Urolithiasis (2024) 52:62
https://doi.org/10.1007/s00240-024-01532-z

RESEARCH

Antioxidant activity and Inhibitory effects of *Cydonia oblonga Miller*. leaves extracts against calcium oxalate stones

Ibtissam Elhadri[1] · Latifa Baddade[1,2] · Mohamed Berkani[1]

Received: 12 October 2023 / Accepted: 16 January 2024

Abstract

Urinary stone disease is a widespread health problem in both adults and children, and its prevalence has been increasing worldwide. Various plants preparations have already been used since ancient times in order to treat urolithiasis. The aim of this study is to evaluate the antioxidant capacity and litholytic effect on kidney stones of *Cydonia oblonga Miller.* leaves. The infusion, methanol and acetone extracts were made from *Cydonia oblonga Miller*. leaf at different concentration. Estimation of mass fractions of total polyphenol, flavonoid, and flavonol contents, as well as the in vitro radical scavenging potential on 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH·) of the investigated extracts was carried out using colorimetric methods. The litholytic property of the extracts was performed by an in-vitro model using experimentally prepared kidney stones-calcium oxalate. As results, the quince leaf extracts revealed stronger antioxidant properties in the DPPH assay, which was proved by the semi-maximal inhibitory concentration values, being about 36.06 ± 3.55, 74.15 ± 6.29, and 142.35 ± 5.09 µg/ml for methanol, acetone and infusion extracts respectively. Furthermore, the tested extracts were found to be more effective in dissolving calcium oxalate stones compared to the control solutions, the mass loss is about 15.13 ± 1.10% with methanol extract, while it is 14.77 ± 1.74% and 11.14 ± 2.86% for acetone and infusion extracts respectively. These findings confirm the quince leaf's richness in phyto-components, offering anti-oxidant property and being able to be used as a remedy for the management of kidney stones by dissolving calcium oxalate stones in the kidneys.

Keywords Calcium oxalate stones · XRD · FT-IR · *Cydonia oblonga Miller*. L · Litholytic activity · Antiradical activity

Introduction

Quince (*Cydonia oblonga Mill*.) is a species of the Rosaceae family, subfamily Maloideae and genus Cydonia. The fruits of the quince are common in Morocco, known as sefarjal, their cultivation is concentrated in several regions: Haouz Marrakech, Meknes, Khenifra, Midelt, Gharb, Beni Mellal and Fez, which were recognized as a Moroccan terroir product. Generally, the quince fruit was consumed fresh, cooked or processed. Interestingly, the different parts of quince (fruits and leaves) are an excellent natural source of nutrients and bioactive compounds (such as phenolic acids, flavonoids, tannins and carotenoid) [1–3] with a beneficial impact on health, including diabetes [4], kidney disease [5], cardiovascular [6], respiratory and gital disorders [7, 8]. They can also prevent or retard hemolysis of the human erythrocyte membrane [9, 10] and damage induced by oxidative stress [11–13].

The urinary lithiasis is a frequent affection resulting from the disorder between inhibitors and promoters of the lithogenesis process, which causes the formation of stones in the kidneys or urinary canal. Epidemiological databases studies showed that renal lithiasis can reaches 4–20% of the population according the countries, and it is characterized by the recidivism after treatment [14–16], with a chemical composition dominated by calcium oxalate in nearly 80% of cases [17].

Calcium oxalate crystals are generally present in three different forms: calcium oxalate monohydrate (COM) or Whewellite, calcium oxalate dihydrate (COD) or

✉ Ibtissam Elhadri
ibtissam.elhadri@usms.ma

[1] Laboratory of Applied Spectro-Chemometry and Environment, Faculty of Sciences and Technologies, Sultan Moulay Slimane University, Beni Mellal, Morocco

[2] Transdisciplinary Team of Analytical Sciences for Sustainable Development, Faculty of Sciences and Technologies, Sultan Moulay Slimane University, Beni Mellal, Morocco

Published online: 10 April 2024

Weddellite, and calcium oxalate trihydrate (COT), in which COM is the predominant component found in the renal calculi [18]. Although new techniques for treatment of urinary lithiasis have been developed as Extracorporeal Shock Wave Lithotripsy (ESWL) and percutaneous nephrolithotomy, but dietary therapy remains the most effective way to eliminate kidney stones and to prevent their recurrence.

Therefore, considering the interesting findings of the pharmacologic studies of *Cydonia oblonga Mill* and their potential therapeutic utility on the hand, and on the other hand the absence of profound works on the medicinal potential of quince as natural treatment of urinary lithiasis, especially in the prevention of calcium oxalate stones, in this context, the aim of our study was undertaken to measuring the dissolving power of experimental urinary stones composed of calcium oxalate and antioxidant activity using three extracts made from Moroccan *Cydonia oblonga M*. leaf.

Materials and methods

Plant materials

Fresh and healthy quince leaf samples were collected from El ksiba (located in central Morocco, 45 km from the city of Beni Mellal, 32° 19′ 48″ North, 6° 21′ 0″ West) in October 2020. The leaves were washed several times with distilled water to remove surface dust, after being dried in an oven at 32 °C for 5 days (in the shade), they were ground with a mortar into a fine powder and then stored in an airtight container until use.

Extraction

For preparing methanolic extract, a quantity of leave-powder was soaked with methanol at a ratio of 1:10 (W/V) and kept under continuous agitation for 1 h at 40 °C, the mixture was filtered using filter paper(0.22 µm), the residue was re-extracted with fresh solvent and the process repeated four times. The filtrates were combined, and concentrated to dryness under reduced pressure (40 °C), [1]. The acetone extraction was done in the same way as the first extraction, using cold acetone for 1 h at room temperature. After, elimination of the solvent from the filtrate. The final extracts were stocked in a refrigerator at 4 °C until the analysis was completed.

The extraction yield in relation to dry matter was variable as follows: 59% and 46% for methanol and acetone respective.

