

Indeed, the alternative treatment using herbal medicine has come into demand in recent years and has renewed interest in the plants that are effective, safe, and culturally acceptable (KHOUCHLAA et al., 2020).

Plants have always played a significant role in traditional medicine in underdeveloped countries and have also been an integral part of local communities' history and cultural practices

(BENCHEIKH et al., 2021). In Algeria, more and more people are turning to traditional medicine to treat this disease (KHITRI et al., 2016).

Development of plant-based medicine as alternative or complementary to the conventional system of medicine have drawn tremendous attention and serves as an immense source of new drug entities (BAWARI et al., 2018). Based on these grounds, *Paronychia argentea* L. was selected for the present study.

Paronychia argentea L. is a perennial plant that belongs to the family of Caryophyllaceae in habitats of the Mediterranean area. (ABDELKHALEK et al., 2021, ARKOUB-HAMITOUCHE et al., 2020). It is the most common plant used abundantly in conventional medicines in Algeria and is popularly known as Arabic tea (Kassaretlahdjer, Fettatlahdjer or Bissatelmoulouk) (ARKOUB-HAMITOUCHE et al., 2020; VEERARAGHAVAN et al., 2020). Extracts from its aerial parts have been frequently used in folk Algerian medicine as diuretic, to treat kidney stones or prostate discomfort, stomach ulcer, anorexia and as an antimicrobial agent (ARKOUB-HAMITOUCHE et al., 2020). The literature review showed the great medicinal bioactivities of *Paronychia argentea*. Its extract has antioxidant, antimicrobial, and nephroprotective activities (ABDELKHALEK et al., 2021).

The main objective is the phytochemical study, exploiting the antioxidant power and contribute to the valorization of improved traditional medicine for the treatment of urolithiasis in Algeria.

2. Materials and methods

2.1. Chemicals

All chemicals used were obtained from Sigma Aldrich, while ethyl acetate, methanol and trisodium citrate dihydrate were procured from Biochem.

2.2. Plant material

Paronychia argentea was collected from the region of Ain Abbassa, 20 Km from the wilaya of Sétif in spring during the month of March. His aerial parts are dried for several days in the dark in a dry, aerated place. After drying, they are ground into a fine powder using a mechanical grinder, which is stored away from light and moisture until use.

2.3. Determination of water content

Moisture content is defined as the loss of weight during drying (YEO et al., 2011). Water content is determined by calculating the difference in weight before and after drying using formula 1:

$$MC\% = \frac{w_1 - w_2}{w_1} \times 100 \quad (1)$$

MC%: moisture content; W1: weight in g of sample before drying (fresh plant); and W2: weight in g of sample after drying (dried plant).

2.4. Extraction

Once the plant product has been properly prepared, it can be used as a raw material to obtain the various extracts. Solid-liquid extraction remains the most widespread technique for recovering and isolating bioactive compounds. This is due to its simplicity of use and relatively high efficiency. For this purpose, we used two different extraction methods:

First method (M1)

The first method involves *successive* maceration with *five solvents* of increasing polarity (petroleum ether, dichloromethane, ethyl acetate, methanol, and water) (DIALLO et al., 2004). Extraction with petroleum ether (PE) is designed to train fats and lipophilic substances, while extraction with dichloromethane (DCM) is used to extract moderately polar compounds. On the other hand, extraction with ethyl acetate (EA) produces an extract rich in polar compounds. Extraction with methanol (MeOH) serves to obtain an extract rich in highly polar compounds, while extraction with water (H₂O) extracts strongly polar compounds.

100g of dry powder is first macerated in 750 ml of petroleum ether. After 24 hours of maceration with soxhlet at the boiling temperature of each extraction solvent, the extracts (EEP, EDCM, EAE1, EMeOH1, and EAQ1) were concentrated under vacuum with a steam rota at temperatures of 37°C, 40°C, 77°C, 55°C, and 95°C, respectively. The extracts obtained were poured into petri dishes and oven-dried (40°C), then preserved in dark vials for later use.

Second method (M2)

In this method, the maceration is repeated with the same solvents, but this time applied directly to the delipidated powder.

100 g of the dry powder was first macerated in 750 ml of EP for 24 hours, then divided into four fractions of 24 g, which were subsequently taken up with 500 ml of DCM, AE, MeOH, and H₂O. Extracts were filtered and evaporated to a dry state, and the residues were weighed.

Yield calculations

The percentage of dry extract was calculated using formula 2:

$$Y\% = \frac{w}{w_0} \times 100 \quad (2)$$

Y: yield expressed in %; W: weight in grams of resulting dry extract; and W₀: weight in grams of plant material to be treated.

2.5. Foam index

The evaluation of saponins consists of calculating the foam index, which is provided by the degree of dilution of an aqueous decoction of a plant that, under given conditions, gives a persistent foam (DOHOU et al., 2003).

2.6. Phytochemical assays

In our work, phytochemical tests for the majority of known secondary metabolites were carried out on the various extracts prepared. Among them, we mention two tests. All samples were analysed in triplicate.

2.6.1. Total polyphenol content

According to the method of Le et al. (2007), total polyphenols were determined using the Folin-Ciocalteu reagent, which forms a redox complex during phenol oxidation (BOIZOT et al., 2006). Absorbance was checked at 760 nm. The calibration curve was plotted with gallic acid at different concentrations (0–100 µg/ml).

2.6.2. Flavonoid assay

The flavonoid content of extracts is determined by the aluminum trichloride method. Absorbance is read at 430 nm. The calibration curve is run with quercetin at different concentrations (0–24 µg/ml), using the same conditions and assay steps. Results are expressed in mg quercetin equivalent per gram of extract (mgEQ/g extract) (CHANG et al., 2002).

2.7. Antioxidant activities

Antioxidant activities were studied on four extracts of *Paronychia argentea* L.: EMeOH1, EMeOH2, EAQ1, and EAQ2 by five different methods. All samples were analysed in triplicate.

Total antioxidant activity

The phosphomolybdenum method was employed to evaluate total antioxidant capacity through the assay of green phosphate/MO5+ complexes (CHEURFA; ALLEM, 2016; CHAOUICHE et al., 2014; HAMED et al., 2022). Absorbance was measured at 695 nm against the blank. Total antioxidant capacity was expressed as milligrams of standard antioxidant equivalent per gram of extract (mg Estd/g extract).

