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Essential Oils from the Leaves of *Ocimum Basilicum* L., *Persicaria Odorata* and *Coriandrum Sativum* L. In Malaysia: Antiurolithic Activity Study Based On Calcium Oxalate Crystallisation

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ABSTRACT

Calcium oxalate is one of the most common components in urolithiasis. Its treatment includes the use of synthetic drugs, ultrasound and surgery. However, cheaper alternative treatment using herbal medicine with less adverse side effect is preferred. Essential oils from Thai basil (*Ocimum bacilicum* L.), Vietnamese coriander (*Persicaria odorata*) and Chinese parsley (*Coriandum sativum* L.) were extracted and investigated for antiurolithic activity based on calcium oxalate crystallisation. Most of the crystals formed in control sample were hexagonal calcium oxalate monohydrate with sizes ranging between 3 to 4 μm. The size of the crystals was found to be slightly reduced in *O. bacilicum* oil (2-4 μm) at high concentration with less aggregation of crystals. Samples with *P. odorata* oil gave smaller crystal size (3 μm) mainly in dehydrate form and the oil was also found to inhibit the aggregation of the crystals at high concentration. *C. sativum* oil enhanced crystallisation (5-6 μm) with increased concentration and showed high aggregation of the crystals. This preliminary study shows the therapeutic potential of these medicinal plants to be used in traditional anti-urolithic therapy.

Keywords: *Calcium oxalate, anti-urolithic, Ocimum bacilicum, Persicaria odorata, Coriandrum sativum*

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INTRODUCTION

Essential oils contain compounds with volatile properties. They consist of a complex mixture of compounds comprising mainly of monoterpenes and sesquiterpenes. Essential oils can be obtained from various parts of aromatic plants including leaves, flowers and stems [1,2]. The oils carry scent and essence that can be commercialized and utilized in cosmetics, perfumes and certain products particularly related to pharmaceuticals and food for functional properties [3]. Essential oils have shown inhibitory effects against mold from food and thus, could be utilized in food preservation [4,5]. Recently, essential oils have also been investigated for their potential as potent inhibitors of SARS-CoV-2 spike protein and coronaviruses [6,7].

Medicinal treatments involving essential oils are also reliable as most of them show antimicrobial, anti-oxidant, anti-inflammatory and antiviral effects that are useful for medicinal purposes [8-10]. Thai basil (*Ocimum bacilicum L.*), Vietnamese coriander (*Persicaria odorata*) and Chinese parsley (*Coriandrum sativum* L.) locally known in Malaysia as *Selasih*, *Kesum* and *Ketumbar* respectively are herbs which are commonly found in Southeast Asian countries, either eaten raw or used in cooking. These plants are also used traditionally in the treatment of several diseases [11-13]. For example, the juice from *O. bacilicum* mixed with honey has been reported to help in expulsion of kidney stones [14]. Ancient Egyptians used *C. sativum* to treat stomach problems, urinary tract infections and digestive problems [15,16]. However, phytochemical and pharmacological studies on *P. odorata* are limited and poorly documented.

Many studies have been conducted to identify the chemical properties and volatile components of essential oils, as well as to test for a variety of biological properties. The chemical components of similar plant may differ from one place to another within the same country, influenced by the grown environment, as well as crop and post-crop processing. Joshi [17] reported that essential oil obtained from hydrodistillation of *O. bacilicum* from North West Karnataka, India mainly consisted of methyl eugenol (39.3%) and methyl chavicol (38.3%). On the other hand, Dris *et al*. [18] reported linalyl acetate (53.89%) and linalool (22.52%) as the main constituents of the plant from the area of Tebessa, Algeria.

Kidney stone disease or urolithiasis is commonly faced by people all around the world and is believed to be increasing globally across sex, race, and age [19]. Urolithiasis can be defined as the formation or aggregation of calcium salts, forming hard, stone-like crystals within the urinary tract. Calcium oxalate and calcium phosphate were identified to be the most common components of kidney stones [20,21]. The constriction of the urinary tract due to the stone's hard structure makes the patients feel intense pain before and after urinating. Urolithiasis is commonly treated using synthetic drugs, surgery, ultrasound and/or extracorporeal shockwave lithotripsy. However, alternative treatments such as using herbal medicines, with cheaper and possibly with less adverse side effects is preferred by many. The Ayurvedic system of medicine also recommends the use of medicinal plants to dissolve urinary calculi and stones.

