

PHYTOCONSTITUENTS EFFECTS OF TRADITIONNALLY HERBES ON DISSOLUTION AND INHIBITION OF KIDNEY STONES (CAOX)

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Abstract

Many developing countries employ medicinal plants for the treatment of kidney stone dissolution. Patients consume aqueous extracts without precise knowledge of the plant's composition. This study aimed to expand the phytochemical knowledge of traditional herbal remedies and evaluate their constituents for the dissolution and inhibition of kidney stones. The herbal plants selected were *Ammi visnaga*, *Nigella sativa*, *Berberis vulgaris*, *Haloxylon scoparium*, *Atriplex halimus* and *Arthrophytum schmittianum*. An extract of each plant was prepared by the infusion method in physiological serum; the extract was filtered and put in the presence of calculi for eight weeks under magnetic agitation. The calculi were weighed biweekly after undergoing a drying process at 40°C for 18 hours, and the pH of the solution was consistently determined. *Ammi visnaga*, *Nigella sativa* and *Haloxylon scoparium* caused significant mass loss and thus had a considerable influence on the dissolution and *in vitro* inhibition of calcium calculi. In conclusion, this study shows that plants containing coumarins, alkaloids, triterpenes and tannins affect the dissolution and inhibition of kidney stone calcium oxalate.

Rezumat

Plantele medicinale se utilizează pentru tratamentul calculilor renali, însă pacienții consumă fitopreparatele fără a cunoaște cu exactitate compoziția acestora. Studiul a evaluat constituenții fitopreparatelor pentru dizolvarea și inhibarea calculilor renali. Plantele medicinale selectate au fost *Ammi visnaga*, *Nigella sativa*, *Berberis vulgaris*, *Haloxylon scoparium*, *Atriplex halimus* și *Arthrophytum schmittianum*. A fost preparat un extract din fiecare plantă prin metoda infuziei în ser fiziologic; extractul a fost filtrat și evaluat în prezența calculilor timp de opt săptămâni sub agitație magnetică. Calculii au fost cântăriți de două ori pe săptămână, după ce au fost supuși unui proces de uscare la 40°C timp de 18 ore, iar pH-ul soluției a fost determinat în mod constant. *Ammi visnaga*, *Nigella sativa* și *Haloxylon scoparium* au provocat pierderi semnificative de masă și, prin urmare, au avut o influență considerabilă asupra dizolvării și inhibării *in vitro* a calculilor de calciu. În concluzie, acest studiu arată că produsele vegetale care conțin cumarine, alcaloizi, triterpene și taninuri influențează dizolvarea și inhibarea calculilor renali de oxalat de calciu.

Keywords: phytoconstituents, dissolution, calcium oxalate, turbidimetric method

Introduction

Kidney stones (KS), also known as renal calculi, are quite prevalent and have had a substantial rise in occurrence in recent times. Approximately 80% of KS consists of calcium oxalate (CaOx), making it a common type. These stones are typically a result of dietary deficiencies, drug usage, and heightened levels of toxicity inside the body [1]. Kidney deposits are accumulations of toxins that develop within the kidney, which has a vital function in the purification and filtration of compounds or chemicals from the blood and urine.

However, when these toxins accumulate in large quantities, they lead to the genesis of KS, and often they are formed when the urine contains a high concentration of calcium, oxalate or phosphorus [2]. Diet is a crucial lifestyle factor that can have a direct impact on health [3]. Many molecules play a protective and inhibitory role against KS development, such as osteopontin and citrate [4, 5], polyelectrolytes [6], amino acids, acid-rich proteins [7], Tamm-Horsfall protein [8] and organic acids [9]. Medicinal herbs have been essential to maintaining and improving human health since ancient times. Numerous studies have confirmed the efficiency of plants on KS in Tunisia, Morocco, Algeria and

other countries, such as *Ammodaucus leucotrichus* [10], *Herniaria hirsuta*, *Ammi visnaga*, *Zea mays* et *Opuntia ficus-india* [11-13], *Tribulus terrestris* [14], *Viburnum opulus* [15], *Rosmarinus officinalis* L., *Origanum compactum* Benth, *Artemisia herba-alba* Asso and *Mentha pulegium* L [16], *Prunus cerasoides* D [17], *Rubia tinctorum* L [18]. Medicinal herbs are sources of vitamins, minerals, and antioxidants; they aid the elimination of crystals outside the body, thus decreasing the creation of KS. In Algeria, there is an increasing interest in medicinal plants and phyto-preparations for treating KS. The purpose of this study was to expand the phytochemical knowledge of traditional plants and to evaluate their components for the dissolution and inhibition of CaOx crystallisation.