Reducing power

The reducing power of Fe³⁺ is determined according to the method described by Yen and Chen (2015). The intensity of the appearing blue-green colour was measured at 700 nm. Ascorbic acid, BHT, and BHA were used as reference antioxidants whose absorbance was measured under the same conditions as the samples.

The EC₅₀ (the sample concentration corresponding to an absorbance of 0.5) is calculated from the curve of absorbance versus sample concentration (BOUGANDOURA et al., 2013; FERREIRA et al., 2007).

Ferrous ion-chelating activity

The antioxidant activity of the extracts, compared with standard chelator (EDTA), was evaluated in terms of ferrous chelation. The complexation of residual iron with ferrozine gives a purple color with an absorption maximum at 562 nm. Chelating activity is expressed as a percentage using equation 1 (DECKER; WELCH, 1990):

$$\text{Chelation activity (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

Ac: standard absorbance; As: sample absorbance.

ABTS.+ cationic radical scavenging

The scavenging capacity of the ABTS.+ cation radical was determined according to the method of Re et al. (1999). Absorbance of the mixture was determined at 734 nm against the blank where the sample was omitted. Antiradical activity is expressed as a percentage of ABTS.+ radical inhibition using formula 3.

$$(\%) \text{ABTS}^+ \text{inhibition} = \frac{A_c - A_e}{A_c} \times 100 \quad (3)$$

Ac: Absorbance of control; and Ae: Absorbance of sample.

The graphs of the variation of the absorbance as a function of the concentration allowed us to determine the IC₅₀ (concentration corresponding to 50% inhibition).

β-carotene bleaching test

The ability of aqueous and methanolic extracts of *Paronychia argentea* L. to prevent β -carotene bleaching is determined according to the method described by Alam et al. (2013), with some modifications. Readings of all samples were performed immediately after ($t = 0$ min) and after 120 min of incubation. Antioxidant activity is expressed as a percentage of inhibition compared with the negative control according to equation 2:

$$I\% = \left[1 - \frac{(As - As_{120})}{(Ac - Ac_{120})} \right] \times 100 \quad (2)$$

As: Initial absorbance of sample; As120: Absorbance of sample at 120 min; Ac: Initial absorbance of negative control; and Ac120: Absorbance of negative control at 120 min.

2.8. *In vitro* study of antilithiasis activity

To assess the anti-lithiasis activity of our extracts, we used three different methods. For the first two techniques, all samples were analyzed in triplicate.

The kinetics of oxalo-calcic crystallization through turbidimetry

With minor modifications to the work of Bensatal and Ouhrani (2008) we perform this analysis without and with an inhibitor-containing extract solution (25, 50, 75, and 100%) against a positive control (1.875 mM trisodium citrate) at the same dilutions. The percentage of inhibition is calculated according to formula 4.

$$I\% = \frac{(T_c - T_i)}{T_c} \times 100 \quad (4)$$

T_c is the turbidimetric slope without inhibitor; T_i is the turbidimetric slope with inhibitor.

The kinetics of oxalo-calcic crystallization through microscopy

In accordance with Agarwal and Varma (2014), the turbid solution (without and with inhibitors) with a determined volume is placed under a microscope for examination. The manipulation is repeated against a positive control (1.875 mM trisodium citrate) at identical dilutions. The percentage of inhibition is calculated using formula 5.

$$I\% = \frac{(TSI - TAI)}{TSI} \times 100 \quad (5)$$

TSI and TAI are the number of calcium oxalate crystals formed in the absence and the presence of the inhibitor, respectively.

Dissolution of calculi tracking

In this method, we *ex-vivo* monitored the effects of various extracts by following the mass variation of natural calculi over seven days for six weeks (HANNACHE et al., 2012). For this purpose, stones were collected from a patient suffering with urinary lithiasis at the Msila Hospital urology department. Their weights varied from 187.4 to 412.6 mg. They were analyzed by Fourier transform infrared spectrophotometry (FTIR) using the KBr pallet. The spectrum was recorded on an FTIR spectrometer (Shimadzu-1800S) between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1} .

Seven Erlenmeyer flasks, including 25 mL of EAQ1, EAQ2, EMeOH1, and EMeOH2, infused in distilled water (INF) and infused in physiological saline at a concentration of 8 mg/mL (INF (NaCl)), were prepared. The seventh Erlenmeyer flask served as the negative control (physiological saline, 9 g/L). Subsequently, the calculi were taken into porous woven fiber pouches and placed in the Erlenmeyer flasks. The efficacy of the extracts is expressed as the dissolution percentage (MEIOUET et al., 2011), calculated by formula 6:

$$a\% = \frac{(W_{\text{initial}} - W_{\text{final}})}{W_{\text{initial}}} \times 100 \quad (6)$$

a% is the dissolution rate of the calculus; W_{initial}, W_{final} are the weights of the calculi before and after incubation in different solutions.

2.9. Statistical analysis

In the various experiments carried out, results are expressed as mean ± SD in order to perform an analysis of variance (ANOVA). Calibration lines are calculated by simple linear regression.

Significant differences between the anti-lithiasis activity results of the different extracts and those of citrate, obtained by microscopy, are determined by a controlled two-factor analysis of variance with a risk of error α set at 5%.

Significant differences between antioxidant and antilithiasis (turbidimetric) activity results are determined by controlled single-factor analysis of variance, followed by the Tukey/Dunnet test for multiple comparisons at the 5% threshold. All statistical calculations were performed using GraphPad Prism.

3. Results and discussion

3.1 Determination of water content

Plants are naturally rich in water, which makes up between 60 and 80% of their composition. However, to ensure optimal preservation, the water content must be kept below or equal to 10%. The plant that we studied had a water content of 12% in the dry matter. Although this value is slightly higher than the ideal range, it still allows for adequate preservation. This is particularly useful when considering the fresh harvest, including flowers rich in volatile compounds (BOURKHISS et al., 2009).

3.2 Analysis of plant extraction

The results of our extractions reveal, firstly, the richness of *Paronychia Argentea* in highly polar compounds. This leads to high yields of EAQ and EMeOH extracts as opposed to EDCM and EAE extracts (Table 1).

Secondly, the yields of the extracts obtained by the second method exceed those of the first. This is attributed to the presence of compounds with polarities close to those observed in the two or three solvents.