Infusion

A standard solution of *C. oblonga* leaves-powder (ca 2.5 g) was prepared in boiling distilled water (150 ml) and allowed to infuse for 60 min. The infusion was filtered under a vacuum passed through a 0.2 m membrane, which was considered to be the crude extract.

Determination of total phenolic content

Total phenols contents in the leaf extracts were determined using Folin-Ciocalteu colorimetric method [19]. The dry extract was diluted to obtain a final absorbance between 0.5 and 1. A standard range was made in aqueous solution (8 concentration points of 0–40 µg/ml) using gallic acid, 0.5 mL of Folin-Ciocalteu reagent (diluted 10 times in ultrapure water) was added to 1 ml of diluted extract or range point. After 5 min, 2 ml of sodium carbonate solution (7.5%) were added. The reaction mixtures stirred thoroughly and incubated for 60 min at room temperature in the dark. Then, the absorbance was measured at 765 nm. The procedure was repeated three times. The concentration of total phenols was calculated from the calibration curve established with gallic acid and was expressed as mg gallic acid equivalent per gram dry weight (dw) extract (mg GAE/g d.w).

Determination of total flavonoid content (TFA)

The aluminum trichloride method [20] was used to quantify flavonoids in each extract of *C. oblonga* leaf with some modifications using rutin as the standard. To 1.5 ml of sample or standard (prepared in methanol) was added 1.5 ml of $AlCl_3$ solution (2% in methanol). After 30 min of reaction, the absorbance was read at 415 nm against a blank sample, which was prepared in the same manner where the sample or standard was substituted by the same amount of distilled water (1.5 ml). The concentration of flavonoids was deduced from a calibration range established with rutin (0–0.05 mg/ml) and was expressed in milligrams of rutin equivalent by a gram dry weight (mg RE/g d.w).

Determination of total flavonol content (TFL)

The total flavonol was measured according to the method described by [21, 22] with some modifications. Briefly, 1 ml of plant extract was mixed with 1 ml of aluminum trichloride (2%) and 3 ml of sodium acetate (5%). The absorbance at $\lambda = 440$ nm was read after 1 h. The same procedure was used for different concentrations of methanolic

rutin solutions (of 0–100 µg/ml). All determinations were made in triplicate. The amount of flavonols in the extracts, in rutin equivalents (RE), was calculated from the rutin calibration curve.

DPPH· radical scavenging activity

The antiradical activity of the different extracts was evaluated against DPPH (1,1-diphenyl-2-picrylhydrazyl) as a relatively stable free radical, according to the protocol described by[23]. Antioxidants reduce the violet-colored diphenyl picryl-hydrazyl to a yellow compound, whose color intensity is inversely proportion to the capacity of the antioxidants present in the solution donate protons.

Briefly, 100 µl of the extracts previously diluted in methanol at different concentration were added to 100 µl DPPH (0.2 mM prepared in methanol). The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. Blank solutions were prepared by mixing 100 µl of methanol with 100 µl of each test sample solution while the negative control was 100 µl of 0.2 mM DPPH solution plus 100 µl ml of methanol. The standard antioxidant solution of ascorbic acid was used as a positive control. Thereafter the absorbance reading was taken at 540 nm against each blank, the test was repeated 3 times. The antiradical activity was estimated according to the equation below:

$$\%\text{Antiradical activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

The IC_{50} values were determined graphically by linear regression.

Evaluation of the litholytic ability on calcium oxalate crystals

The ability of the different extracts to dissolve calcium oxalate crystals was evaluated according to the protocol described by [24] with slight modifications.

Calcium oxalate stones synthesis

$CaCl_2$ and $(NH_4)_2C_2O_4$ stock solutions with the concentration of 0.1 mol/l were prepared in an aqueous solution of sodium chloride (NaCl) at 0.15 mol/l and pH was adjusted to 6.0 by sodium acetate 0.2 mol/l, identical volume (0.5 ml) of calcium chloride solution and ammonium oxalate solution was placed in microcentrifuge tubes (2 ml) previously weighted, after incubating for 30 min without stirring in the water bath of 37 °C the calcium oxalate CaOx was precipitated. Thereafter, the tubes were centrifuged at 16,000*g* using a high-speed microcentrifuge, and the supernatant was removed, the precipitates were washed using ethanol, stirred by vortex for a few seconds, and centrifuged again as detailed earlier. The tablet weights were quantified by difference after allowing it to dry in oven at 40 °C for 18 h and weighted again.

The ability of extracts to dissolving calcium oxalate tablets

1 ml of each extract at different concentrations ranging from 0.5 to 3 mg/ml for the infusion extract and 0.5–1.5 mg/ml for the acetone and methanolic extracts at pH 6 were added to the calcium oxalate tablets and the mixture was left under mild mixing by vortex for 3 days at 37 °C. At the end of this experiment the tubes were centrifuged and the tablets rinsed, dried, and balanced as described above.

The dissolution activity was determined by calculating the dissolution rate of the tablets after the time spent in the experimental medium by comparing their final weight with their initial weight with the following formula:

$$a\% = \frac{W_{\text{initial}} - W_{\text{final}}}{W_{\text{initial}}} \times 100$$

where $a\%$: dissolution rate; W_{initial} and W_{final} are the weights of the tablet before and after incubation with the extract.

Each experiment was repeated three times in the same conditions, and the results were expressed by calculating the mean ± SD of the values obtained.

Characterization

X-ray powder diffraction (XRD): This method was used to determine the phase composition of the crystals. The diffractograms presented in this manuscript were all recorded using a powder diffractometer in the range of 10–60° 2θ with a step of 0.02° 2θ. Phase identification was performed using the PDF-2 database of the ICDD (version 2016).

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDXS): Morphological studies and elemental analysis of the crystals formed were realized using a scanning electron microscope (SEM) equipped with an energy dispersive X-ray spectroscopy (EDS).