Materials and Methods

Stones

Six stones, of whewellite and weddellite types, coming from the urology service of the University Hospital Centre in Sidi Bel Abbes, Algeria, have been compared with the stone studied by Daudon *et al.* [2].

Plant material

The dried plants were purchased from the herbarium in Algeria. A voucher specimen was deposited in the herbarium of the Department of Pharmacy, Faculty of Medicine, Djillali Liabes University, Algeria, and was identified, authenticated, and certified by Dr. A. Selka. Then, the herbs were washed in running water and dried at room temperature away from light. The samples were crushed and sieved to obtain a homogeneous granular structure and stored in glass vials for further analysis.

Extraction method

A saline solution was prepared by adding 9 g NaCl to 1 L of distilled water, and 3 g of powders from each plant were added to 100 mL of boiling saline water; it was infused for 30 minutes and then filtered. The stone was located in a porous bag and then immersed in the extract solution under constant agitation. The codes for the six aqueous extracts (AqE) are as follows: *Arthrophytum chmittianum*-AqEAc; *Berberis vulgaris*-AqEBv; *Atriplex halimus*-AqEAh; *Ammi visnaga*-AqEAv; *Haloxylon scoparium*-AqEHs; *Nigella sativa*-AqENs. For each experiment, every 15 days, the pH of the solution was measured. The loss mass of the renal calculi was evaluated by weighing and drying them in a drying stove at 40°C for 18 hours [19].

Qualitative phytochemical assessment

The above-mentioned extracts were subjected to phyto-chemical screening using the standard protocol and reagents [20]. Mayer's test was used to detect alkaloids. A few drops of Mayer's reagent were added to 1 mL of extract, and the presence of alkaloids was indicated by the formation of a yellowish or white precipitate. A frothing test was used to identify saponins; exactly 0.5 g of the extract was dissolved in distilled water in a

test tube, and frothing that persisted after warming was considered preliminary evidence for saponins. The Staisny reaction (10 mL formalin + 5 mL chlorhydric acid) was used to detect hydrolyzable and condensed tannins. 15 mL of Staisny's reagent was added to 30 mL of extract, and the mixture was heated in a water bath for 15 to 30 minutes. The presence of condensed tannins is revealed by the appearance of a precipitate. Following filtration, the filtrate was saturated with 10 mL of sodium acetate solution, followed by 1 mL of 1% FeCl₃ solution. The presence of hydrolysable tannins is indicated by the formation of a blue-blackish stain. For steroids, 2 mL of chloroform and concentrated H₂SO₄ were added to 5 mL of AqE. The presence of steroids was indicated by the appearance of a red colour in the lower chloroform layer. Triterpenoids were found by treating 5 mL of each extract with 2 mL of chloroform, allowing it to evaporate on the water path, and then boiling it with 3 mL of concentrated H₂SO₄. A grey colour appeared, indicating the presence of triterpenoids. To detect coumarins, 3 mL of 10% NaOH was added to an aqueous extract. Positive results showed a yellow colour. Reducing sugars were identified using the Fehling test, which involved mixing 1 mL of Fehling A and B solutions and boiling them for one minute. An equal volume of test solution was added, after 5 - 10 minutes in a boiling water bath, a yellow precipitate was first observed, followed by a brick red precipitate. Glycosides were determined by mixing 5 mL of each extract with 25 mL of a 10% v/v H₂SO₄ solution. This mixture was heated to boiling for 15 minutes before cooling and neutralising with a 10% w/v NaOH solution. 5 mL of Fehling solution was added to it, and the presence of glycosides was indicated by the red brick precipitate.