A comparison with a previous study on the same species in the Mechria region revealed differences in yields as a function of the extraction method used (MOHAMMEDI, 2013).

According to Majhenic et al. (2007), the content of dry extracts varies not only between different plants of the same family but also depending on the extraction parameters such as temperature, solvent, particle size, and solvent diffusion coefficient (MAJHENIC et al., 2007). In addition, a similar study carried out in the Marrakech region, on a related species led to comparable results in terms of index (BRAHIM et al., 2015).

Table 1 - Yields obtained from two extraction methods

The extract	Weight of dry residue (g)		Weight of the dry extract (g)		Yeld%	
	M1	M2	M1	M2	M1	M2
EEP	100	100	3.4	3.4	3.4	3.4
EDCM	96.4	24.4	0.9	0.21	0.9	0.9

EAE	93.4	23.7	1	0.4	1.07	1.6
EMeOH	87.7	24	10.4	3.8	11.8	12.8
EAQ	78.8	25.1	5.1	4.9	6.4	17.9

3.3 Determination of foaming index

A persistent foam formation reaching 1 cm with a foam index of 222.222 attests to the saponin richness of our plant, a result very similar to that observed in this region of Morocco.

3.4 Phytochemical analysis

Examining the *total polyphenol* results, it is clear that some extracts contain high polyphenols, such as EMeOH2, EMeOH1, EAE1, EAQ1, and EAQ2, while others have lower values (EDCM and EAE2). These differences are due to the medium polarity of DCM and ethyl acetate.

Statistical analyses have confirmed significant differences in the total polyphenol contents among the various extracts at a 5% significance level. Previous studies have demonstrated variable polyphenol contents depending on the extraction method used (GONÇALVES et al., 2013).

In particular, extracts EAE2 and EMeOH1 exhibit high *flavonoid* contents, while extracts EDCM and EAE1 show lower values.

3.5. Antioxidant activities

Total antioxidant capacity

The total antioxidant capacity of the extracts studied is expressed as ascorbic acid, BHT, and BHA equivalents, derived from the calibration curves of each standard (Table 2).

Table 2 shows that EMeOH2 has the highest antioxidant power (272.22 µg EVc/1mg extract), followed by EMeOH1 and EAQ2 (259.35 and 257.03 µg EVc/1mg extract), respectively, while EAQ1 has the lowest power (182.40 ± 2.99 µg EVc/1mg extract), with all three standards. This observed potency may be due essentially to the richness of the extracts in polyphenol and flavonoid content.

Table 2 - Total antioxidant activity of various extracts of *Paronychia argentea* L.

Extracts	Ascorbic acid (µg EVc/1mg extract)	BHT (µg EBHT/1mg extract)	BHA (µg EBHA/1mg extract)
EMeOH2	272.22±3.33 ns	266.39±3.33 ns	276.48±3.33 ns
EMeOH1	259.35±5.69 ns	253.52±5.69 ns	262.68±5.69 ns
EAQ2	257.03±10.26 ns	251.20±10.26 ns	260.37±10.26 ns
EAQ1	182.40±2.99***	176.57±2.99***	185.74±2.99***

Statistical variations between extracts reveal:

- The existence of a highly significant difference ($P < 0.05$) between EAQ1 and the three extracts: EAQ2, EMeOH1, and EMeOH2.
- A non-significant difference ($P < 0.05$) between extracts EAQ2, EMeOH1 and EMeOH2.

It should be noted that the total antioxidant effect exerted by these extracts is very considerable compared with many results, citing as an example the work carried out by Abbasi et al. (2015) on *Silene conoidea* L. and *Stellaria media* L. (family Caryophyllaceae), in the same family as *Paronychia*

argentea L., which showed that the antioxidant power of the acetone extract was greater than that of the aqueous extract for *Silene conoidea* L. and *Stellaria media* L.

Ferric reducing power

The methanolic extracts had the best *reducing power*, with EMeOH2 being the best reductant of ferric ions ($EC_{0.5} = 342 \mu\text{g/ml}$), followed by EMeOH1. Aqueous extracts showed low reducing power (Table 3).

Statistical analysis shows that all $EC_{0.5}$ values for the four extracts differ significantly at the 5% level from each other and from the three standards.

Sait et al. (2015) reported *the reducing power* of ethanolic, infused, and decocted extracts of the same species, but from western Bejaia. The ethanolic extract proved to be the best reducer, with an $EC_{0.5}$ of $188 \mu\text{g/ml}$, while the infused and decocted extracts had $EC_{0.5}$ of around $357 \mu\text{g/ml}$ and $518 \mu\text{g/ml}$, respectively.

The difference in potency between their extracts and ours can be attributed to climatic factors and the extraction method used.

Chelation power of ferrous ions

This method uses ferrous ion chelation by extracts to inhibit the formation of the Fe (II)-Ferrozine complex after Fe^{2+} treatment. EDTA is the most commonly chelating agent added in medicines and cosmetic to prevent oxidation (LOMBARDO et al., 2020). The results show that EDTA exerts a 100% chelating effect at a concentration of $60 \mu\text{g/ml}$, while the aqueous extracts, EAQ1 and EAQ2, exert maximum chelating effects of 86% and 82%, respectively, at a concentration of $150 \mu\text{g/ml}$.

Table 3 reveals that the two extracts EAQ1 and EAQ2 show good chelating activity, with EC_{50} values of $38.34 \mu\text{g/ml}$ and $70.34 \mu\text{g/ml}$, respectively, which are not statistically different from EDTA at the 5% level. However, the two methanolic extracts show a very weak chelating effect ($EC_{50} = 2821.54 \mu\text{g/ml}$ and $3150.55 \mu\text{g/ml}$ for extracts EMeOH1 and EMeOH2, respectively), demonstrating the existence of a highly significant difference ($P < 0.05$).

It's very important to point out that the EAQ1 ($38.34\% \pm 0.48 \mu\text{g/ml}$) and EAQ2 ($70.34\% \pm 0.23 \mu\text{g/ml}$) extract results are so similar to those obtained by Gonçalves et al. (2013), on the same species from the Algarve region (southern Portugal), for hot-infused ($39.80\% \pm 8.66 \mu\text{g/ml}$) and cold-infused ($67.29\% \pm 4.49 \mu\text{g/ml}$), respectively.

