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RESEARCH ARTICLE

In-vitro evaluation of some traditional medicinal plants on calcium oxalate urolithiasis

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ABSTRACT

The prevalence of urolithiasis in Middle East countries is predominantly high. Traditional medicinal plants play a role in the prevention and management of urolithiasis. The effect of *Petroselinum crispum*, rind of *Citrus sinensis* L., rind of *Citrus limon* L., *Ammi visnaga* (L.) LAM., *Tamarindus indica* L., *Nigella sativa* L., *Cymbopogon proximus* Hochst. ex A. Rich., *Hibiscus sabdariffa* L., *Hordeum vulgare* L., and *Cymbopogon schoenanthus* (L.) Spreng. on calcium oxalate crystallization was investigated *in-vitro*. The antispasmodic activity on acetylcholine-induced contraction in rat ileum was also screened *ex-vivo*. A significant reduction in the mean diagonal of calcium oxalate crystals was observed for *P. crispum*, *C. proximus*, *N. sativa*, and *C. limon* (4.41, 4.71, 5.44, and 5.67 μm, respectively) compared to the negative control (12.19 μm). *H. sabdariffa* exhibited antinucleation effect however, *P. crispum* showed a marked inhibition of calcium oxalate aggregation at 10 mg/mL after 60 min compared to the negative control. A remarkable *ex-vivo* antispasmodic activity was observed for *C. proximus*, *C. schoenanthus*, and *A. visnaga* extracts. Our results provide scientific evidence for the traditional use of the studied plant-derived products as potential therapies for calcium oxalate urolithiasis.

Keywords: Calcium oxalate aggregation; Kidney stones; Renal stone; Urolithiasis; Traditional medicinal plants.

INTRODUCTION

One of the most common problems related to the kidney, in which women and men are suffering alike, is kidney stones or urolithiasis. Urolithiasis (renal lithiasis) is a globally distributed disease; however, its prevalence varies from one country to another. The prevalence rates of urolithiasis in the Middle East countries have been reported to be predominantly high (Amir et al. 2018). There are different types of crystals that can build up and lead to kidney stone formation, including calcium, uric acid, struvite, and cysteine. However, calcium oxalate stone takes the lead (Amir et al. 2018). High consumption of food rich in oxalates (such as nuts, rhubarb, beet, and spinach), protein, and salt increase the risk of calcium oxalate stone. Moreover, many other factors also can increase the risk, such as low water-intake, obesity, and digestive diseases, like "Inflammatory Bowel Disease" IBD, which affect the body's fat absorption ability and thus calcium attaches to fat leaving oxalate free taken to the kidney (Hueppelshaeuser et al. 2012; Nouvenne et al. 2008).

Currently, the treatment of kidney stones is varied according to the size and cause of stones. Small stones can be passed by drinking a lot of water, but this process may be accompanied by pain, so pain relief medications and smooth muscle relaxants that relax the ureter muscle to ease the passage of stone may be advised (Pickard et al. 2015). Nevertheless, in the case of large stones, there is no way to help the stone to pass the ureter spontaneously and without injury, so surgery should be performed by extracorporeal shock wave lithotripsy, percutaneous nephrolithotomy, or by using ureteroscope (Srisubat et al. 2014). Unfortunately, there is no satisfactory medicine to dissolve kidney stones, despite significant progress in medical treatment. Traditional medicines are still preferred by many patients to treat several medical conditions. Particularly, plant-derived natural products are used by several cultures forming a crucial starting point for recent innovations in drug discoveries (Cragg and Newman 2013). The primary mechanisms of these plants and their phytoconstituents in controlling urolithiasis include antispasmodic activity, inhibitory effect on crystallization,

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nucleation, and aggregation of crystals, in addition to their diuretic and antioxidant activity (Nirumand et al. 2018). Several medicinal plants extract have been reported to exhibit *in-vitro* anti-crystallization activities, such as *Kalanchoe pinnata, Emblica officinalis* (Sohgaura et al. 2018), pseudo-stem of *Musa* sp. (Abu Zarin et al. 2020), *Herniaria hirsute* (Atmani and Khan 2000), *Plantago major* (Aziz et al. 2005), *Bergenia ciliata* (Saha and Verma 2013), *Tribulus terrestris* (Aggarwal et al. 2010), and *Bergenia ligulata* (Bashir and Gilani 2009; Garimella et al. 2001). Several traditional medicinal plants are frequently used by patients in the Middle East region for the management of urolithiasis, including *Petroselinum crispum*, rind of *Citrus sinensis* L., rind of *Citrus limon* L., *Ammi visnaga* (L.) LAM., *Tamarindus indica* L., *Nigella sativa* L., *Cymbopogon proximus* Hochst. ex A. Rich., *Hibiscus sabdariffa* L., *Hordeum vulgare* L., and *Cymbopogon schoenanthus* (L.) Spreng. Some of these natural products have scientific basis of such use, whereas others do not. Therefore, this study aimed to evaluate the anti-urolithiatic activity of these plants' extracts by investigating their ability to inhibit the aggregation of calcium oxalate crystals using the turbidimetric method by comparing the optical density (OD) at 620 nm. Additionally, the morphological changes and sizes of the formed crystals in presence and absence of extracts were also examined by an optical microscope. Furthermore, the antispasmodic activity was evaluated by observing the *ex-vivo* smooth muscle relaxant effect on acetylcholine (Ach)-induced contraction on rat ileum.