FT-IR: Fourier Transform Infrared (FT-IR) spectrophotometric analysis of the synthesized CaC_2O_4 was performed using an FT-IR spectrometer (Perkin–Elmer) with a resolution of 4 cm^{-1} in the range of 400–4000 cm^{-1}.

Table 1 Total amount of plant phenolic compounds, flavonoids and flavonols

Extracts	Total phenolic (mg GAE/g)[a]	Total flavonoids (mg RE/g)[b]	Total flavonols (mg RE/g)
Methanol extract	268.9 ± 3.55	29.7 ± 0.12	10.7 ± 0.38
Acetone extract	131.3 ± 4.09	40.3 ± 1.47	11.2 ± 0.24
Infusion extract	81.9 ± 1.91	6.8 ± 0.29	6.6 ± 0.03

Values are represented as means ($n = 3$) ± SD

[a]mg GAE/g: mg gallic acid equivalents per g dry weight plant extract

[b]mg RE/g: mg rutin equivalents per g dry weight plant extract

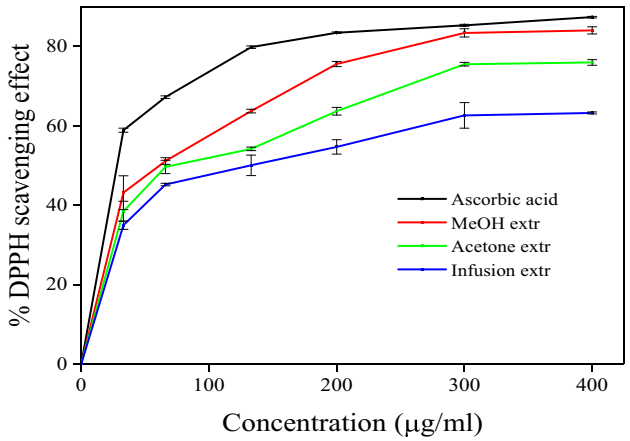

Fig. 1 Antioxidant activity of different extracts from *C. oblonga M.* leaf, evaluated by the DPPH radical scavenging test. Data show mean ± SD of three separate experiments

Results

Contents of total polyphenol, flavonoid and flavonol

Quantitative determinations of polyphenols, flavonoids and flavonols content in quince leaves extracts were achieved by spectrophotometric analysis.

Table 1 shows significant variations in total phenols contents (TPCs) of quince leaves when extracted with different solvents. Nevertheless, the minimum TPCs were observed in aqueous extract (81.9 ± 1.91 mg GAE/g) and the maximum values were obtained for methanolic extract with 268.9 ± 3.55 mg GAE/g dry weight plant extract. The flavonoidic content (TFA) was almost the double in extract where acetone had been used. Flavonols levels (TFL) in all *C. oblonga* leaves extracts have similar values but in small amounts ranged between 11.2 ± 0.24 and 6.6 ± 0.03 mg RE/g dry weight plant extract.

Table 2 IC50 values determined in the DPPH assay of *C. oblonga M.* leaf extracts

	IC_{50} (DPPH) values µg/ml
Methanol extract	36.06 ± 3.55
Acetone extract	74.15 ± 6.29
Infusion extract	142.35 ± 5.09
Ascorbic acid	2.048 ± 0.372

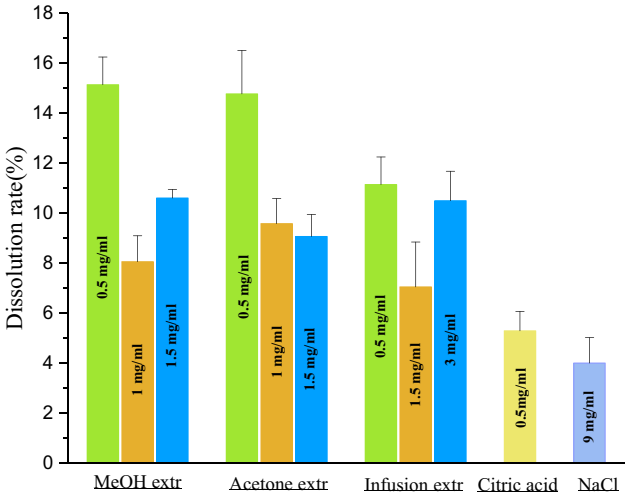

Fig. 2 Percent dissolution of calcium oxalate tablet, after 3 days in contact with the different concentrations of *C. oblonga M.* leaf extract, citric acid and NaCl solution. Values are represented as mean ± standard deviation ($n = 3$)

DPPH· radical scavenging activity

Quince leaf extracts have been evaluated for their DPPH radical scavenging ability, the latter was expressed as percent inhibition and median inhibitory concentration (IC50) values and the obtained results were compared with those of ascorbic acid. As showing in Fig. 1 all the investigated extracts exhibited significant increase antiradical activity in a concentration-dependent manner. Interestingly, the methanolic extract (83.40%) exhibited a strong antioxidant activity more than that of acetone and aqueous extract (75.52 and 62.62% respectively) at the same concentration 0.3 mg/ml. The calculated IC50 values for quince leaf extracts ranged from 36.06 ± 3.55 to 142.35 ± 5.09 µg/ml (Table 2), while for ascorbic acid it was found a 2.048 ± 0.372 µg/ml.

Quince leaf extracts effect against artificial renal stones

The dissolving activity of calcium oxalate (CaOx) tablets with quince leaves extract was assessed after 72 h of incubation. The results collected via this assay (Fig. 2) show a partially dissolution of the tablets with all the investigated extract, but the weight loss was not dose-dependent.

At increasing doses of *C. oblonga* leaf extracts, there was less effect of calculus weight reduction. At 0.5 mg/ml both alcoholic extracts presented numerically more weight loss than that caused by infusion extract (15.13 ± 1.10%, 14.77 ± 1.74% and 11.14 ± 2.86% respectively), the values decreased to 10.60 ± 0.34%, 10.07 ± 0.87% and 10.50 ± 3.46% for methanolic, acetonic and infusion extract at dose of 2 and 3 mg/ml respectively. However, no significant effect was seen at medium concentration. In addition, citric acid (3 mM) and saline solution (NaCl 9 mg/ml) caused very weak variation of stone mass, which generated 5.3% and 4.01% dissolution respectively.