ABTS.+ cationic radical scavenging

The antioxidant activity of the various extracts is deduced from their ability to trap the ABTS.+ radical obtained from ABTS, compared to the standard antioxidant BHT.

The first observation to be deduced from these results is that all the extracts studied show considerable anti-radical activity and are capable of scavenging the ABTS.+ radical (Table 3).

The two extracts, EMeOH1 and EMeOH2, revealed potent inhibitory activity with IC_{50} of 145.117 and 142.364, respectively, while the EAQ1 and EAQ2 extracts represented lower inhibitory activity with IC_{50} of 675.400 and 320.824, respectively.

The results of the antiradical power of *Paronychia argentea* L. prove that it exhibits a powerful power compared to other species in the Caryophyllaceae family. Balamurugan et al. (2013) found that *Polycarpaea corymbosa* L.'s methanolic extract inhibits free radicals by 29.83% at $800 \mu\text{g/ml}$.

β-carotene bleaching assay

The bleaching kinetics of β-carotene in the absence and presence of *Paronychia argentea* L. extracts and standard antioxidants (BHT) were monitored.

The mean values for percent bleaching inhibition of β-carotene SD are summarized in Table 3.

Table 3 - EC0.5, EC50, IC50, and percentage inhibition values for extracts and standards

Sample	FRAP EC0,5 (μg/ml)	Ferrous chelation EC50 (μg/ml)	ABTS.+ IC50 (μg/ml)	β-carotene Inhibition (%)
EMeOH2	342.33 ± 0.004***	3150.55 ± 1.38***	142.364 ± 0.939 ns	87.583 ± 2.427 ns
EMeOH1	574.33 ± 0.008***	2821.54 ± 0.46***	145.117 ± 0.476 ns	79.066 ± 1.294***
EAQ2	1595.35 ± 0.3***	70.34 ± 0.23ns	320.824 ± 0.818***	85.317 ± 3.318 ns
EAQ1	1799.45 ± 0.1***	38.34 ± 0.48ns	675.400 ± 0.873***	74.627 ± 4.95***
Ascorbic acid	106.48 ± 0.004***			
BHT	152.55 ± 0.004 ns		47.654 ± 0.347 ns	88.635 ± 3.332 ns
BHA	142.55 ± 0.002 ns			
EDTA		15.35 ± 0.0007ns		

These results enabled us to conclude that the EMeOH2, EAQ2, and EMeOH1 extracts exert a strong inhibition power of β-carotene bleaching of the order of 87.583%, 85.317%, and 79.066%, respectively. These values are very close to those of BHT (88.635%), validated by a non-significant difference at the 5% threshold. EAQ1 extract, in turn, exerts a considerable power of the order of 74.627%, with a significant difference at the 5% level.

According to Arkoub-Hamitouche et al. (2020), the antioxidant properties of *P. argentea* aqueous and alcoholic extracts have been documented *in vivo* and *in vitro*. They demonstrated strong prevention of lipid peroxidation as well as a chemoprotective impact by quenching free radicals. This antioxidant efficacy is mostly attributable to their phenolic components (phenolic acids, tannins, and flavonoids), which appear to be critical given the involvement of free radicals in the onset and progression of degenerative diseases such as arthritis, cancer, and Alzheimer's disease, among others.

3.6. *In vitro* study of antilithiasis activity

The kinetics of oxalo-calcic crystallization using turbidimetry

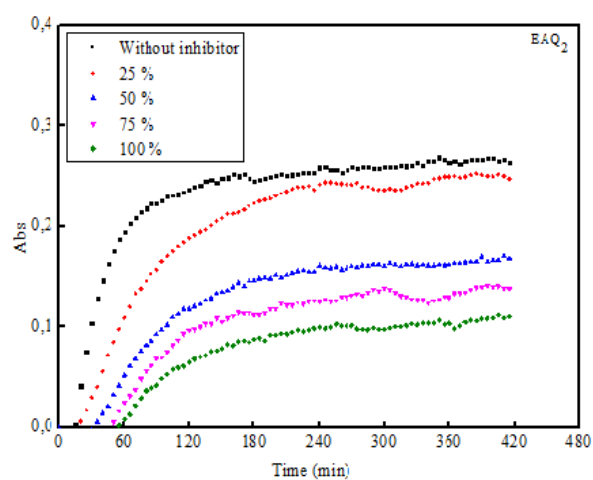
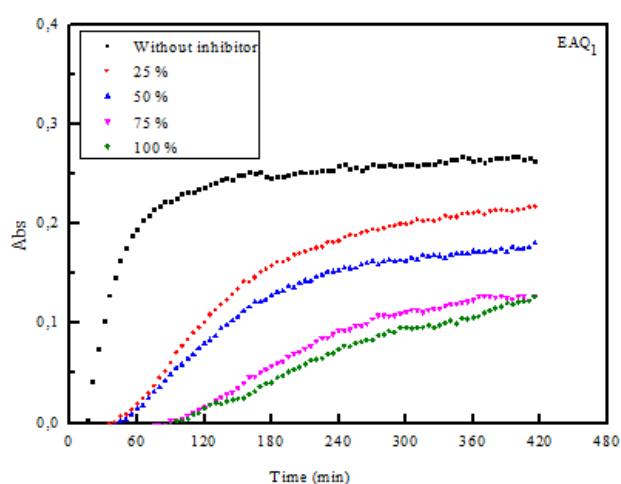
To assess and compare the inhibitory impact of the extracts and monitor the crystallization progression, it is essential to conduct a trial without an inhibitor, serving as a reference. Figure 1 presents the variation in absorbance of calcium oxalate as a function of time, showing the general appearance of crystallization, with the growth stage and crystal aggregation clearly visible. The germination stage, generally defined by a very short induction time, appears in this test within the first 15 seconds.

To evaluate the inhibitory potential of various extracts on crystallization, we tested different concentrations (25%, 50%, 75%, and 100%) alongside a reference, sodium citrate. We concluded that all curves showed a trend towards crystallization based on the curves obtained (Figure 1). The tested extracts demonstrated varying degrees of inhibitory effect, and the profiles of the anti-lithiasis power obtained indicate that their inhibitory capability is dose-dependent.

The evaluation of the inhibitory power is determined by the percentage of inhibition. The various inhibition percentages, as well as the slope values and regression coefficients, are listed in Table 4.