CaOx formation

An investigation was conducted to assess the impact of additives on the kinetics of CaOx crystallisation in a water-based solution. We quantified the turbidity of the suspension resulting from the combination of the precipitating reagents. The optical density (OD) of the crystals formed was measured at three seconds intervals, at 620 nm, using a Uvikon 930 spectrophotometer (Kontron Instruments). OD was directly proportional to the mass of crystals formed *per* unit volume of calcium chloride and sodium oxalate; 20×10^{-3} M and 1×10^{-3} M solutions were buffered at pH = 5.5 and brought to an ionic strength of 0.15 M with sodium chloride. A calcium chloride solution of 6×10^{-3} M was prepared from a 20×10^{-3} M solution. Then, a 1.5 mL aliquot of this solution was transferred into a 10 mm light bath maintained at 37°C. After obtaining a stable baseline absorbance value for 10 seconds, crystal formation was induced by adding an equal volume of freshly prepared sodium oxalate solution. Thus, the final concentrations in the test were 3×10^{-3} M and 0.5×10^{-3} M for calcium and oxalate, respectively, with a CaOx product of 1.5×10^{-6} mol²/L [21].

Statistical analysis

The results were expressed as mean \pm standard error ($X \pm ES$) and were analysed using SPSS software (SPSS 15.0; IBM Inc., Chicago, IL, USA).

Results and Discussion

The ability of the extracts to dissolve the stones for 8 weeks is presented in Table I.

Table I

Evolution of the stone's weight during 8 weeks in extract plant

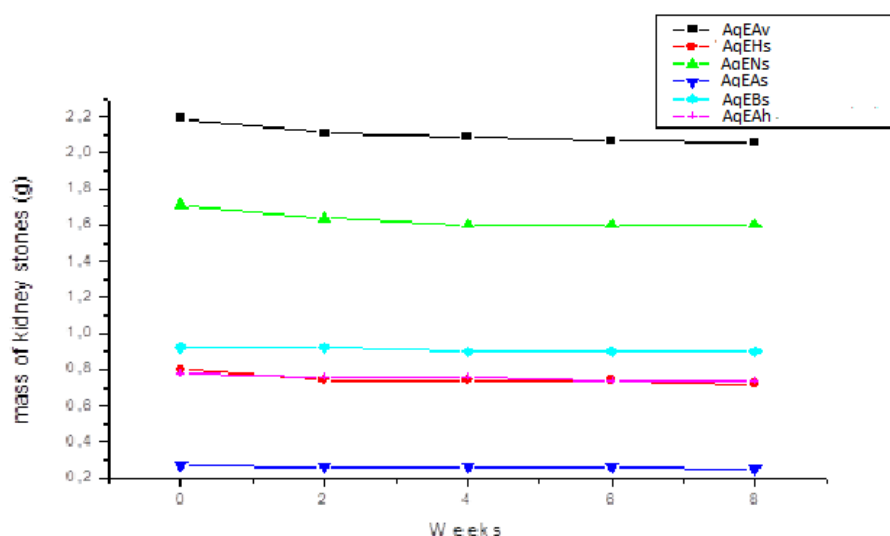
Net weight /week	Stone 1 AqEAv	Stone 2 AqEHs	Stone 3 AqENs	Stone 4 AqEAc	Stone 5 AqEBv	Stone 6 AqEAh
Net weight (g)	2.19	0.80	1.71	0.27	0.92	0.78
2 nd week (g)	2.11	0.74	1.64	0.26	0.92	0.76
4 th week (g)	2.09	0.74	1.60	0.26	0.90	0.76
6 th week (g)	2.07	0.74	1.60	0.26	0.90	0.74
8 th week (g)	2.06	0.72	1.60	0.25	0.90	0.74
A loss of mass (mg)	130 \pm 28%	80 \pm 7%	110 \pm 13%	20 \pm 1%	20 \pm 1%	40 \pm 4%

AqE: Aqueous extract; AqEAv: *Ammi visnaga*; AqEHs: *Haloxylon scoparium*; AqENs: *Nigella sativa*; AqEAs: *Arthrophytum schmittianum*; AqEBv: *Berberis vulgaris*; AqEAh: *Atriplex halimus*

The stone loses anywhere from 20 mg to 130 mg of weight. Regarding the studied plant extracts, the dissolution kinetics seem to behave differently (Figure 1). For eight weeks, AqEAv, AqENs and AqEH

were very effective and noticed a notable stone mass loss of 130 mg, 110 mg and 80 mg, respectively.