MATERIAL AND METHODS

Plant material

The plant samples, including the aerial parts of parsley (*Petroselinum crispum* (Mill.) Fuss), orange rind (fruit rind of *Citrus sinensis* L.), lemon rind (fruit rind of *Citrus limon* L.), khella fruits (*Ammi visnaga* (L.) LAM.), peeled fruits of tamarind (*Tamarindus indica* L.), black seeds (*Nigella sativa* L.), the aerial parts halfa bar (*Cymbopogon proximus* Hochst. ex A. Rich.), roselle (the calyces of *Hibiscus sabdariffa* L.), barley grains (seeds of *Hordeum vulgare* L.), and the aerial parts of camel grass (*Cymbopogon schoenanthus* (L.) Spreng.) were purchased in their entire form from an herbal market at Al-Kharj, KSA (24.1576° N, 47.3248° E), Table 1 and Fig 1. The authentications of the purchased plants were confirmed by Prof. Fatma M. Abdel Bar (Professor of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University). Voucher specimens were deposited in the herbarium of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University under the codes from (#16735-16745). Fresh samples (parsley, orange rind, and lemon rind) were spread on trays, shade-dried, then the dried plant samples were ground using an electric homogenizer and kept in an airtight container for further use.

Extraction

Generally, 100 g of powdered plant samples were placed in 600-mL beakers and extracted by cold maceration method using methanol (300 mL, three times), followed by water extraction (1 x 300 mL) to ensure exhaustion of polar active constituents. The combined hydro-alcoholic extracts were concentrated using an R-210 Professional Rotary Evaporator (BÜCHI Labortechnik GmbH, Germany), then transferred to pre-weighed porcelain dishes and left to dry in air, Fig 1.

Anti-nucleation assay

Synthetic urine

Synthetic urine was freshly prepared and adjusting the pH to 6.0 according to the reported method (Burns and Finlayson 1980; Yousefi Ghale-Salimi et al. 2018).

Microscopic evaluation of calcium oxalate crystallization

For sample preparation, stock solutions of the plants' extracts were prepared in DMSO, followed by dilution with synthetic urine and filtration using filter paper. A solution of 10 mg/mL of extracts in each case was used for calcium oxalate induction by adding 0.1 M sodium oxalate (40 µL/mL) and incubating for 24 hours. A negative control is prepared similarly but without plant extracts. Potassium sodium hydrogen citrate (Uralyt-U®, MADAUS GmbH, Germany) was used as a standard (10 mg/mL) solution in synthetic urine. The formed precipitate was examined using an objective lens (40x) on OPTIKA® microscope provided with ProView software (B-3W Windows tablet PC with B3 camera, Italy).

Spectrophotometric turbidimetric assay

The reported method by Yousefi Ghale-Salimi et al. (2018) was used with some modifications. Sample solutions were prepared by the same method used for microscopic evaluation followed by serial dilutions using freshly prepared synthetic urine to obtain 10, 5, 2.5, and 1.25 mg/mL concentrations, separately in each case. Potassium sodium hydrogen citrate (100, 50, 25, and 10 mg/mL) and magnesium citrate (10, 5, 2.5, and 1.25 mg/mL) were used as positive controls. Two separate inspections were performed using spectrophotometric determination on a UV-visible spectrophotometer at 620 nm (UV1800 Spectrophotometer 2450, Shimadzu, Japan), on adding sodium oxalate solution: firstly, to determine the effect of different concentrations of the plant extracts and/or standards on induced calcium oxalate precipitation, and secondly, to compare the rate of nucleation of induced calcium oxalate, at 10 mg/mL of extracts and/or standards, over time (at zero, 5, 10, 15, 30, and 60 minutes). Turbidity (%) was calculated relative to negative control from equation [1]:

Fig 1. Photographs summarizing the preparation of different plants' extracts. a) Photographs of the studied traditional anti-urolithiatic plants; b) Extraction of the plant materials; c) Evaporation of the extracts.