Table 4 - Turbidimetric parameters of oxalo-calcic crystallization with various inhibitors

The extract	The concentration (%)	The slope	R	Inhibition (%)
EMeOH1	25	0.0015	0.996	74
	50	0.0014	0.983	76
	75	0.0013	0.974	78
	100	0.0011	0.987	81
EMeOH2	25	0.0013	0.992	79
	50	0.0010	0.973	83
	75	0.0009	0.970	85
	100	0.0007	0.976	87
EAQ1	25	0.0012	0.994	79
	50	0.0009	0.992	84
	75	0.0006	0.993	90
	100	0.0004	0.991	92
EAQ2	25	0.0023	0.989	61
	50	0.0014	0.982	76
	75	0.0012	0.984	80
	100	0.0010	0.978	82
Citrate	25	0.0008	0.98	85
	50	0.0006	0.986	89
	75	0.0001	0.989	98
	100	0.0001	0.972	99



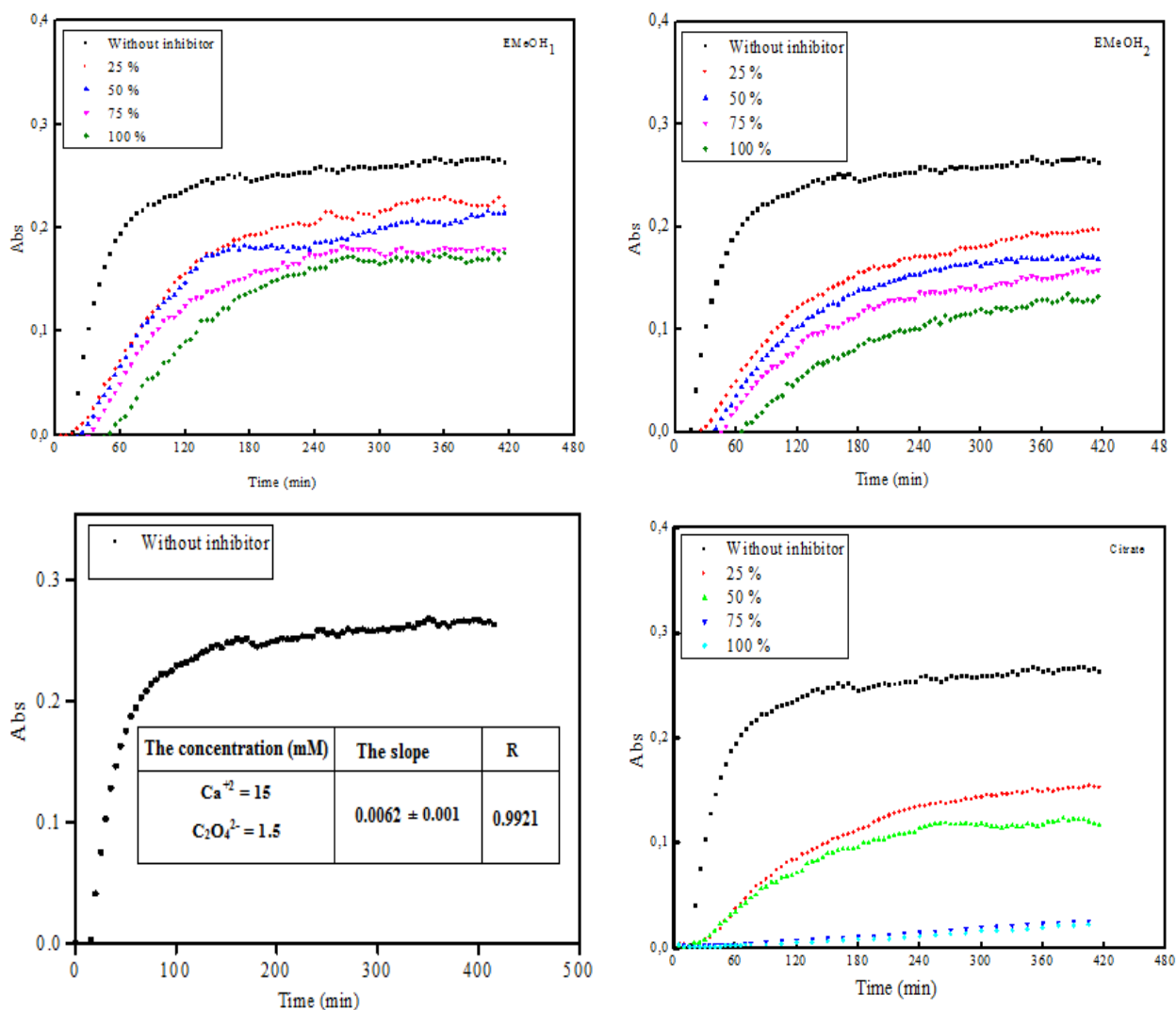


Figure 1 - Inhibitory effect on oxalo-calcic crystallization over time at various concentrations (25%, 50%, 75%, and 100%) for extracts EAQ1, EAQ2, EMeOH1, EMeOH2, and citrate.

The decrease in turbidimetric slope directly reflects inhibitory effectiveness. The minimum value (0.0004) was observed with EQA1 at a concentration of 100%, yielding an inhibition percentage of 92%, which is relatively close to that of citrate (at the same concentration), showing almost complete inhibition (99%). It's notable that the analysis of variance ($p < 0.05$) indicates a significant difference between them.

The extracts EMeOH2 (75% and 100%) and EAQ1 (75%) also exhibited significant anti-lithiasic activity, with inhibition percentages of 85, 87, and 90%, respectively. It's worth noting that EAQ1 at 75% is more effective than EMeOH2 at 100%. A significant difference was observed both among the inhibitory effects of the extracts themselves and between the extracts and citrate, as confirmed by statistical analysis at a 5% significance level.

Following oxalo-calcic crystallization through microscopy

The evolution of the anti-lithiasic power can be monitored through an optical microscope, which provides visual access to the different stages of oxalo-calcic crystallization. This method can be used to study the inhibitory effect of different extracts based on two controlled parameters: time and inhibitor concentration.

