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Growth characterization of calcium hydrogen phosphate dihydrate crystals influenced by *Memecylon edule* extract

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Abstract
To investigate the inhibitory effect of ethanol extract of leaves of *Memecylon edule* on the growth of calcium hydrogen phosphate dihydrate (CHPD) crystals. Calcium hydrogen phosphate dihydrate (CHPD) crystals were grown by the single diffusion gel growth technique and the inhibitory effect of ethanol extracts of leaves of *Memecylon edule* on the growth of CHPD crystals has been studied. The grown crystals were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Powder X-Ray diffraction (XRD) for further confirmations. With an increase in the concentration of ethanol extract of *Memecylon edule*, the weight of the formed crystals were gradually reduced from 2.55 g to 0.13 g (leaves) for the CHPD crystals, respectively. The crystals harvested from the CHPD were characterized by Fourier Transform Infrared Spectroscopy (FTIR) to confirm the functional groups, and Powder X-Ray Diffraction technique (XRD) analyses to confirm the crystalline phases of the CHPD and hydroxyapatite (HAP) crystals. Results obtained indicated that *Memecylon edule* (leaves) has the potential to inhibit the formation of calcium hydrogen phosphate dihydrate crystals. This study confirms that using ethanol extract of leaves of *Memecylon edule* can promote the formation of hydroxyapatite (HAP) crystals and reduce the nucleation rate of CHPD crystals, a major component of calcium urinary stone.

Keywords: Calcium phosphate, hydroxyapatite, *Memecylon edule*, Fourier transform infrared spectroscopy (FTIR), powder x-ray diffraction (XRD)

Introduction
A large number of people are suffering from problems due to urinary stones (Mohamed *et al*., 2007) [1]. Urinary stone is formation of urinary calculi at any level of urinary tract. It is estimated that 12% of world population experiences renal stone disease with a recurrence rate of 70-80% in male and 47-60% in female (Tiwari *et al*., 2012) [2]. Urinary stones have been found to contain calcium phosphate, calcium oxalate, uric acid and magnesium ammonium phosphate with apatite and sruvites predominating (Beghalia *et al*., 2008; Aggarwal *et al*., 2000) [3, 4]. Epidemiological data collected during several decades showed that the majority of stones, up to 80%, are composed mainly of calcium oxalate (CaOx) (Daudon *et al*., 2004) [5]. Calcium containing stones are the most common comprising about 75% of all urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) and a mixture of both (45%) (Joshi *et al*., 2005; El-Shall *et al*., 2004) [6, 7]. Calcium oxalate stones are found in two different varieties, calcium oxalate monohydrate or whewellite and calcium oxalate dihydrate or weddellite (Bouropoules *et al*., 2004; Anjian *et al*., 2007; Monje *et al*., 2002; Aggarwal *et al*., 2000; Yongtai *et al*., 2008; Sheng *et al*., 2005) [8-13]. Calcium phosphate is present in urinary calculi as either apatite (Ca_{10} $(PO_4)_6(OH)_2$ or brushite ($CaHPO_4.2H_2O$) (Rajendran *et al*., 2010; Doddametikurke *et al*., 2007; Madhurambal *et al*., 2009) [14-16]. These calcium oxalate and calcium phosphate chemicals are part of a person's normal diet and make up important parts of the body, such as bones and muscles (Prasobh *et al*., 2011) [17]. Urinary stones are characterized by high recurrence rate therefore requiring a preventive treatment using medicinal plants (Fouad *et al*., 2004; Bensatal *et al*., 2008) [18, 19].

Memecylon edule Rox belongs to the family Melastomataceae and a valuable Indian ethnomedicinal plant (Elavazhagan *et al*., 2010) [20]. The leaves of *M. edule* was said to heal the burning wounds without scar. The anti-inflammatory, analgesic and antioxidant activities of the leaves used in traditional medicine in reliving inflammation and pain (Nualkeiu *et al*., 2009) [21]. Decoction of stem has also been relief fever symptoms of common diseases such as common cold, measles and chicken box (Joshi and Joshi, 2003) [22]. Decoction of stem has also been relief fever symptoms of common diseases such as common cold, measles and chicken box (Harathi *et al*., 2015) [23]. The studies of chemical constituents show the presence of Furfural, 2-Cyclopenten-1-one, 2-hydroxy-, 1-Benzoyl-3-amino-4-cyano-3-pyrroline,