Characterizations

XRD

The phase identification using the Powder Diffraction File (PDF 2) indicated that calcium oxalate monohydrate is the major phase in all collected CaOx tablets. Whereas the XRD patterns of synthesized CaOx crystals showed strong diffraction peaks at 2 theta 14.919°, 24.381°, 30.099° and 38.200°, which are assigned to (100), (040) and (200) planes of whewellite (COM), respectively (Fig. 3). On the other hand, Rietveld refinement of obtained crystals in absence of quince leaf extracts was performed in order to further improve the crystal phase analysis. The Rietveld refinement parameters of constituent phases are listed in Table 3. The results of quantitative phase analysis of crystal using the Rietveld whole powder profile fitting structure refinement method showed that the crystal is monophasic i.e., 100% COM. The X^2, R_p, R_{wp} and R_{exp} values from powder diffraction data of the sample are most similar to earlier reports [25, 26], indicating the goodness of fit regardless of the number of constituent phases. The final Rietveld refinement plots is illustrated in Fig. 4 below.

Table 3 Finishing Rietveld refinement parameters and unit cell sizes of synthesized CaC_2O_4 stone

Constituent phases	$CaC_2O_4.H_2O$ (COM)
Crystal system	Monoclinic
Space group	P 2_1/c
Unit cell dimensions	a = 6.2760 Å; b = 14.5912 Å; c = 10.1614 Å; β = 109.028°
Cell volume	879.6750 Å^3
X^2	1.32
Rf^2	0.0593
Rp	0.0623
Rwp	0.0742
Rexp	0.0621

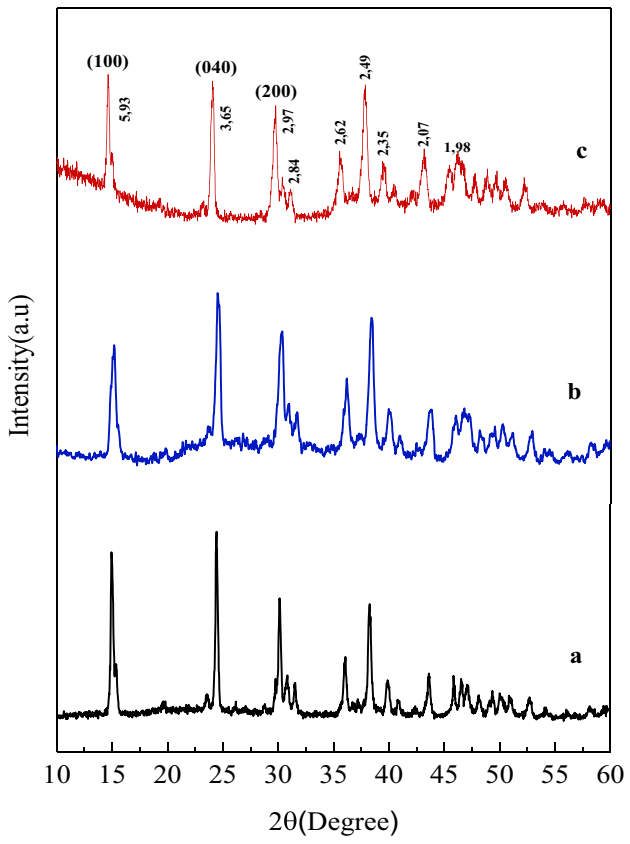

Fig. 3 Typical X-ray diffraction plots of CaC_2O_4 samples collected in the presence of methanolic extract (**a**), acetonic extract (**b**) and infusion extract (**c**) of *C. oblonga M* leaf

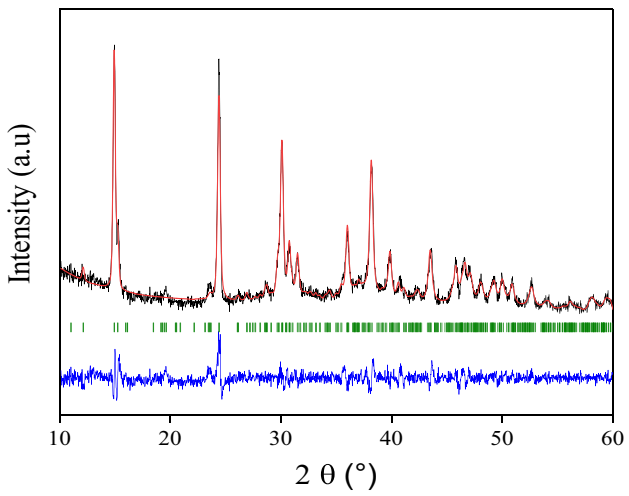

Fig. 4 Final Rietveld refinement profile of the synthesized calcium oxalate tablet. Observed (black), calculated data (red) and difference between them (blue)