Furthermore, the impact of AqEAh, AqEAs and AqEBv on stone dissolution is ineffective with 40 mg, 20 mg and 20 mg stone mass loss, respectively.

**Figure 1.**

Effect of the six medicinal plant extracts on the *in vitro* dissolution of whewellite and wheddellite stones

The initial pH levels of the AqE of all plants were predominantly acidic, with the exception of *Atriplex halimus*, which exhibited a slightly basic pH of 7.39.

After an eight week immersion period, the pH of the stones increased, resulting in a consistent characteristic observed in all the extracts (Table II, Figure 2).

Table II

Evolution of pH solutions for 8 weeks

Selected plants	AqEAv	AqEHs	AqENs	AqEAs	AqEBv	AqEAh
initial pH	5.66 \pm 0.27	5.25 \pm 0.30	5.92 \pm 0.10	5.22 \pm 0.40	5.47 \pm 0.22	7.39 \pm 0.25
2 nd week	9.03 \pm 0.23	9.14 \pm 0.33	8.62 \pm 0.06	8.18 \pm 0.45	8.03 \pm 0.21	8.81 \pm 0.30
4 th week	8.90 \pm 0.20	8.94 \pm 0.20	8.76 \pm 0.06	8.50 \pm 0.40	8.50 \pm 0.22	9.11 \pm 0.28
6 th week	8.97 \pm 0.21	8.94 \pm 0.25	8.76 \pm 0.05	8.81 \pm 0.41	8.85 \pm 0.23	9.16 \pm 0.31
8 th week	9.05 \pm 0.15	8.69 \pm 0.10	8.99 \pm 0.05	8.91 \pm 0.35	8.96 \pm 0.25	9.36 \pm 0.30

AqE: Aqueous extract; AqEAv: *Ammi visnaga*; AqEHs: *Haloxylon scoparium*; AqENs: *Nigella sativa*; AqEAs: *Arthrophytum schmittianum*; AqEBv: *Berberis vulgaris*; AqEAh: *Atriplex halimus*

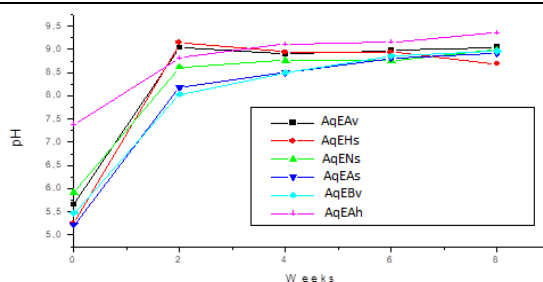


Figure 2.

Evolution of the extract's pH during eight weeks in the presence of renal stones (whewellite and weddellite)

Table III illustrates the results of phytochemical screening, which indicate the presence of various compounds in the six aqueous extracts. The plants used in the preparations were found to be particularly rich in catechic tannins, saponins, sterols and a small number of alkaloids in both AqEAs and AqENs. Alkaloids are abundant in AqEBs, AqEHs and AqEAs, while AqEAh lacks alkaloids. Terpenes and sterols are strongly present in AqEAv, AqEHs, AqEAs and AqENs. Nevertheless, a weak presence in AqEBv and AqEAh was detected. Tanins and saponins are present only in the AqEAh.