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Some plant-derived compounds such as saponins, lupeol, lupeol linoleate as well certain medicinal plant extracts were found to able to dissolve or inhibit the formation of kidney stones and were used in traditional antiurolithic therapy [22-25]. Some drugs used for renal and hepatic diseases especially against cholesterol stones in the gall bladder and the bile duct contain *alpha*-pinene and *beta*-pinene18 [26]. Terpene oils formulated with a mixture of essential oil components such as pinene, camphene, borneol, anethole, fenchone and cineol were found to facilitate the passage of kidney stones without side effects [27]. The use of *O. bacilicum*, *P. odorata* and *C. sativum* in folk medicine and the lack of reports on their anti-urolithiatic activity prompted our evaluation on the essential oils extracted from the leaves of these plants on crystallisation of calcium oxalate.

EXPERIMENTAL

Plant Materials and Extraction of the Oils

Thai basil (*Ocimum bacilicum* L.), Vietnamese coriander (*Persicaria odorata*) and Chinese parsley (*Coriandrum sativum* L.) were purchased from local market in Kuantan, Pahang, Malaysia. The samples were identified by Dr Shamsul Khamis, a botanist from Universiti Putra Malaysia (UPM). Voucher specimens (PIIUM 0219, PIIUM 0220 and PIIUM 0221, respectively) were deposited in the Herbarium, Kulliyyah of Pharmacy, International Islamic University Malaysia (IIUM). The leaves (500 g for each sample) were cleaned and placed into round-bottom flasks and subjected to hydrodistillation using Clevenger apparatus for a minimum of 3 hours [28]. The oil was extracted from the aqueous mixture with dichloromethane. The solvent was then removed from the oils using rotary evaporator (water bath, 27 ^{o}C) and the oils are then stored at 4 ^{o}C.

Gas Chromatography-Mass Spectrometry Analysis

The extracted essential oils are subjected to GC-MS analysis to identify the essential oils components. The samples were analysed by a PerkinElmer Clarus SQ 8T GC-MS apparatus equipped with capillary column Elite-5MS (30 m × 0.32 mm internal diameter, 0.25 µm phase thickness). Helium was set up at a flow rate of 1.2 mL/min and used as the carrier gas. The samples were injected in the split mode with injector set at 200 ^{o}C. The oven temperature was set at an initial temperature of 40 ^{o}C for 2 min and then programmed to heat up to 250 ^{o}C at 5 ^{o}C/min. The components were identified by comparing and the mass spectral data with the National Institute of Standards and Technology (NIST) 2.0 MS libraries.

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Crystallisation Assay

The crystallisation assay was carried out according to previous methods [24]. A volume of urine was collected from a healthy subject in a polypropylene bottle; sodium azide was added as preservative and then stored at -80 ºC. The urine sample (2 mL) was placed into falcon tubes and allowed to warm up to 37 ºC. Approximately 50 µL of the essential oils was added into each tube and tubes without the oils were used as control. Sodium oxalate (50 µL) was added into each tube and the samples were incubated at 37 ºC for 30 minutes. The optical density (at 620 nm) for each sample was then measured using a Lambda 35 UV/VIS spectrophotometer (Perkin Elmer).

Scanning Electron Microscopy Analysis

The calcium oxalate crystals were centrifuged and filtered using a 0.8 µm membrane (Millipore). Coating process was initiated and the size, including morphology of the crystals was observed under a scanning electron microscope (ZEISS EVO 50).