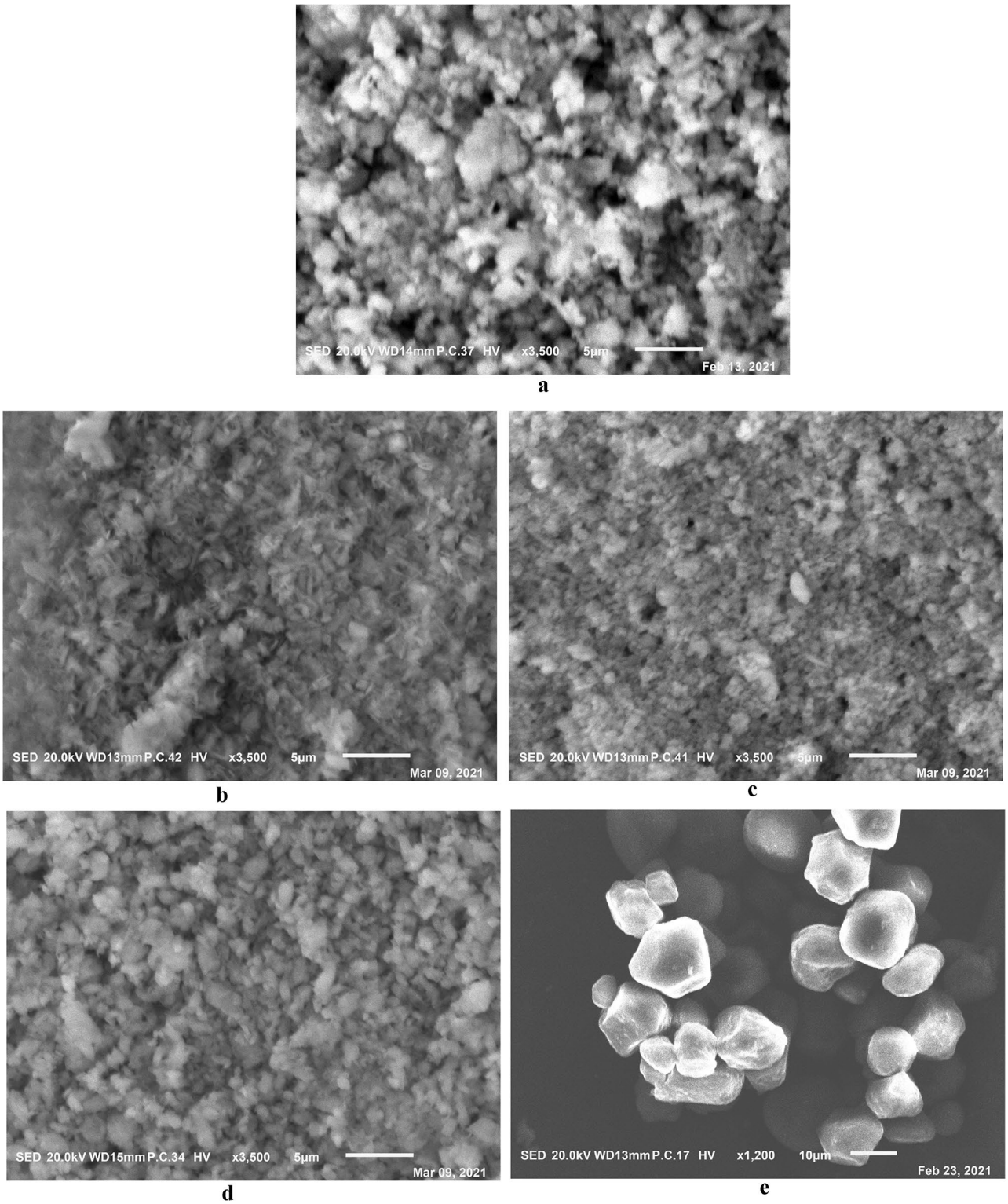

Fig. 5 SEM micrographs of CaC_2O_4 tablets collected in the absence (**a**) or presence (**b**–**e**) of different concentrations of *C. oblonga M*. leaves extracts

SEM

Morphological examination of the synthesized calcium oxalate stones was performed with SEM. As shown in Fig. 5a, irregular crystals of CaC_2O_4 are formed in the absence of the extracts, which are probably obtained by aggregates of numerous small crystals. In Fig. 5b and c, a significant predominance of single whewellite crystals with hexagonal pattern and multi- twinning aggregates can be clearly observed from the acetone and infusion extracts compared to the precipitate collected from the methanolic extract, whewellite continues to prevail in addition to single weddellite crystals in a tetragonal bipyramidal form (Fig. 5d and e). It is noteworthy noted that in the presence of all the studied extracts significantly reduces the aggregation level of crystalline CaOx, potentially allowing the small supercritical nuclei to grow to a larger size.

EDS

The relative elemental composition of the synthesized crystals was determined with EDS. As can be seen in Table 4 oxalate concentration increase in the all samples treated with the investigated extracts in contrast the calcium concentration decreases. Our findings shows that all studied *C. oblonga* leaf extracts displayed better performance in dissolution of calcium stone (Fig. 6).

FT-IR

For all samples the infrared spectra were registered and the spectra of methanolic, acetonic and infusion samples at the initial concentration (0.5 mg/ml) are shown in Fig. 7.

According to Fig. 7, multiple low intensity bands are observed between 3060 and 3500 cm^{-1}, typical of the OH stretching of water [27], corresponding to whewellite (COM) crystals, which are also differentiated by the presence of two intense signals at 1662/1610 and 1376/1315 cm^{-1}, due to the antisymmetric and symmetric ν_s(COO–) stretching mode of coordinated oxalate groups, respectively [28]. Furthermore, other peaks appear in the fingerprint region, the band at 517 cm^{-1} is due to in-plane bending of O=C=O, the bands at 780 cm^{-1} and 661 are due to the C−H bending and out-of-plane bending mode of O−H, respectively [29], and two weak signals at 947 and 885 cm^{-1} attributed to water releases that are unique to COM.

Table 4 (%) Atomic distribution of C, O and Ca elements in CaOx crystals

Extracts	Concentration of extracts	Atomic percent		
		C	O	Ca
Control	–	13.44	60.68	25.88
Methanol extract	$C_1 = 0.5$	29.81	63.48	6.71
	$C_2 = 1$	27.22	64.14	8.64
	$C_3 = 1.5$	33.61	61.34	5.05
Acetonic extract	$C_1 = 0.5$	32.50	62.27	5.23
	$C_2 = 1$	27.40	62.37	10.23
	$C_3 = 1.5$	30.72	61.05	8.23
Infusion extract	$C_1 = 0.5$	26.18	61.07	12.74
	$C_2 = 1.5$	30.28	60.48	9.24
	$C_3 = 3$	30.65	61.35	8.01