$$\% \text{ Turbidity} = OD_{sample}/OD_{control} * 100 \quad [1]$$

Where OD_{sample}: Absorbance of sample; $OD_{control}$: Absorbance of control

Screening of the smooth muscle relaxation effect

EmkaBath2 (Emka technologies, France) was used to record the inhibitory effects of different plant extracts on acetylcholine (Ach, LOBA Chemie, India)-induced contraction on isolated rat ileum (adult male Wistar rats), using the previously published methods with minor modification (Ibrahim et al. 2019; Pavlović et al. 2012; Razafindrakoto et al. 2016). Tyrode solution was prepared according to published procedure (Ibrahim et al. 2019). Atropine sulfate (LOBA Chemie, India) was used as a positive control. The protocol methodology used in this study was approved by the Bioethical Research Committee (BERC-002-03-21) in College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA.

Statistical analysis

Results are expressed as mean ± S.D, from four observations. Values were analyzed using GraphPad Prism version 9.2 using two-way ANOVA followed by Dunnett's multiple comparisons as compared to negative control.

Table 1: List of selected traditional plants used in the treatment of urolithiasis and their main phytochemicals

Common name	Scientific name	Plant family	Part used	Main phytochemicals
1. Parsley	*Petroselinum crispum* (Mill.) Fuss	Apiaceae	Aerial parts	Essential oil (e.g. apiol), flavonoids, phenolics, carotenoids, and furanocoumarins, (Chauhan and Aishwarya 2018)
2. Black seeds	*Nigella sativa* L.	Ranunculaceae	Seeds	Essential oil (e.g. thymoquinone), alkaloids, terpenoids, coumarins, and flavonoids (Ahmad et al. 2021)
3. Halfa bar	*Cymbopogon proximus* Hochst. ex A. Rich.	Poaceae	Aerial parts	Alkaloids, essential oil (e.g. piperitone and δ-2-carene), sesquiterpenes (e.g. proximadiol), flavonoids, carotenoids, and tannins (Avoseh et al. 2015; El-Askary et al. 2003)
4. Roselle	*Hibiscus sabdariffa* L.	Malvaceae	Calyces	Polyphenols (e.g. anthocyanins), flavonoids, and organic acids (Riaz and Chopra 2018).
5. Orange rind	*Citrus sinensis* L.	Rutaceae	Fruit rind	Essential oil (e.g. limonene), flavonoids, and (e.g. hesperidin) (Aghel et al. 2008; González-Mas et al. 2019).
6. Khella	*Ammi visnaga* (L.) LAM.	Apiaceae	Fruits	γ-Pyrones and coumarins, including furanochromone derivatives (e.g. khellin and visnagin) (Khalil et al. 2020)
7. Barley	*Hordeum vulgare* L.	Gramineae	Seeds	Flavonoids (e.g. vitexin, saponaretin, quercetin, rutin, and kaempferitrin), lignans, tocopherols, and phenolic acids (Kobus-Cisowska et al. 2020; Lahouar et al. 2014; Seikel and Bushnell 1959).
8. Tamarind	*Tamarindus indica* L.	Fabaceae	Peeled fruits	Phenolics (e.g. catenin, procyanidin B2, epicatechin), organic acids, triterpenes (e.g. lupanone and lupeol), pyrazines, and thiazoles (Bhadoriya et al. 2011; Kuru 2014)
9. Lemon rind	*Citrus limon* L.	Rutaceae	Fruit rind	Flavonoids (e.g hesperidin, diosmetin-7-rutinoside), limonoids, terpenoids, carotenoids, coumarins, furanocoumarins, and essential oils (e.g. limonene) (Klimek-Szczykutowicz et al. 2020)
10. Camel grass	*Cymbopogon schoenanthus* (L.) Spreng.	Poaceae	Aerial parts	Essential oil (e.g. piperitone and limonene), alkaloids, flavonoids, tannins, saponins, and steroids (Al-Snafi 2016; Avoseh et al. 2015; Ponce-Monter et al. 2008)

RESULTS AND DISCUSSION

For many years, plants constituted the main basis of different traditional systems. Many promising drug discoveries relied on the long history of use of traditionally used plants and their diverse array of secondary metabolites, which are responsible for their drug-like properties or what is known as the "predrug stage". Nevertheless, a lot of herbs used in folk medicine lack sufficient information on their pharmacological basis and mechanism(s) of action (Pan et al. 2013). In this regard, the current study aimed at investigating the potential anti-urolithiatic activities of several plants that are traditionally used in Saudi Arabia and in other regions in the world.