Nucleation Assay

The assay was carried out following the methods described by Atmani and Khan [24]. Stock solutions of 3.0 mmol/L calcium chloride and 0.5 mmol/L sodium oxalate were prepared in a buffer containing Tris (0.05 mol/L) and sodium chloride (0.15 mol/L) at pH 6.5. The solution was filtered thrice using a 0.22 µm membrane. The essential oil (100 µL) was mixed with 950 µL of calcium chloride. Then, 950 µL of sodium oxalate was added to start the crystallisation. The solution was stirred at 800 rpm using magnetic stirrer on a hot plate with temperature maintained at 37 ºC. The optical density of the samples was measured at 620 nm using Lambda 35 UV/VIS spectrophotometer (Perkin Elmer).

RESULTS AND DISCUSSION

The main compounds identified for all three essential oils were estragole, cyclopropane and nonanedioic acid (Table 1). The percentage of estragole was the highest in *O. basilicum* (93.1%), *P. odorata* (87.7%) and *C. sativum* (48.1%). Estragole was previously reported as the main constituent of *O. basilicum*, [2]. However, the main constituents reported for *P. odorata* were dodecanal, decanal and sesquiterpenes [29-31], while the essential oil extracted from the leaves of

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C. sativum were mainly aldehydes such as 2*E*-decenal, decanal and alcohols such as linalool, 2*E*-decen-1-ol and *n*-decanol [32-34].

Table 1: Percentage of detected compounds from *Coriandrum sativum* L., *Ocimum basilicum L.* and *Persicaria odorata*

Compound	***C. sativum* L. (%)**	***O. basilicum* (%)**	***P. odorata* (%)**
Estragole	48.1	93.1	87.7
Cyclopropane	5.8	5.7	7.2
Hexadecanoic acid	1.4	-	-
5-Amino-4-cycnopyrazole	6.5	0.015	-
Camphene	1.9	-	-
Hystrene	8.3	-	0.2
Menthol	11.2	-	-
3-Cyclohexene-1-ethanol	8.9	0.035	0.3
Nonadedioic acid	7.9	1.15	4.6

The crystallisation assay was performed by initiating the occurrence of nuclei in urine by supersaturating it with calcium oxalate. After nuclei formation, the increased number of formed crystals indicates the growth of crystal and aggregation among each other, which would eventually lead to urolithiasis [35]. Crystallisation of calcium oxalate can be measured by obtaining the absorbance or optical density of the crystals at 620 nm using spectrophotometer [24]. A high value of absorbance indicates high turbidity of the sample and high number of calcium oxalate crystals in the urine.

Figure 1 shows that the optical density of calcium oxalate crystals increases with the concentration of the oils (62.5, 125, 250, 500 and 1000 µg/mL). The absorbance of the samples was all higher than the control with *O. basilicum* oil giving higher turbidity than the other two oils. The results show that all three oils promote crystallisation by producing more crystals in urine at different rates. This result is supported by the negative value of nucleation inhibition assay (Figure 2), which indicates that the oils may contain nucleating agents.

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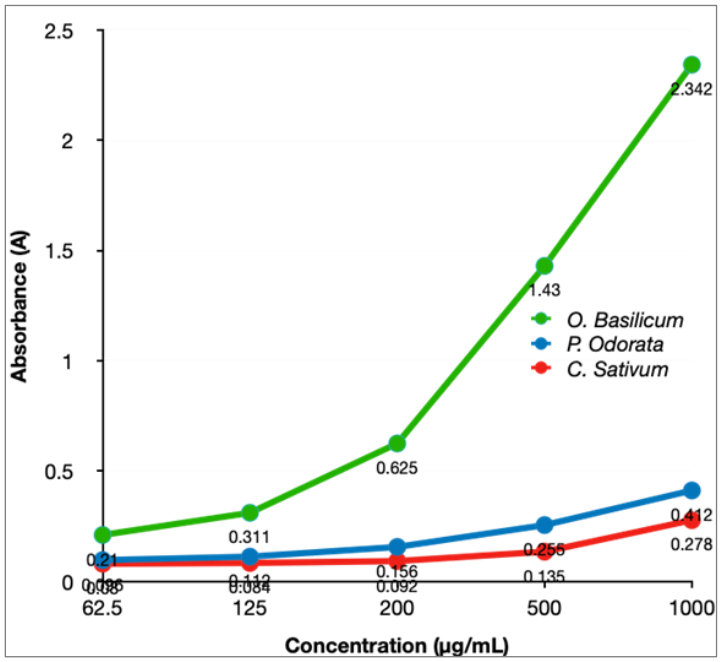