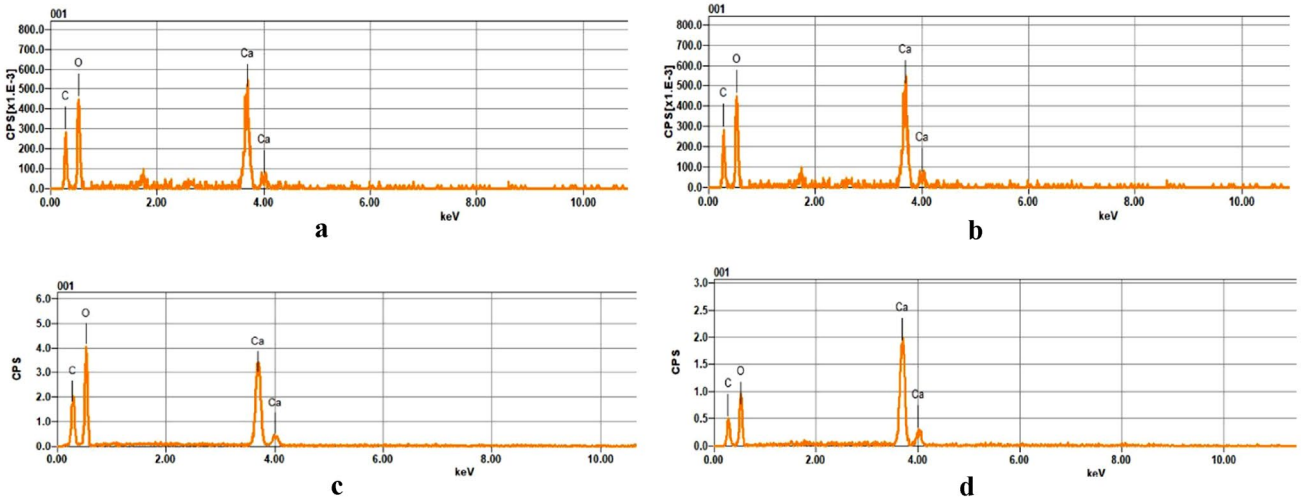

Fig. 6 Energy-dispersive X-ray spectra of synthesized calcium oxalate tablets, **A** standard, **B** acetone extract (1 mg/ml), **C** methanol extract (1 mg/ml) and **D** infusion extract (3 mg/ml)

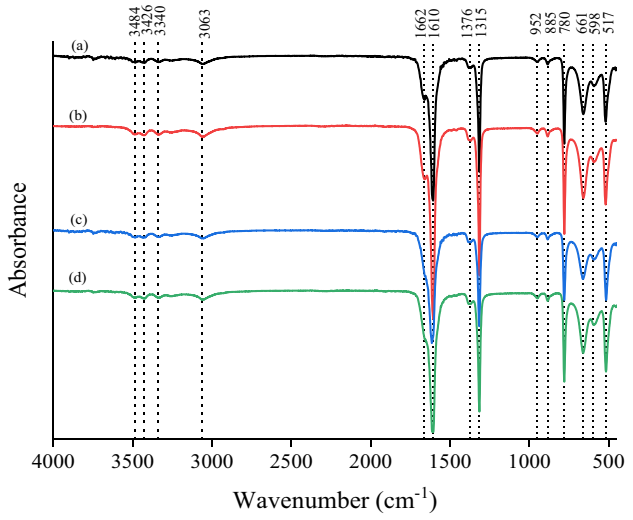

Fig. 7 FT-IR spectra of the synthetic calcium oxalate stones in absence (**a**), presence of acetone (**b**), methanol (**c**), and infusion leaf extract (**d**)

Discussion

Various extraction solvents were investigated. Analysis of chemical composition of *C. oblonga M.* leaves shows that the contents of TCP, TFA and TFL varied according to the polarities of the solvents employed. Our findings revealed that methanol was better than the other solvents for extracting phenolic compounds. These results are in agreement with previous studies reporting that methanol is good for extracting polyphenols from plant materials due to their good solubility and higher polarity [11, 30]. The total phenolic content determined in the methanolic extract of quince leaves was lower than that found by [2, 3] but higher than that identified by Zhang et al. [31] and [1]. For flavonoid and flavonol contents, the results obtained indicate that acetone is more efficient, followed by methanol and water on the extraction of these compounds. Value obtained for total flavonoid in acetone quince leaves extract was significantly comparable with those published by [1] for pulp and peel quince. However, the use of water does not give good extraction results.

Antioxidant activity of *C. oblonga Miller* leaf extracts was evaluated using DPPH assay and the results were compared with those of ascorbic acid. In the presence of DPPH· free radical, the H atom is transferred to the latter to transform it to stable DPPH molecule, which causes decrease in the concentration of the free radical and also the absorbance until the exhaustion of the hydrogen donor antioxidant (Iqbal et al. [32]). In the present study both methanol and acetone quince leaf extracts presented similar antiradical activities but greatly higher than that of infusion extract. Considering the important total phenolic content of methanolic extract, its lowest IC50 value (36.06 µg/ml) was expected, whereas the IC50 values were 74.150 and 142.35 µg/ml for the acetone and infusion extracts, respectively. Our results are close to those declared by [12] which obtained a value of IC50 at 38.4 µg/ml for methanolic Tunisian quince leaf extract [9] tested the scavenging ability against DPPH radicals of green tea and 12 quince leaf samples collected from different places of Portugal, IC50 values reported (21.6 µg/ml and 12.7 µg/ml respectively) revealing that green tea possesses more antioxidant capacity than quince leaf methanolic extracts. As mentioned by [10] a significant antioxidant activity were observed for pulp, peel, and seed of quince fruit with IC50 values 0.6, 0.8 and 12.2 mg /ml, respectively, but far lower than antiradical activity reported for leaf extract. Some authors have justified the higher antioxidant properties of *C. oblonga M.* leaf to their higher composition, in terms of organic acids and phenolic compounds (mono, dicaffeoylquinic acids, quercetin and kaempferol derivatives) [2, 3].