Microscopic evaluation of calcium oxalate crystallization

In susceptible patients, calcium oxalate nucleation started in supersaturated urine as the first step towards urolithiasis, since the developed nuclei either grow and/or aggregate to a large size causing a pathological condition. Morphologically, the *in-vitro* synthesized calcium oxalate crystals were detected by microscopic investigation in three well-defined types: (1) envelope (bipyramid)-shaped, COD crystals, (2) weddellite COD-W crystals, and (3) thin hexagonal lozenge, COM-TL crystals, Fig 2, as described before by several studies (Daudon et al. 2016; Guerra et al. 2006; He et al. 2010).

Previously He et al. (2010) reported that more calcium oxalate dihydrate (COD) are detected in the urine of a healthy person. Nevertheless, more calcium oxalate monohydrate (COM) has been observed in the urine of a lithogenic patient. Also, COM has been reported as the most thermodynamically stable type found in lithogenic patients than COD, as it shows a superior affinity for adhering to renal tubular cells causing the formation of urinary stones (Verkoelen et al. 1995). However, COM have several crystal forms, for instance, in the absence of any additives (such as NaCl), Sun et al. (2017) identified two distinct types of *in-vitro*-synthesized COM crystals by the scanning electron microscope (SEM); a) calcium oxalate monohydrate-thin lozenge (COM-TL) which is

very weak, fragile, and has blunt edges; b) calcium oxalate monohydrate-hexagonal lozenge (COM-HL) which is thick and has sharp edges compared to COM-TL and can easily cause cell membrane rupture (Sun et al. 2017). In our study, COM-TL crystals were detected in large numbers in samples treated with the standards (magnesium citrate and potassium sodium hydrogen citrate), as observed in Fig 5e. This suggested that citrates act by modifying calcium oxalate crystals (COD or COM) to less harmful types/shapes. This result was in full agreement with the previously published data describing the effect of citrate on alteration of the shape of COM in diluted urine compared to the negative control (Guerra et al. 2006). Additionally, healthy person has a narrow crystal size distribution (20-250 nm) with less liability to aggregate or to transform to the harmful, COM form (He et al. 2010).

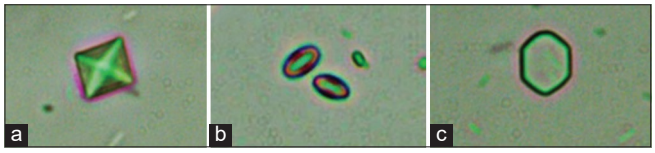

Fig 2. Different microscopically identified crystal shapes of calcium oxalate. (a) Envelope (pyramid)-shaped crystals of calcium oxalate dihydrate (COD); (b) Weddellite crystals of calcium oxalate dihydrate (COD-W); and (c) Thin hexagonal lozenge (COM-TL) crystals of calcium oxalate monohydrate.

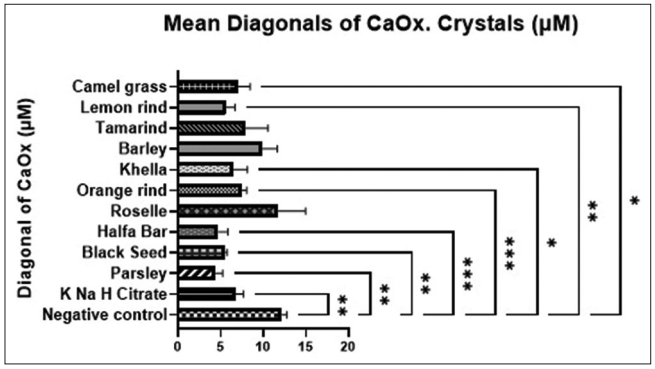

Fig 3. Average size calcium oxalate crystals for the investigated plant extracts (10 mg/mL) using light microscope. The results are expressed as diagonals in mm ± SD. Induction of calcium oxalate crystallization was induced in artificial urine by 40 µL of 0.1 M sodium oxalate per mL in presence (test) or absence (negative control). K Na H Citrate (potassium sodium hydrogen citrate, Uralyt-U®) was used as a standard. All measurements are taken after 24 hours of treatment. Significance levels *: $p > 0.05$; **: $p > 0.01$; ***: $p > 0.0001$.