Figure 1: The optical density of induced whole urine treated with different concentration of essential oils

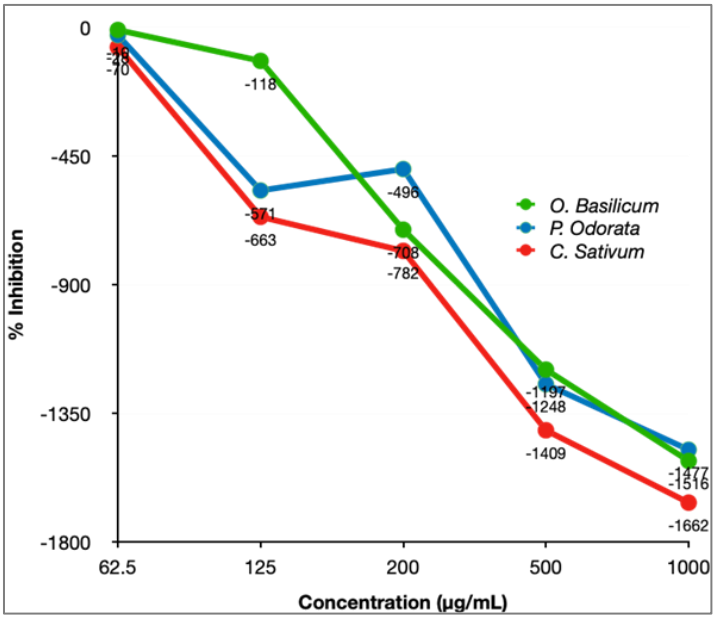

Figure 2: Percentage of inhibition of *Ocimum basilicum L.*, *Persicaria odorata*, and *Coriandrum sativum L.* oil on nucleation of calcium oxalate

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The size and morphology of the control and treated crystals were observed by Scanning Electron Microscopy (SEM). Most of the crystals formed were hexagonal calcium oxalate monohydrates (COM) and only a few calcium oxalate dihydrates (COD) were observed. Most of the crystals in the control sample observed were calcium oxalate monohydrates (Figure 3) with sizes ranging between 3 to 4 µm.

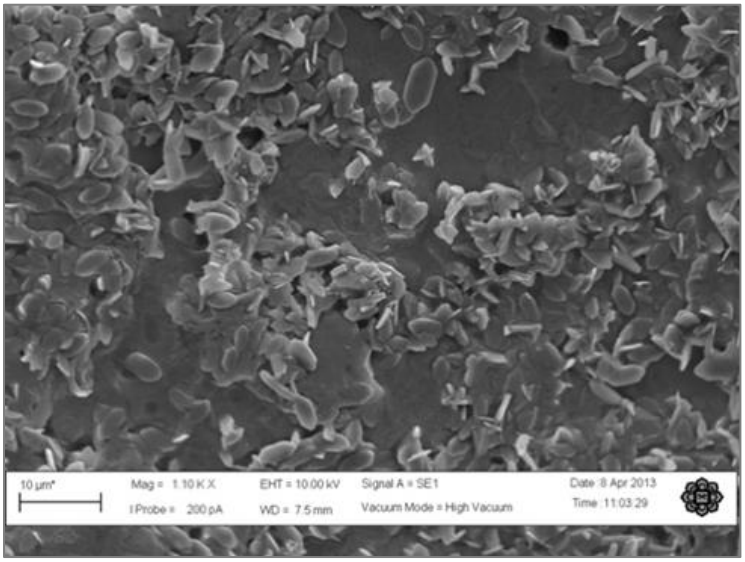

Figure 3: Morphology of calcium oxalate monohydrate, COM (control)