Table III

Chemical screening results of aqueous extracts of medicinal plants used for dissolving kidney stones (weddellite and whewellite)

Compound	AqEAv	AqEHs	AqENs	AqEAs	AqEBv	AqEAh
Alkaloids	+	++	+	++	++	+
Saponins	+++	+	+	+	+	
CT		-			-	
HT	+	+	+	+	+	
Steroids		-	-	-	-	-
Triterpenoids		+	+	+	+	++
Coumarins	++	+	-	+	+	-
RC		+		+	+	+
Glycosides		+	+	+	+	+++

CT: Condensed tannins; HT: Hydrolysable tannins; RC: Reducing compound; AqE: Aqueous extract; AqEAv: *Ammi visnaga*; AqEHs: *Haloxylon scoparium*; AqENs: *Nigella sativa*; AqEAs: *Arthrophytum schmittianum*; AqEBv: *Berberis vulgaris*; AqEAh: *Atriplex halimus*

The crystallisation of CaOx was investigated in the presence of AqEAv, AqENs and AqEHs at 37°C. The effect of the extract on crystal growth rate is evaluated by comparing growth inhibition of calcium oxalate crystallisation in the absence ($OD_{control}$) and

in the presence of the extract (OD_{Test}). The CaOx crystallisation inhibitory activity of extract plants increased with increasing concentrations of the extract in a dose-dependent manner, between 0.3 and 0.5 mL (Table IV).

Table IV

Effect of various plant extracts on calcium oxalate crystallisation at 37°C

Amount of additive	Mean	D_0/D_T	SD	Coeff V (%)	I (%)
AqENs (mL)					
0.1	0.01970	1.76	7.683 E-4	3.90	87.32
0.3	0.00862	4.01	4.482 E-4	5.20	94.45
0.5	0.00626	5.52	3.462 E-4	5.53	95.97
AqEHs (mL)					
0.1	0.01904	8.19	9.843 E-4	5.16	87.78
0.3	0.00393	8.80	3.055 E-5	7.77	97.46
0.5	0.00283	12.22	3.035 E-5	1.07	98.17
AqEAs (mL)					
0.1	0.11554	1.35	4.662 E-4	3.00	25.93
0.3	0.01589	9.81	4.830 E-4	3.03	89.81
0.5	0.01514	10.30	4.960 E-4	3.27	90.29

SD: Standard Deviation; Coeff V: Coefficient of variation; AqE: Aqueous extract; AqEAv: *Ammi visnaga*; AqEHs: *Haloxylon scoparium*; AqENs: *Nigella sativa*; AqEAs: *Arthrophytum schmittianum*; AqEBv: *Berberis vulgaris*; AqEAh: *Atriplex halimus*

In the presence of all extracts, the slope of CaOx growth decreased, as shown in Figures 3, 4 and 5. The kinetics of CaOx crystal growth are proportional to the nature of the extract and its volume. At 0.1 mL of AqEAv, the percentage of inhibition is 87.32%, AqENs 87.74% and AqEHs 25%. However, at 0.3 mL of AqEAv, the percentage of inhibition is 94.45%, AqENs is 97% and AqEHs is 89%. Under the conditions of the study, the effect of AqENs at 0.5 mL is more

marked than that of AqEAv and AqEHs. In addition, AqEAv inhibition percentage on CaOx is 95.97% at 0.5 mL of extract, while AqEHs is 90.29%.

Many previous studies regarding the composition of herbal plants, dissolution, or inhibition of kidney stones were based on the organic or inorganic compounds of aqueous extracts.

Our results showed that the extracts obtained from six species are effective against kidney stones. The effect

of the plants increases in the following order: *Ammi visnaga*, *Nigella sativa*, *Haloxylon scoparium*, *Arthrophytum schmittianum*, *Berberis vulgaris* and *Atriplex halimus*. The responsible effect on the dissolution of kidney stones was mainly due to the bioactive compounds inherent in aqueous extracts, such as the principal compounds of AqEAv, two majority substances belonging to the group of furanochromones as khellin and visnagin [22], khellinol, ammiol, khellol glycoside and khellinin. AqEAv also contained fixed oils and coumarins, the main one being the pyranocoumarin visnadin [23]. *Ammi visnaga* is a medicinal plant that has been used for the treatment of several diseases, including urolithiasis (kidney stones) [11 - 24].