In the current study the shape and size of the formed crystals upon induction of calcium oxalate crystallization by adding sodium oxalate (40 µL of 0.1 M solution/mL of artificial urine), in the presence or absence of different plant extracts (10 mg/mL), were examined microscopically, Table 2 and Fig 3. Negative control (untreated sample) showed the presence of large COD (Mean diagonal size, 12.2 µm) and COM aggregates, Fig 5f. Nevertheless, mostly all samples treated with the investigated plant extracts showed marked crystal size reduction and/or morphological modification relative to the negative control, Figs 4 and 5. Regarding COD crystal size, the standard drug, potassium sodium hydrogen citrate showed significant ($p < 0.001$) reduction of crystal diagonal (6.7 µm) compared to negative control. Parsley and halfa bar showed remarkable anti-aggregation activity with a significant reduction ($p < 0.05$ and $p < 0.001$, respectively) in mean crystal size diagonals (4.4 and 4.7 µm, respectively), followed by khella ($p < 0.05$) and lemon rind ($p < 0.001$) which showed crystal diagonals of 6.4 and 5.7 µm, respectively, compared to the negative control. However, hibiscus showed no significant effect on mean calcium oxalate crystal diagonal (11.7 µm), when compared to the negative control (12.2 µm), Table 2. Regarding the shape of the produced calcium oxalate crystals, the non-harmful crystal type (COD) was detected as the dominant one in treated samples with most of the

Table 2: Size reduction of calcium oxalate crystals in presence of tested plant extracts and their *ex-vivo* spasmolytic effect

Sample name	Diagonal (µM)[a]	Screening of muscle relaxation activity using *ex-vivo* Ach-induced contraction on rat ileum[b]
Parsley	4.41±0.4**	No marked inhibition
Black seeds	5.44±0.16**	No marked inhibition
Halfa bar	4.71±0.57***	Complete relaxation at 200 µg/mL
Roselle	11.68±1.64^{ns}	Significant relaxation at 200 µg/mL
Orange rind	7.52±0.27***	Week inhibition at 200 µg/mL
Khella	6.44±0.86*	Complete relaxation at 500 µg/mL
Barley	9.82±0.93	Significant relaxation at 200 µg/mL
Tamarind	7.89±1.33	No marked inhibition
Lemon rind	5.67±0.50**	Significant relaxation at 200 µg/mL
Camel grass	7.08±0.70*	Complete relaxation at 100 µg/mL
K Na H citrate	6.74±0.46**	ND
Neg. control	12.19±0.30	ND

[a]Tested at 10 mg/mL, and K Na H citrate: Potassium sodium hydrogen citrate (Uralyt-U®), was used as standard. Results are expressed as diagonals in µM ± S.D as determined by light microscope, after 24 hours of treatment. ND: Not determined. Significant levels; *: $P > 0.05$; **: $P > 0.01$; ***: $P > 0.0001$.using one-way ANOVA followed by Dunnett's multiple comparisons as compared to negative control.
[b]Tested at final concentrations of 100, 200, 300, and 500 µg/mL.

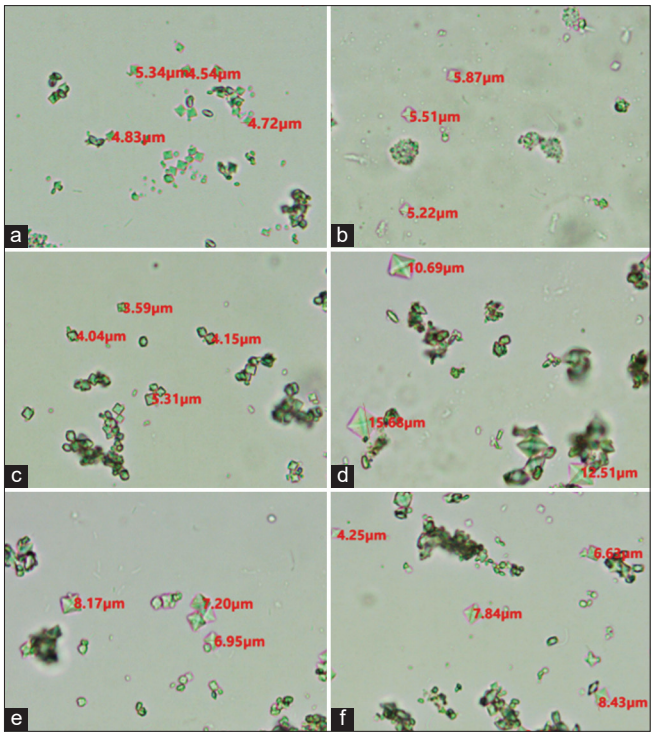

Fig 4. Complete microscopic views (x40) of randomly selected fields of calcium oxalate crystals from turbidimetric assay. All measurements are taken after 24 hours of treatment of artificial urine with 40 µL of 0.1 M sodium oxalate/mL in presence of 10 mg/mL of extracts; (a) Parsley; (b) Black seed; (c) Halfa bar; (d) Roselle; (e) Orange rind; and (f) Khella.