Figure 2 depicts photos of the crystallization reaction taken at 30 seconds, 5, 15, 30 minutes, and 1 hour, leading to the following deductions:

The reaction is very rapid, marked by the appearance of small crystals within the first 30 seconds (Photo 2a), which is explained by the reduced germination time. The size of these crystals increases over time, accompanied by a decrease in their numbers. Photo 2d illustrates the aggregation of already-formed crystals, while Photo 2e shows almost complete aggregation.

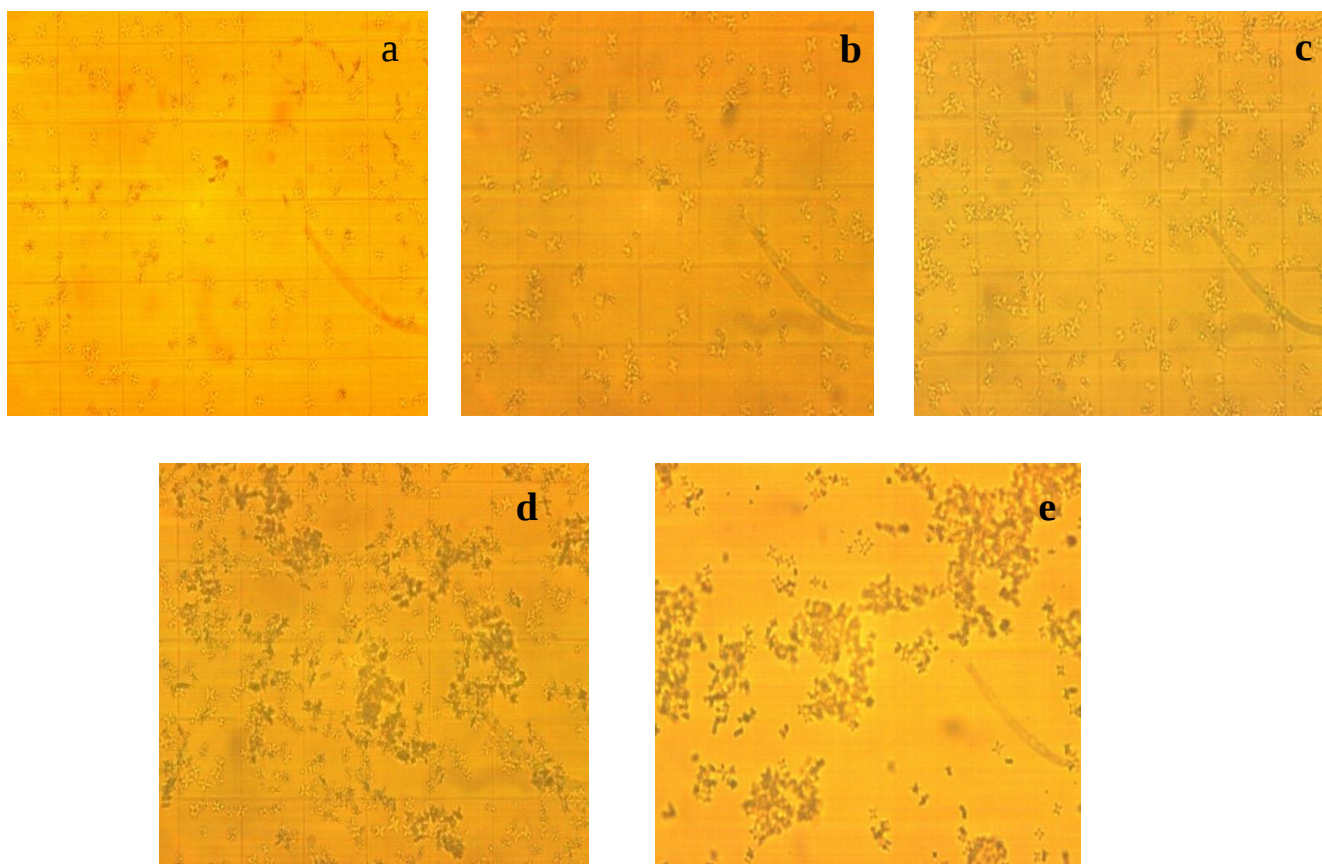


Figure 2 - Images of oxalo-calcic crystallization without inhibitor taken by optical microscope (X 40) after 30 s (a), 5 min (b), 15 min (c), 30 min (d), and 1 hour (e).

We selected the extracts EAQ1, EAQ2, EMeOH1, and EMeOH2 to evaluate their inhibitory power, comparing them with the effect of citrate on oxalo-calcic crystallization.

Microscopic observation revealed that the extracts reduce the size of crystals with a significant decrease in their number, and they also prevent aggregation (Figure 3). The inhibitory power is estimated by calculating the inhibition percentage of each extract at different stages of crystallization and concentrations and comparing them to citrate (Table 5).

It is confirmed that at a high concentration, the inhibitory power of citrate shows inhibition percentages higher than 94%. At low concentrations (25% and 50%), the EMeOH2 extract exhibits a significant percentage of inhibition (91% and 93%) after 1 hour, with no significant difference at the 5% threshold compared to citrate.

The extracts EAQ1, EMeOH1, and EMeOH2 at concentrations of 75% and 100% demonstrated strong inhibitory power, with inhibition percentages of 90% and 97%, respectively. The majority of these percentages show no significant difference compared to citrate ($p < 0.05$). The

analysis of variance for two controlled factors reveals their significant influence on the experimental results, with a highly significant interaction between the two factors ($p < 0.05$).