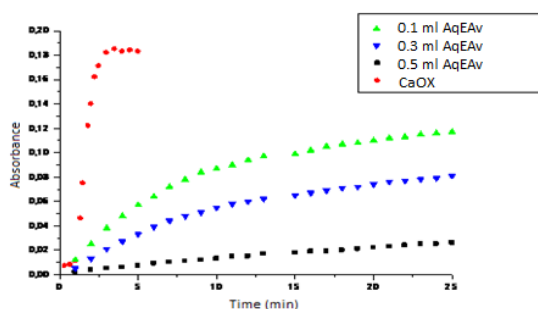


Figure 3.

CaOx crystallisation in the presence of different volume of AqEAv at 37°C

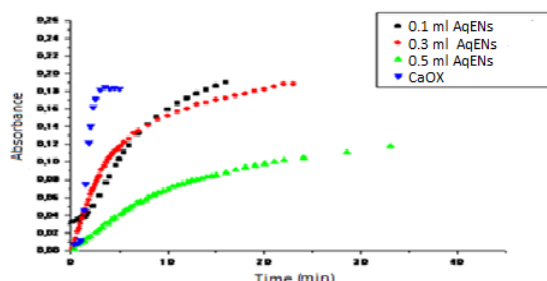


Figure 4.

CaOx crystallisation in the presence of different volume of AqENs at 37°C

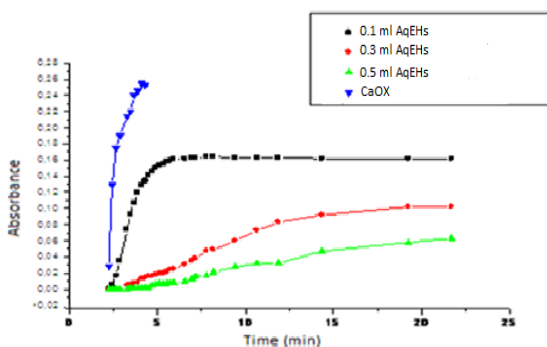


Figure 5.

CaOx crystallisation in the presence of different volume of AqEHs at 37°C

Nigella sativa is an essential source of protein and minerals, such as phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), copper (Cu), iron (Fe), zinc (Zn) and copper (Cr) [25]. The *Nigella* seed proteins are composed of 17 amino acids. The primary present amino acid is glutamic acid, followed by arginine, aspartic acid, leucine and glycine. *Nigella sativa* seeds are rich in unsaturated fatty acids, mainly linoleic acid (58%), oleic acid (22%), eicodadienoic acid (3%) and dihomolinoleic acid (12%) and saturated fatty acids (palmitic and stearic acid) [26, 27]. *Nigella sativa* demonstrated therapeutic properties due to prominent constituents such as nigellicine, nigellidine, thymoquinone, p-cymene, carvacrol, trans-anethole and 4-terpineol [28, 29]. The aqueous extract of *Nigella sativa* is composed of alkaloids, anthraquinones, flavonoids, saponins and tannins [30]. The functional and antioxidant properties of galacturonic acid and glucuronic acid were reported from the aqueous extract seeds of *Nigella sativa* [31]. AqENs seeds are rich in gummy and mucilaginous compounds and possess anxiolytic and anti-inflammatory activities [32].

Haloxylon scoparium (Pomel) in AqE was found to contain alkaloids, triterpenoids and saponins, with a notable abundance of alkaloids [20]. The aerial part of *Haloxylon scoparium* contains various mineral elements, including iron, potassium, magnesium, phosphorus, sodium, copper, calcium, strontium, selenium and zinc [33].