Addition of *O. basilicum* oil at 62.5 µg/mL, did not change the size of crystals (Figure 4a). However, increasing the concentration to 1000 µg/mL reduced the size of COM to 2-4 µm (Figure 4b). The increase in turbidity of the urine is dependent on the number of crystals but not on the size of the crystals [36]. In addition, increasing turbidity values reflects an increasing number of particles, increase in nucleation and crystal particle density [37]. The results also show that *O. basilicum* oil promotes crystallisation as the number of crystals increases but the size of crystals was reduced at higher concentrations. The oil was also found to promote aggregation of calcium oxalate crystals (Figure 4a) at low concentrations, forming stone-like structures compared to the control sample (Figure 3). However, as the concentration was increased to 1000 µg/mL, less aggregated crystals were formed (Figure 4b), showing that *O. basilicum* oil inhibited the aggregation of crystals or was able to break down aggregated crystals at higher concentrations.

The size of crystals in samples with *P. odorata* oil at low concentration (62.5 µg/mL) was bigger, between 5-7 µm, which consisted of COM form (Figure 4c). The crystals were also well aggregated compared to control but less aggregated compared to crystals treated with *O. basilicum* oil at the similar concentration. However, when the oil concentration was increased to 1000 µg/mL,

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a mixture of COD and COM was formed. The COD crystal size was about 3 µm, while the size of COM was relatively small and could not be estimated due to the inconsistency and irregularity of shape (Figure 4d). In addition, the crack in the bigger crystals could be observed and this might be due to the dehydration processes [38]. Thus, in this case increasing the concentration of oils also breaks down the crystals to smaller sizes. The formation of COD with smaller size (3 µm) is advantageous as this form of crystals is less tightly bound to epithelial cell surfaces compared to COM particles [39]. Therefore, the oil might contain substances that induce the formation of COD.

The size of the crystals treated with 62.5 µg/mL of *C. sativum* oil was smaller (2-6 µm) but the number of particles was higher than the control sample (Figure 4e). The COM crystals formed were less aggregated compared to control and sample treated with *O. basilicum* oil at the same concentration. However, at much higher concentration the size of crystals was bigger (5-6 µm) and highly aggregated compared to other samples (Figure 4f). The aggregation of crystals produced defined form of stones that could be found in urolithiasis patients. This study shows that *C. sativum* oil promotes the precipitation of calcium oxalate particles in urine and has no inhibitory effect on the aggregation of crystals.

Although the three essential oils promoted crystallisation of calcium oxalate, they also inhibited the aggregation of the crystals. The different proportions of compounds reflect the higher aggregation inhibitory activity of *O. basilicum* oil compared to the other two oils. Thus, *O. basilicum* oil may exhibit antiurolithic effect by inhibiting the aggregation of calcium oxalate crystals, which is the critical phase of kidney stone formation.