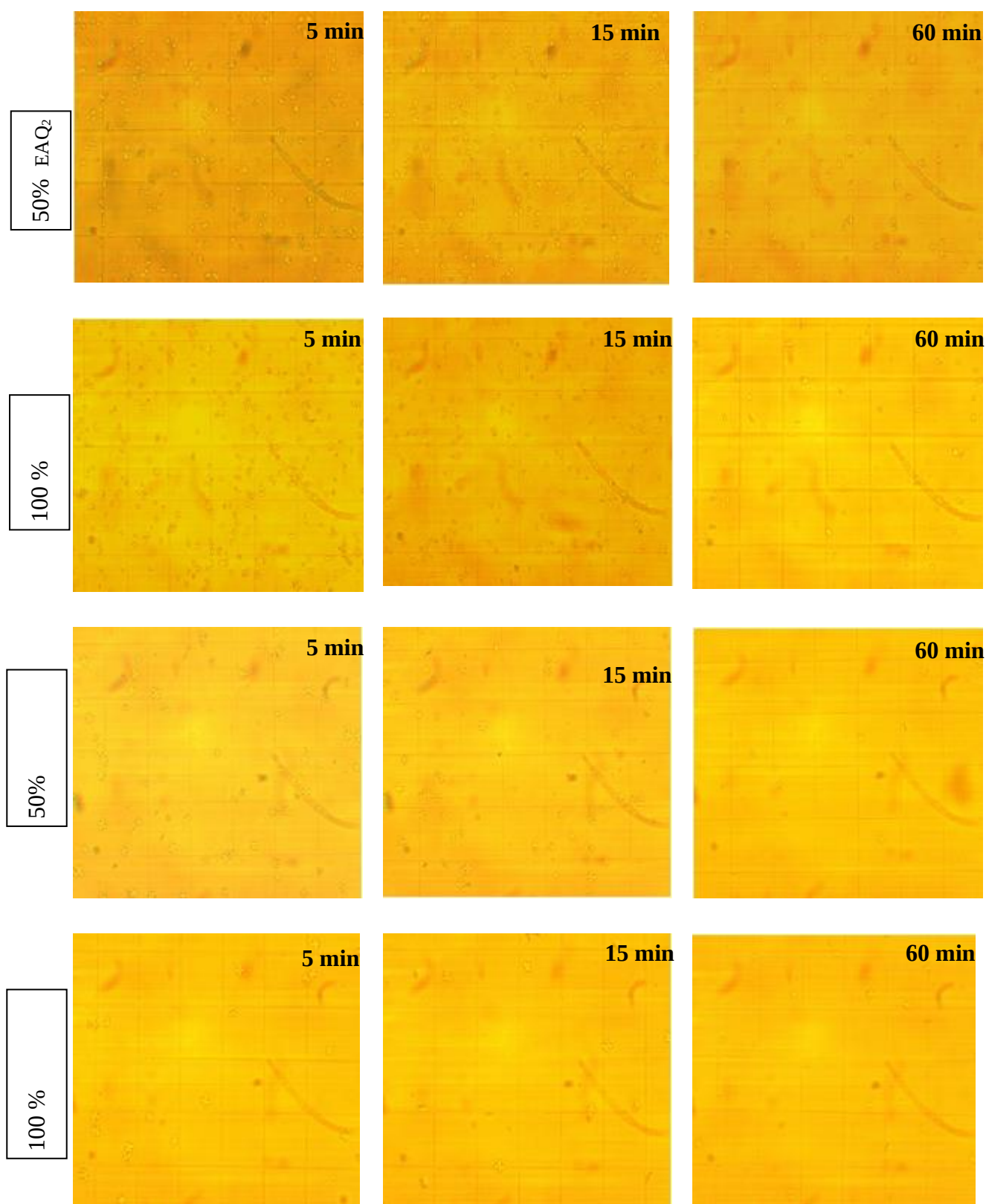


Figure 3 - A photograph showing the effect of EMeOH and EAQ extracts at concentrations of 50 and 100% on oxalocalcic crystallization for 5, 15, and 60 min, taken with an optical microscope (X40).

Table 5 - Percentage inhibition of various inhibitor

	Extract	Time				
		30 s	5 min	15 min	30 min	1 h
25	EAQ1	13 ± 0.57***	32 ± 2.22***	49 ± 1.14***	56 ± 2.07***	60 ± 0.68***
	EAQ2	40 ± 0.38***	46 ± 1.34***	61 ± 0.65***	68 ± 0.91***	73 ± 1.42***
	EMeOH1	40 ± 1.23***	47 ± 1.34***	60 ± 0.56***	64 ± 0.91***	82 ± 1.06 ns
	EMeOH2	35 ± 1.23***	44 ± 0.30***	64 ± 0.65***	74 ± 0.68***	91 ± 0.68 ns
	Citrate	61 ± 0.76 ns	66 ± 0.53 ns	75 ± 0.49 ns	84 ± 0.91 ns	89 ± 1.18 ns
50	EAQ1	31 ± 1.53***	64 ± 0.30***	70 ± 1.31***	79 ± 1.22***	82 ± 1.04***
	EAQ2	54 ± 0.44***	65 ± 0.81***	73 ± 0.65***	75 ± 0.59***	80 ± 0.79**
	EMeOH1	52 ± 0.01***	56 ± 0.53***	66 ± 0.49***	74 ± 0.68***	82 ± 1.46***
	EMeOH2	51 ± 0.96***	56 ± 0.53***	73 ± 0.48***	80 ± 0.56**	93 ± 0.68 ns
	Citrate	81 ± 0.58 ns	83 ± 1.63 ns	92 ± 0.24 ns	92 ± 1.22 ns	95 ± 0.68 ns
75	EAQ1	47 ± 2.11***	64 ± 0.81***	86 ± 0.65***	87 ± 0.58**	90 ± 0.68 ns
	EAQ2	63 ± 0.38***	72 ± 1.11***	76 ± 0.60***	81 ± 0.34***	89 ± 0.68***
	EMeOH1	57 ± 0.30***	70 ± 0.80***	73 ± 0.24***	81 ± 1.22***	94 ± 0.39 ns
	EMeOH2	69 ± 0.58***	73 ± 0.61***	86 ± 0.65***	91 ± 0.58*	94 ± 0.93 ns
	Citrate	90 ± 0.38 ns	90 ± 0.30 ns	94 ± 0.43 ns	96 ± 0.58 ns	97 ± 0.86 ns
100	EAQ1	68 ± 0.58***	72 ± 0.53***	91 ± 0.56**	92 ± 0.58**	96 ± 0.39 ns
	EAQ2	73 ± 0.58***	85 ± 0.50***	84 ± 0.49***	85 ± 0.60***	89 ± 0.01***
	EMeOH1	63 ± 0.38***	79 ± 0.92***	82 ± 0.89***	89 ± 0.34***	97 ± 0.68 ns
	EMeOH2	86 ± 0.50***	85 ± 0.53***	90 ± 0.65**	95 ± 0.30 ns	95 ± 0.40*
	Citrate	95 ± 0.22 ns	94 ± 0.30 ns	98 ± 0.34 ns	98 ± 0.58 ns	99 ± 0.39 ns

* significant difference between extracts and citrate, ns difference not significant ($p < 0.05$).