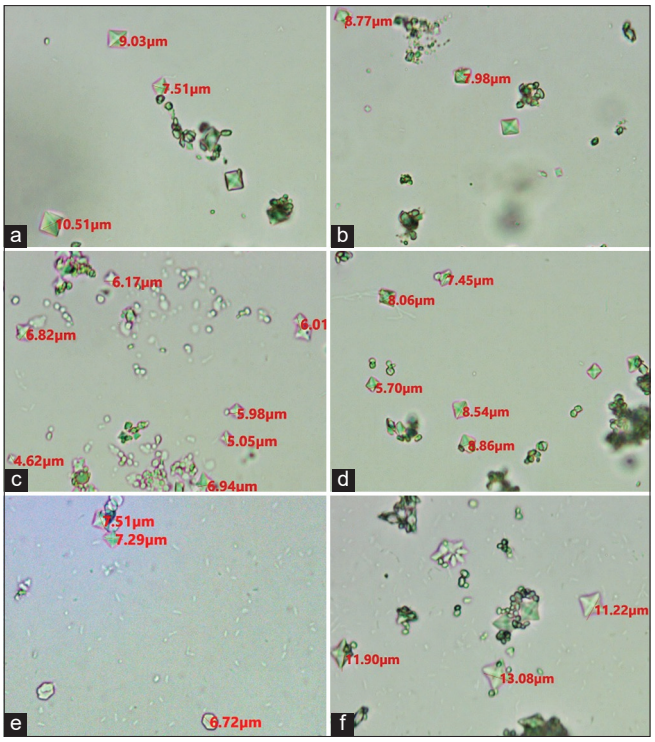

Fig 5. Complete microscopic views (x40) of randomly selected fields of calcium oxalate crystals from turbidimetric assay. All measurements are taken after 24 hours of treatment of artificial urine with 40 µL of 0.1 M sodium oxalate/mL in presence of 10 mg/mL of extracts; (a) Barley; (b) Tamarind; (c) Lemon rind; (d) Camel grass; (e) Potassium sodium hydrogen citrate (Uralyt-U®, 10 mg/mL) as a standard; and (f) Negative control (no added sample).

tested plant extracts, including parsley, halfa bar, khella, and lemon rind than COM crystals, Figs 4 and 5.

Nirumand et al. (2018) discussed the possible pharmacological mechanisms of the anti-urolithiatic action of plant polyphenols, including their inhibition of calcium oxalate deposition and crystal growth. In other words, the crystal growth-inhibitors bind to certain faces of the crystals and discourage the attachment of other molecules, in a way that it reduces the rate of crystal growth (Farmanesh et al. 2014). This indicated that the obtained anticrystallization activity is related to the presence of phenolic secondary metabolites that *in-vitro* hinder calcium oxalate crystal growth, Table 1.

Spectrophotometric turbidimetric assay

The number of formed crystals is an estimate of turbidity and measured as absorbance (OD) at 620 nm (De Bellis et al. 2019). Consequently, the effect of different plant extracts on nucleation and aggregation of induced calcium oxalate crystals was monitored by OD measurement at 620 nm for 1 h. (at 0, 5, 10, 15, 30, and 60 min) after addition of 0.1 M sodium oxalate solution (40 µL/mL of artificial urine), at room temperature. Except for hibiscus, most of the tested plant extracts showed no marked effect on nucleation (0-5 min) however, they promoted more turbidity in a dose-response manner indicating suppression of aggregation (after 60 min), particularly at high concentrations, Fig 6.

Therefore, an observed inverse relationship between the mean diagonal of calcium oxalate crystals for tested plant extracts and their calculated % turbidity. This can be explained by the fact that inhibition of aggregation of calcium oxalate crystals in artificial urine treated by the active plant extracts yields a greater number of small-sized crystals that eventually caused the scattering of light and consequently high absorbance values (i.e., high %turbidity). However, aggregation of such crystals allows light transmittance and causes lower absorbance values (i.e., low %turbidity). As for parsley at 10 mg/mL concentration, it showed the highest % turbidity (233.6%) followed by khella (193.6%), camel grass (163.2%), tamarind (162.4%), barley (132.3%), halfa bar (131.0%), and lemon rind (130.7%). It is worth noting that the same extracts showed a remarkable reduction in calcium oxalate crystal size compared to control which indicated their dual anti-urolithiatic action.

Effect on the rate of nucleation and aggregation

As mentioned above, any increase in absorbance will reflect an increased number of calcium oxalate particles over time.