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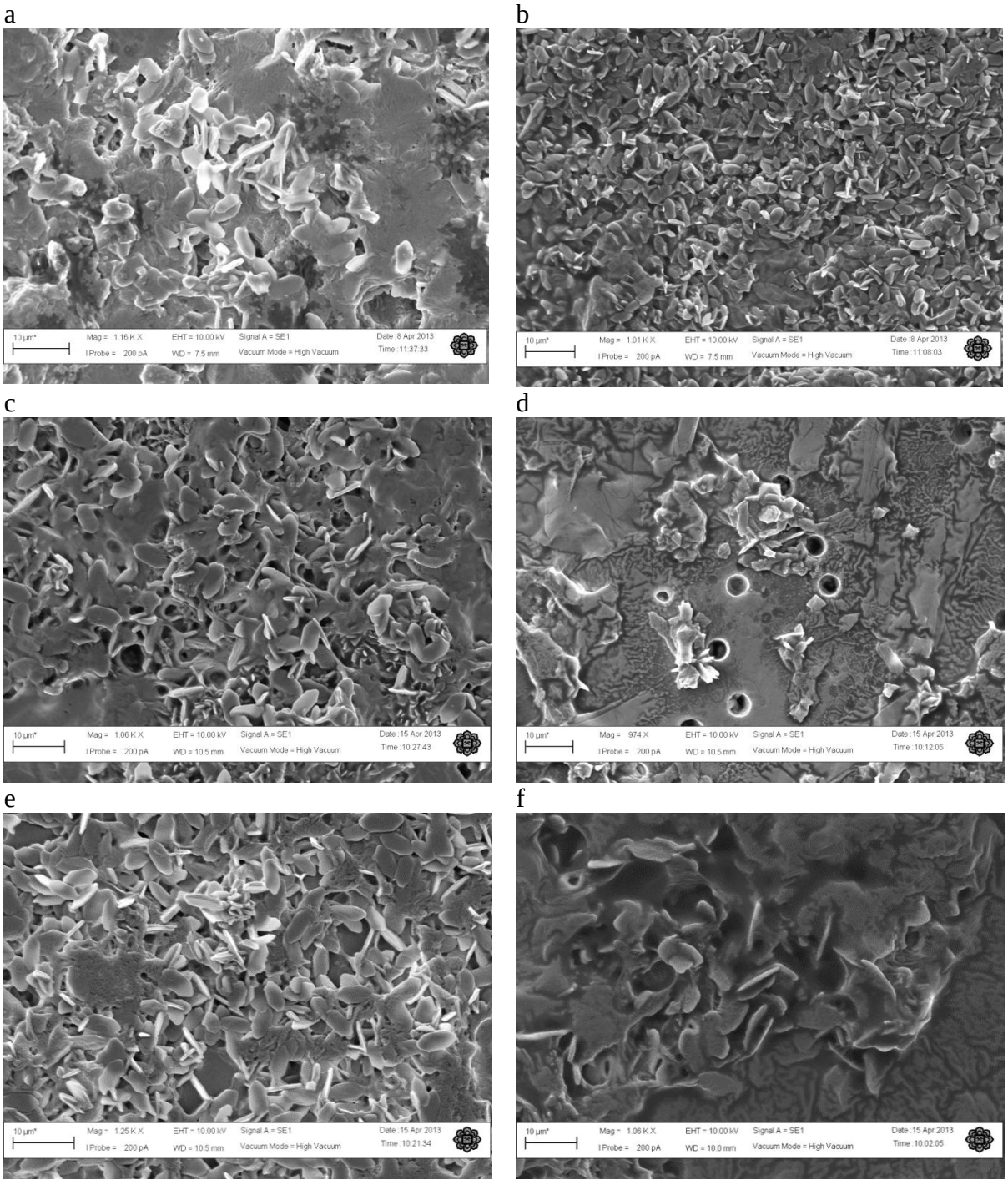

Figure 4: The SEM monographs of calcium oxalate formed within urine that treated with a. *O. basilicum* L. (62.5 µg/mL); b. *O. basilicum* L. (1000 µg/mL); c. *P. odorata* (62.5 µg/mL); d. *P. odorata* (1000 µg/mL); e. *C. sativum* L. (62.5 µg/mL) and f. *C. sativum* L. (1000 µg/mL)

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CONCLUSION

The oils from the leaves of *O. basilicum*, *P. odorata* and *C. sativum* were extracted and analysed, with estragole found to be the major component of all three essential oils. The oils were found to promote crystallisation of calcium oxalate. *O. basilicum* oil induced the highest number of crystals in urine, but the size of crystals was reduced with increasing concentration of the oil. This situation could prevent urinary stone formation by inducing the excretion of small particles from the kidney. The oils showed different inhibitory effects on aggregation of the crystals, which could be due to different amounts of components in each oil particularly estragole that could affect the aggregation of crystals. Although *P. odorata* oil gave bigger crystal sizes, it induced the formation of calcium oxalate dihydrate at higher concentrations, which may indicate that the oil contains substances that can inhibit calcium oxalate monohydrate formation. Inhibiting the early phase of urolithiasis is very important to prevent formation of crystals that lead to later phases of crystallisation. The effectiveness of *O. basilicum*, *P. odorata* in reducing the size and inhibiting aggregation of calcium oxalate crystals could provide a basis for the use of the oils in the treatment of urolithiasis.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

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