The dissolution process of urinary stones (*ex-vivo* method)

To enhance comprehension of our plant's inhibitory effect, we initiated a third method. This method involves monitoring the mass loss of urinary calculi under the influence of various extracts during a 6-week incubation period.

The infrared analysis results (Figure 4) of urinary stones from patients with urinary diseases indicate the presence of oxalate. This was confirmed by the appearance of the following peaks:

- The oxalate anion's C=O antisymmetric and symmetric stretching modes [$\gamma_s(\text{CO})$ and $\gamma_s(\text{CO})$], in the wave numbers 1700-1600 cm^{-1} and 1400-1200 cm^{-1} , respectively. For hydrated compounds, the HOH bending mode also contributes in the $\gamma_s(\text{CO})$ region (CASADIO et al., 2019 ; MAMMATE et al., 2022). The bending mode $\delta(\text{OCO})$ in the range of 830-770 cm^{-1} was identified (SILVERSTEIN et al., 1962).

According to Roy et al. (2012), other low-energy vibrations are related to mixed vibrations that arise from stretching and bending modes, including water in the lattice and ring deformation. Similarly, the higher wavenumber broad absorption around 3450 cm^{-1} is related to the OH group due to the presence of water (ROY et al., 2012).

This finding helps to identify urinary stones as *oxalocalcic* type, providing important information for anti-lithiasis testing.

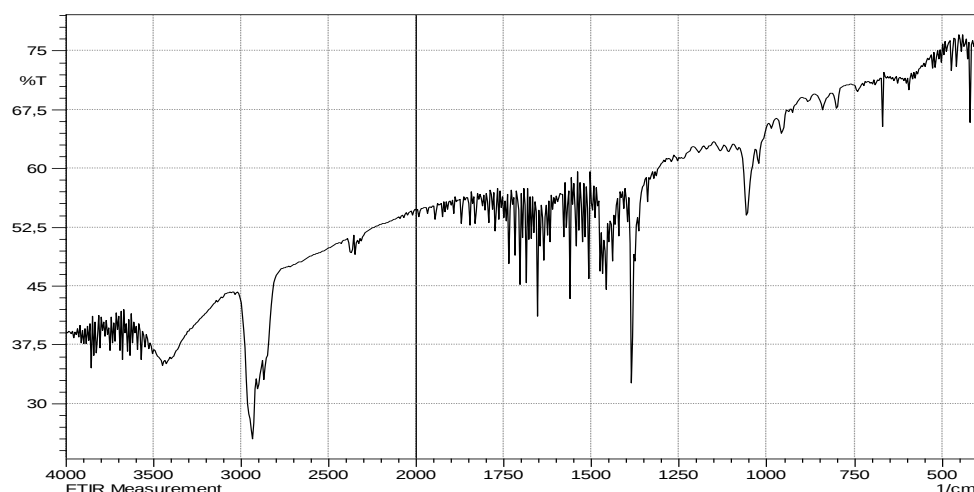


Figure 4 - The infrared spectrum corresponds to oxalocalcic urinary lithiasis.

The calculated dissolution rates, expressed as percentages, are summarized in Table 6.

Table 6 - Dissolution rate of urinary stones from various extracts

The extract	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
INF (NaCl)	2.06	4.9	9.4	11.5	13.1	14.8
INF	2.02	6.7	7.8	10.4	21.8	25.4
EAQ1	2.00	5.1	7.3	25.05	32.9	36.8
EAQ2	5.1	7.1	10.07	39.4	47.9	52.3
EMeOH1	4.2	7.9	8.5	18.2	24.7	31.3
EMeOH2	3.1	8.3	15.04	20.6	34.2	42.6
NaCl(9 g/l)	1.7	5.7	9.4	10.5	12.2	13.1

Examination of these results indicates that dissolution kinetics appear to vary depending on the incubation extract.

During the first two weeks, mass loss varies slightly. Except for the extract prepared by infusion in physiological medium, there is a significant loss of mass after three weeks. The EAQ2 and EMeOH2 extracts proved to be the most effective for stone dissolution, achieving dissolution rates of 52.3% and 42.6%, respectively.

According to Gürocak et al. (2006), although the exact mechanism of action for each remedy remains unknown, commonly recognized extracts help prevent the formation of urinary stones (GÜROCAK et al., 2006). They accomplish this by altering the ion composition of the urine or by acting as diuretics. Some of these extracts also contain *saponins* that can break down mucoproteins, substances that promote crystal formation. In addition, some herbal remedies have antimicrobial properties, protecting a layer of mucus that prevents stones from adhering and causing urolithiasis.

The process of dissolving oxalic stones in the presence of extracts from our plant seems to be related to the action of some active compounds. A plausible hypothesis is the formation of *oxalate-active compound complexes*. The phytochemical study of the aerial part of our plant reveals the presence of flavonoids, polyphenols, tannins, saponins, etc. These active compounds can form different complexes, such as oxalate-flavonoid, oxalate-tannin, oxalate-polyphenol, and oxalate-saponin.

The formation of these complexes seems to be controlled by hydrogen bonds and hydrophilic interactions between the functional groups of the active compounds and the oxalate ion (-OOC-COO-) derived from calcium oxalate, as suggested by Mecheri et al. (2023) and Meiouet et al. (2011)

(urinary stones in these references are based on cystine). These findings open interesting perspectives for understanding the mechanisms of oxalic stone dissolution through the specific properties of the active compounds of *Paronychia argentea* L.

According to the study of El Habbani et al. (2021), the complexes formed are more soluble than calcium oxalate, leading to the dissolution of stones while maintaining high levels of dissolved calcium oxalate in the solution. A mechanism of action was also suggested by them, involving an interaction between calcium oxalate and the molecules.

Conclusion

All the results found prove the valuable interest of the Arab population for the plant *Paronychia argentea* L. which is widely used as tea for the treatment of several diseases. This plant's extracts can be excellent medicines for dissolving oxalocalcic stones. All antioxidant activities yielded positive results, indicating significant potential for the use of the extract in various contexts. The exciting prospect of *in vivo* testing promises to validate and build on these encouraging results.

Conflicts of interest

The authors declare no conflicts of interest regarding the work presented here.

Authors' contribution

Haffar Hichem - execution of the experiment and work corrections; Chetouani Asma - work corrections and revision of the text.

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