In this work, we also performed in vitro litholytic activity on the synthesized oxalocalcic stone by comparing different quince leaf extracts with standard. Our study results showed considerable dissolution effect at low extract concentrations, especially for methanol and acetone quince leaf extracts, while citric acid at 3 mM caused slight dissolution of the tablets, it might be attributed to citrate acts at the nucleation and/or crystallization level to inhibit stone formation and not after their development. However, and in accordance with previous studies chromatographic analysis of quince revealed the presence of significant quantities of polyphenolic compounds such as 3-*O*-caffeoylquinic, 4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic and 3,5-*O*-dicaffeoylquinic acids. Quince leaves also have the highest content of quercetin and kaempferol derivatives such as quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-glucoside [2, 3], kaempferol-3-*O*-rutinoside and kaempferol-3-*O*-glucoside [2, 3], which clearly indicates that tablet dissolution is due to the additive and/or synergistic effects of phytochemicals, through the formation of CaOx-active ingredient complexes, whose stability is maintained by hydrogen and hydrophilic bonds between the functional groups of the active ingredients and the carboxyl functions of the calcium oxalate molecule. The complexes formed are more soluble than calcium oxalate itself. In addition, studies have revealed that phytochemicals also act as a true inhibitor of calcium oxalate, being able, at least in vitro, to bind to the surface of crystals, reducing their size and modifying their structure. All quince leaf extracts examined are more effective to dissolving calcium oxalate tablets compared to the effects shown by [33] who tested several plant extracts on CaOx and cystine stones. The in vitro study realized by [34] carried out on the dissolution of oxalo-calcium stone in the presence of *A. unedo* leaf extracts during eight weeks showed

better dissolution rate compared to that of our result, but it was smaller than dissolving effects reported in other study [35]. Although, several work which examined the extracts of medicinal plants to dissolve urinary calculi showed an interesting dissolving effect for cystinic lithiasis [35, 36].

Conclusion

In our in vitro study, the direct effect of three extracts of *C. Oblonga M.* leaf on experimental kidney stones was evaluated. In agreement with the results and discussion, it can be concluded that quince leaf has a litholytic effect on kidney stones by reducing super saturation and particle size as demonstrated by SEM images and EDS analysis. This characteristic is beneficial in the preventive treatment of urinary lithiasis, in order to favor the excretion of small crystals, avoiding their retention in the collecting tubes, in the renal papilla or at the level of a calyrial fold, first step of the lithiasis process. *C. oblonga M.* leaf could therefore constitute interesting curative and/or prophylactic treatments for lithiasis patients. Other beneficial properties such as antioxidant activity cannot be ignored. To our knowledge, this is the first study concerning the litholytic effect of quince leaf. Our results bring attention to the effect of *Cydonia oblonga Miller* leaf extracts, which should be taken account of in any application, including the development of a new anti-Urolithitic in the pharmaceutical sector and the preservation of fresh nutrition in the food industry. However, further experimental in vivo studies should be carried out to extract and purify phytochemicals compounds to test their effect on urinary lithiasis and assess the safety profile and potential toxicity of Moroccan *Cydonia oblonga Miller* leaf extracts.

Author contributions I.E and L.B conceived of the presented idea. I.E developed the theory, performed the computations and wrote the main manuscript text. M.B supervised the findings of this work.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Stojanović BT, Mitić SS, Stojanović GS et al (2017) Phenolic profiles and metal ions analyses of pulp and peel of fruits and seeds of quince (*Cydonia oblonga Mill.*). Food Chem 232:466–475. https://doi.org/10.1016/j.foodchem.2017.04.041
2. Oliveira AP, Pereira JA, Andrade PB et al (2007) Phenolic profile of *Cydonia oblonge Miller* leaves. J Agric Food Chem 55:7926–7930. https://doi.org/10.1021/jf0711237
3. Oliveira AP, Pereira JA, Andrade PB et al (2008) Organic acids composition of *Cydonia oblonga Miller* leaf. Food Chem 111:393–399. https://doi.org/10.1016/j.foodchem.2008.04.004
4. Srirama R, Senthilkumar U, Sreejayan N et al (2010) Assessing species admixtures in raw drug trade of Phyllanthus, a hepatoprotective plant using molecular tools. J Ethnopharmacol 130:208–215
5. Jouyban A, Shoja MM, Ardalan MR et al (2011) The effect of quince leaf decoction on renal injury induced by hypercholesterolemia in rabbits: a pilot study. J Med Plant Res 5:5291–5295
6. Vaez H, Hamidi S, Arami S (2014) Potential of *Cydonia oblonga* leaves in cardiovascular disease. Hypothesis 12:1–10
7. Janbaz KH, Shabbir A, Mehmood MH, Gilani AH (2013) Insight into mechanism underlying the medicinal use of *Cydonia oblonga* in gut and airways disorders. J Anim Plant Sci 23:330–336
8. Minaiyan M, Ghannadi A, Etemad M, Mahzouni P (2012) A study of the effects of *Cydonia oblonga Miller* (Quince) on TNBS-induced ulcerative colitis in rats. Res Pharm Sci 7:103
9. Costa RM, Magalhães AS, Pereira JA et al (2009) Evaluation of free radical-scavenging and antihemolytic activities of quince (*Cydonia oblonga*) leaf: a comparative study with green tea (*Camellia sinensis*). Food Chem Toxicol 47:860–865. https://doi.org/10.1016/j.fct.2009.01.019
10. Magalhães AS, Silva BM, Pereira JA et al (2009) Protective effect of quince (*Cydonia oblonga Miller*) fruit against oxidative hemolysis of human erythrocytes. Food Chem Toxicol 47:1372–1377. https://doi.org/10.1016/j.fct.2009.03.017
11. Alesiani D, Canini A, D'Abrosca B et al (2010) Antioxidant and antiproliferative activities of phytochemicals from Quince (*Cydonia vulgaris*) peels. Food Chem 118:199–207. https://doi.org/10.1016/j.foodchem.2009.04.098
12. Benzarti S, Hamdi H, Lahmayer I et al (2015) Total phenolic compounds and antioxidant potential of quince (*Cydonia oblonga Miller*) leaf methanol extract. Int J Innov Appl Stud 13:518–526
13. Din Ganaie MU, Behl T, Nijhawan P et al (2020) Investigation of anti-depressant effect of aqueous and ethanolic extract of *Cydonia oblonga* in rats. Obes Med 18:100202. https://doi.org/10.1016/j.obmed.2020.100202
14. El Habbani R, Chaqroune A, Arrayhani M et al (2016) Epidemiological study on urinary stones in the region of Fez and the risk of recurrence. Progres en Urologie: Journal de l'association Francaise d'urologie et de la Societe Francaise d'urologie 26:287–294
15. Daudon M, Bader CA, Jungers P et al (1993) Urinary calculi: review of classification methods and correlations with etiology. Scan Microsc 7:32
16. Elliot JS, Rabinowitz IN (1980) Calcium oxalate crystalluria: crystal size in urine. J Urol 123:324–327
17. Castiglione V, Jouret F, Bruyere O et al (2015) Epidémiologie de la lithiase urinaire en Belgique sur base d'une classification morpho-constitutionnelle. Nephrol Ther 11:42–49
18. Daudon M, Letavernier E, Frochot V et al (2016) Respective influence of calcium and oxalate urine concentration on the formation of calcium oxalate monohydrate or dihydrate crystals. C R Chim 19:1504–1513
19. María R, Shirley M, Xavier C et al (2018) Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. J King Saud Univ Sci 30:500–505. https://doi.org/10.1016/j.jksus.2017.03.009
20. Miliauskas G, Venskutonis PR, Van Beek TA (2004) Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 85:231–237. https://doi.org/10.1016/j.foodchem.2003.05.007
21. Awah FM, Uzoegwu PN, Ifeonu P et al (2012) Free radical scavenging activity, phenolic contents and cytotoxicity of selected