The bark and root of *Berberis vulgaris* are commonly used in the treatment of various diseases, including diabetes, parasitic infections and Alzheimer's disease [34, 35, 36, 37]. *Berberis vulgaris* root bark is a rich source of alkaloids; in addition to berberine, three new alkaloids were investigated: bersavine, muraricine and berbostrejdine [37]. Other compounds were identified, including 8-oxo berberine, berbedine, berbamine, aromoline, obamegine and palmitine, representing tannins, triterpenes and coumarins. Phenolic compounds have been extracted from *Berberis vulgaris* root bark, including N-(p-trans-coumaryl) tyramine, cannabisin G and lyonirosinol [38]. The major components of the bark root extract of *Berberis vulgaris* were tetracosanoic acid, methyl ester, phthalic acid, diisooctyl ester, 1,2-bis(trimethylsiloxy)ethane and 1,2-benzene dicarboxylic acid, diisononyl ester [39]. Among the identified components, three of them, comprising 55.99% of the extract, are fatty acids (aliphatic compounds). *Berberis vulgaris* bark root has also been shown to prevent stone formation in rats [40].

Arthrophytum scoparium is a halophyte used as a traditional medicinal plant that has been reported to have several bioactive compounds such as coumaric acid, cinnamic acid, chrysoeriol, cyanidine, catechol and caffeoylquinic acid. It is also rich in alkaloids such as salsolinol, isosalsoline, N-methylisosalsoline, salsolidine, carnegine, N-methylcorydaldine, tryptamine,

N-methyltryptamine, norsynephrine and flavonol glycosides [41].

Atriplex halimus is native to the Mediterranean and is frequently found on marginal soils and degraded lands [42]. The leaves of *Atriplex halimus* are a halophyte widely used in traditional medicine in Algeria for the treatment of different types of diseases, such as diabetes [43], rheumatism and hydatid cyst, against the protozoa of *Echinococcus granulosus* [44], as it has antioxidant and anti-inflammatory effects [44, 45]. It is a source of plants rich in bioactive compounds, as the most essential phenolic acids are gallic acid, chlorogenic acid, caffeic acid, catechin, vanillic acid, berberine, p-coumaric, trans-cinnamic acid, ferulic acid, 3-hydroxy-4-methyl cinnamic acid (isoferulic acid), carboxylic acid, salicylic acid, rutin, m-anisic acid (3-methyl benzoic acid), myricetin and quercetin [44]. Dessena *et al.* reports that *A. halimus* leaves showed the highest content of all elements P, Na, K, Ca, Mg, Cu, Zn and Mn, except for iron [46].

Certain small molecules and ions, such as sodium and citrate, have the ability to inhibit the formation of calcium oxalate monohydrate crystals and influence the dissolution of CaOx crystals [47]. Hydroxyl acids are anticipated to create calcium metal ion complexes [48]. Over a period of two weeks, the pH of the solution in contact with the aqueous extracts increased, indicating the hydrolysis of CaOx x H₂O and CaOx x 2H₂O, resulting in the formation of Ca(OH)₂.

Plants are rich in organic acids with various health benefits and can inhibit stone formation and dissolve kidney stones [49].

Phytochemicals such as polyphenols, alkaloids, and triterpenes have been found to possess antioxidant properties and exhibit inhibitory and dissolution effects on kidney stones [16-50]. Alkaloids are the second most bioactive found in plants, addressing their protective effects against kidney stone inhibition [51]. In Algeria, the most active plants are *Ammodaucus leucotrichus*, *Ajuga iva*, *Erica multiflora* and *Stipa tenacissima* [10]. In Morocco, various plants, *Herniaria hirsute*, *Opuntia ficus-india*, *Zea mays* and *Ammi visnaga*, are proposed against the dissolution of stones [13]. *D. biflorus* seeds are widely used in South Asia, India, and Pakistan [52]. The extract of *C. album* has been found to have a positive inhibitory effect on the *in vitro* crystallisation of CaOx and CHPD crystals [53]. Furthermore, *T. arjuna* shows promise as a potential inhibitor of CaOx crystallisation [54].

Conclusions

The findings verify that *Ammi visnaga*, *Nigella sativa* and *Haloxylon scoparium* aqueous extracts can dissolve stones and prevent CaOx crystallization *in vitro*. The identification of bioactive compounds in these herbs

has great promise for the development of a more effective urolithiasis preventive treatment.

Conflict of interest

The authors declare no conflict of interest.

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