A previous study by Mittal et al. (2016), conducted a control experiment to study the absorbance-time relationship of the formed calcium oxalate crystals after oxalate addition and have analyzed the results by linear regression analysis. They defined the maximum slope of increase in absorbance at 620 nm as the slope of nucleation (S_N), which determined the maximum rate of development of new particles. At the equilibrium point (at saturation), the rate of nucleation will be equal to the rate of growth. Similarly, the maximum slope of decrease in absorbance at 620 nm was defined as the slope of aggregation (S_A), which described the maximum rate of crystal growth or aggregation. In this conducted control experiment, a steep increase in absorbance (S_N) was reached followed by a gradual decrease due to crystal aggregation (S_A) (Mittal et al. 2016). In Fig 7, the effect of the tested extracts and standard at 10 mg/mL on the slopes of absorbance by time upon addition of 0.1 M oxalate solution (40 μL/mL of artificial urine) has been studied. The results indicated that only hibiscus (roselle) exhibited antinucleation effect as revealed from the slope of nucleation (S_N) which is almost comparable to the positive control. From the other side, other tested extracts (e.g., halfa bar, black seed, roselle, lemon rind, parsley, and camel grass) inhibited the aggregation of calcium oxalate crystals as obvious from the increased absorbance at 60 min (increased slope of aggregation, S_A) as compared to the negative control, Fig 7. This anti-aggregation effect can be explained by the presence of plant phytoconstituents, including polyphenols (such as flavonoids) and terpenoids (such as saponins) that were able to prevent calcium oxalate crystals from further adherence and growth (Ahmed et al. 2018; Atmani et al. 2006; Nirumand et al. 2018).

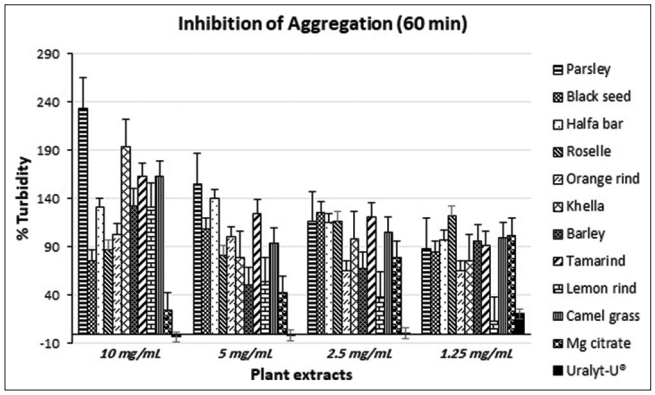

Fig 6. Inhibition of aggregation calculated as the increase of % turbidity (±SEM) from the turbidimetric spectrophotometric assay at 620 nm, after 60 min of induction of calcium oxalate formation with 0.1 M sodium oxalate (40 mL/mL of artificial urine). Plants extracts and standards were investigated at 10, 5, 2.5, and 1.25 mg/mL.

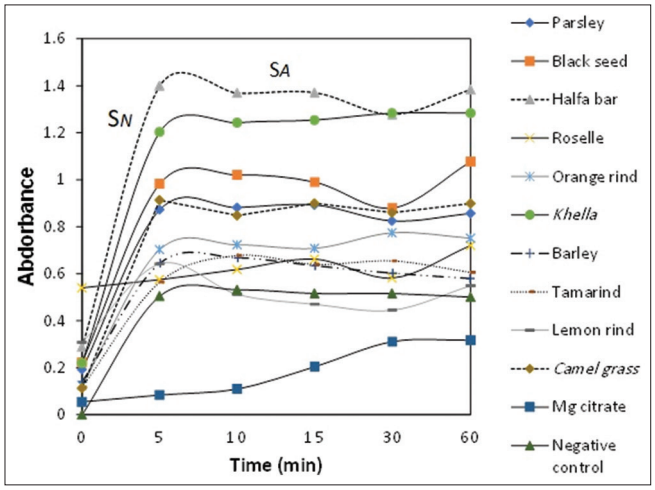

Fig 7. The effects of tested extracts and Mg citrate standard on the slopes of absorbance-time graphs, at a concentration of 10 mg/mL in artificial urine, after adding 40 μL/mL of 0.1 M sodium oxalate. Where S_N is the slope of increase of absorbance with time (nucleation rate) and S_A is the slope of decrease of absorbance with time (aggregation rate) for three independent observations.

Antispasmodic activity

Renal colic can be described as the worst pain that the patient will ever suffer. The passage of the renal calculi is usually associated with acute pain due to obstruction of the urinary stream and by the increased pressure created on the wall of the urinary tract causing ureteral smooth muscle spasm and increased peristalsis. Hence, the use of effective antispasmodic agents plays a crucial role in the treatment of such conditions (Golzari et al. 2014). Consequently, the studied plant extracts were preliminarily screened for their spasmolytic effect on Ach-induced contraction in rat ileum, Table 2.