Nigerian medicinal plants. Food Chem 131:1279–1286. https://doi.org/10.1016/j.foodchem.2011.09.118
22. Kumaran A, Joel Karunakaran R (2007) In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT Food Sci Technol 40:344–352. https://doi.org/10.1016/j.lwt.2005.09.011
23. Rondón M, García I, Cornejo X et al (2015) Phytochemical screening and antioxidant activity of seven medicinal plants species from Ecuador. Pharmacologyonline 3:19–28
24. Saso L, Valentini G, Leone MG et al (1998) Development of an in vitro assay for the screening of substances capable of dissolving calcium oxalate crystals. Urol Int 61:210–214. https://doi.org/10.1159/000030331
25. Ghosh S, Basu S, Chakraborty S, Mukherjee AK (2009) Structural and microstructural characterization of human kidney stones from eastern India using IR spectroscopy, scanning electron microscopy, thermal study and X-ray Rietveld analysis. J Appl Crystallogr 42:629–635. https://doi.org/10.1107/S0021889809016446
26. Orlando MTD, Kuplich L, de Souza DO et al (2008) Study of calcium oxalate monohydrate of kidney stones by X-ray diffraction. Powder Diffr 23:S59–S64. https://doi.org/10.1154/1.2903738
27. Leroy C (2017) Oxalates de calcium et hydroxyapatite: des matériaux synthétiques et naturels étudiés par techniques RMN et To cite this version : HAL Id : tel-01443727 Oxalates de calcium et hydroxyapatite : des matériaux
28. Ghosh S, Bhattacharya A, Chatterjee P, Mukherjee AK (2014) Structural and microstructural chracterization of seven human kidney stones using FTIR spectroscopy, SEM, thermal study and X-ray Rietveld analysis. Zeitschrift fur Kristallographie 229:451–458. https://doi.org/10.1515/zkri-2014-1725
29. Bhattacharyya S, Mandal AK, Singh SK (2014) Analysis of the chemical composition of urinary calculi using Fourier transform infrared spectroscopy: a preliminary study. J Postgrad Med Educ Res 48:128–131. https://doi.org/10.5005/jp-journals-10028-1117
30. Chirinos R, Rogez H, Campos D et al (2007) Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruíz & Pavón) tubers. Sep Purif Technol 55:217–225. https://doi.org/10.1016/j.seppur.2006.12.005
31. Zhang L, Rocchetti G, Zengin G et al (2021) The Uhplc-Qtof-Ms phenolic profiling and activity of *Cydonia oblonga* mill. Reveals a promising nutraceutical potential. Foods 10:1230. https://doi.org/10.3390/foods10061230
32. Iqbal E, Kamariah AS, Linda BLL (2015) Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. J King Saud Univ Sci 27:224–232. https://doi.org/10.1016/j.jksus.2015.02.003
33. Khouchlaa A, Talbaoui A, El Yahyaoui El Idrissi A et al (2017) Détermination des composés phénoliques et évaluation de l'activité litholytique in vitro sur la lithiase urinaire d'extrait de *Zizyphus lotus* L. d'origine marocaine. Phytothérapie 1–6
34. Kachkoul R, Squalli Housseini T, Mohim M et al (2019) Chemical compounds as well as antioxidant and litholytic activities of *Arbutus unedo* L. leaves against calcium oxalate stones. J Integr Med 17:430–437. https://doi.org/10.1016/j.joim.2019.08.001
35. Ammor K, Mahjoubi F, Bousta D et al (2020) In vitro litholytic activity of extracts and phenolic fractions of some medicinal plants on urinary stones. Mediterr J Chem 9:468–477. https://doi.org/10.13171/mjc9602001101135ka
36. Meiouet F, El Kabbaj S, Daudon M (2011) Étude in Vitro De L'Activité Litholytique De Quatre Plantes Médicinales Vis-À-Vis Des Calculs Urinaires De Cystine. Prog Urol 21:40–47. https://doi.org/10.1016/j.purol.2010.05.009