The results (Fig 8) showed that camel grass, halfa bar, and khella showed remarkable relaxation of induced contraction at cumulative doses of 100, 200, and 500 μg/mL, respectively. Also, roselle, barley, and lemon rind showed significant relaxation at 200 μg/mL. Therefore, barley, halfa bar, lemon rind, camel grass, and khella have dual mechanisms of their action in ameliorating the symptoms of urolithiasis, including the anti-aggregation and the antispasmodic mechanisms. It is worth noting that some of these herbs are known for their antispasmodic activity due to the established identity of bioactive compounds, such as khellin and visnagin (khella) (Bhagavathula et al. 2014) and proximadiol (halfa bar) (Abdel-Moneim et al. 1969; El-Askary et al. 2003). For camel grass, Pavlovic et al. (2016) suggested some of the essential oil components to be responsible for spasmolytic activity, including the major component, piperitone (Ponce-Monter et al. 2008), and two minor components; limonene (Cardoso Lima et al. 2012) and β-eudesmol (Morita et al. 1996).

CONCLUSION

Urolithiasis is a globally distributed disease with calcium oxalate as the most common type of kidney stones. Folk

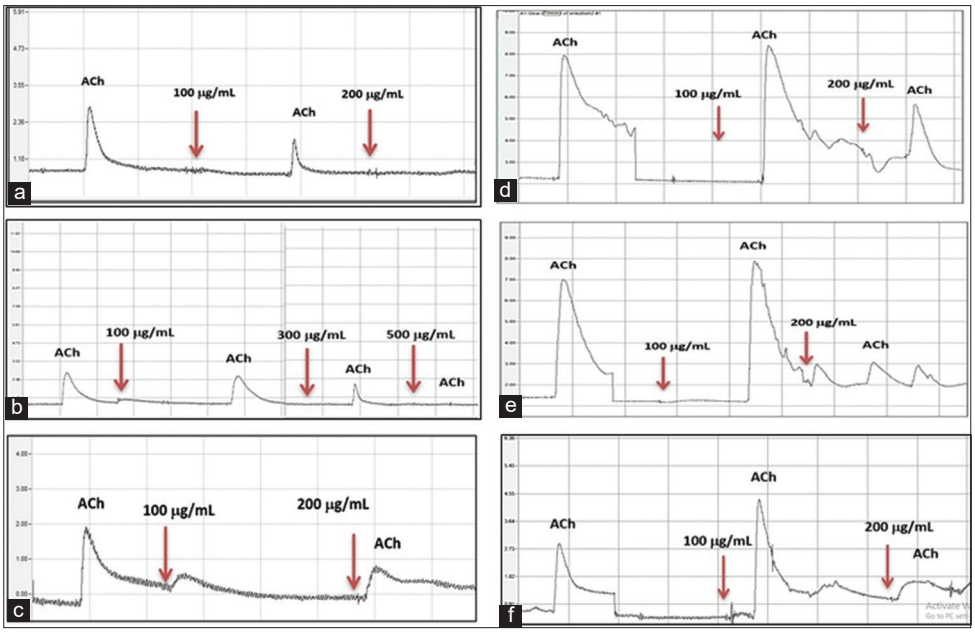

Fig 8. Inhibition of *ex-vivo* Ach-induced contraction on rat ileum. a) Halfa bar: showed remarkable at a final concentration of 100 µg/mL and complete inhibition after cumulative addition of 200 µg/mL; b) *Ammi visnaga* showed inhibition at final concentration 100 µg/mL and 300 µg/mL and complete inhibition at 500 µg/mL cumulative addition; c) Camel grass: showed significant relaxation at 100 µg/mL; d) Hibiscus: showed inhibition only after cumulative addition of 200 µg/mL; e) Barley: showed inhibition only after cumulative addition of 200 µg/mL; f) Lemon rind: showed inhibition only after cumulative addition of 200 µg/mL.

medicine is a rich source of diverse traditional plants that are commonly used for the treatment of urolithiasis. The current study targets the formation of calcium oxalate crystals by preventing their aggregation. We investigated the anti-urolithiatic activity of ten commonly used traditional natural products to uncover the scientific evidence for such use. Tamarind, camel grass, lemon rind, and barley extracts showed inhibition of calcium oxalate aggregation. Whereas parsley, halfa bar, khella, and lemon rind showed a significant reduction in calcium oxalate crystal size. Furthermore, halfa bar, khella, and camel grass extracts showed *ex-vivo* inhibition of Ach-induced contraction on rat ileum. Some of the potential bioactive compounds responsible for the obtained activity have been discussed. This study opens doors for future studies on identification of new phytochemicals for treatment and/or prevention of calcium oxalate urolithiasis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Authors' contributions

Fatma M. Abdel Bar designed the research. Fatma M. Abdel Bar, Amjad J. Majrashi, Mariam F. Al-Dossery conducted the experiments. Fatma M. Abdel Bar, Ahmed I. Foudah and Amal A. Galala analysed the data and wrote the manuscript. All authors read and approved the written manuscript.

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