2(3H)-Furanone, 3-acetyldihydro-, Phentermin-propionyl, cis-1,2-Dihydrocatechol, 1,2-Butanediol, 1-phenyl-, Hydrouracil, 1-methyl-, Methyl 2-furoate, Levoglucosenone, 1-Deoxy-d-altritol, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Benzoic acid, 2-hydroxy-, methyl ester, 1,4:3,6-Dianhydro-à-d-glucopyranose, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 2-Methoxy-4-vinylphenol, Hydroquinone, Methyl-à-d-ribofuranoside, 1,2,3-Benzenetriol, 1,3-Cyclohexanediol, 4,6-dimethyl-2-nitro-, diacetate (ester), (1à,2á,3à,4á,6á)-, Dodecanoic acid, D-Allose, Benzeneacetic acid, 4-hydroxy-3-methoxy-, 2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-, n-Hexadecanoic acid, cis-9-Hexadecenal (Manjckam *et al*., 2007; Murugan *et al*., 2006; Murugesan *et al*., 2011) [24-26]. Phenolic phytochemicals have antioxidative, antidiabetic anticarcinogenic, antimicrobial, antiallergic, and antimutagenic and anti-inflammtory (Rajendraprasad *et al*., 2006) [27]. In the present investigation, the effects of ethanol extract of leaves of *Memecylon edule* are used as additives to induce the nucleation and growth of CHPD crystals by single diffusion gel growth technique and are reported for the first time. This study incorporated a multidisciplinary approach in characterizing CHPD crystals grown *in vitro* to help formulate prevention or dissolution strategies in controlling calcium urinary stone growth.

Materials and Methods

Materials and instruments

Anhydrous ethanol, calcium chloride, magnesium acetate, oxalic acid, sodium metasilicate, orthophosphoric acid were all purchased from sigma-aldrich (New Delhi, India) analytical grade. Fourier Transform Infrared (FTIR) spectra were recorded with a nominal resolution of 4 cm^{-1} and a wave number range from 400 to 4000 cm^{-1} using the KBr pellet technique. Powder X-Ray Diffraction (XRD) was performed with a PW1710 based type set up using Cu radiation.

Collection of plant material

The leaves of *Memecylon edule* were collected in the month of June from the srirangam, Trichy, Tamil Nadu, India. The plant was identified and leaves of *Memecylon edule* were authenticated and confirmed from Dr. S. John Britto, Director, Rapinat Herbarium, St. Joseph's College, Tiruchirapalli, and Tamil Nadu for identifying the plants.

Preparation of ethanol extracts

The leaves of *Memecylon edule* were washed in running water, cut into small pieces and then shade dried for a week at 35-40ºC, after which it was grinded to a uniform powder of 40 mesh size (Joshi *et al*., 2005) [6]. The ethanol extracts were prepared by soaking 100 g each of the dried powder plant materials in 1 L of ethanol using a soxhlet extractor continuously for 10 hr. The extracts were filtered through whatmann filter paper No. 42 (125mm). The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The filtrate was condensed using a rotary evaporator and the residue 1.2 g (leaves) obtained were used to prepare the series (0.15, 0.25, 0.50, 0.75 and 1.0%) of supernatant concentrations for *in vitro* studies (table 1).

Growth of CHPD crystals

Glass test tubes were used as a crystallization apparatus and the single diffusion reaction technique was employed. 1M Ortho phosphoric acid was mixed with the sodium metasilicate ($Na_2SiO_3 \bullet 9H_2O$) solution (density 1.04g/cm^3 at pH 9.4), so that the pH of the mixture was maintained at 5 and left undisturbed for 2-3 days. After gelation took place, a supernatant solution of 1 M calcium chloride ($CaCl_2$) was gently poured onto the set gel. After adding the supernatant solution, the test tubes were capped airtight. All experiments were conducted at a temperature of 37 ± 2°C. The grown CHPD crystals were characterized using FTIR, powder XRD techniques to verify the structure and proper formation of the grown crystals

Table 1: Supernatant solutions added to the set gels for CHPD crystals.

Supernatant Solutions (SS) (Groups and Treatments)	Compositions
I (Control)	10 ml of 1 M calcium chloride
II (Distilled water)	5 ml of 1 M calcium chloride+5 ml of distilled water
III (0.15% methanol extract)	5 ml of 1 M calcium chloride+5 ml of 0.15% of ethanol extract of leaves of *Memecylon edule* separately
IV (0.25% methanol extract)	5 ml of 1 M calcium chloride+5 ml of 0.25% ethanol extract of leaves of *Memecylon edule* separately
V(0.50% methanol extract)	5 ml of 1 M calcium chloride+5 ml of 0.50% of ethanol extract of leaves of *Memecylon edule* separately
VI(0.75% methanol extract)	5 ml of 1 M calcium chloride+5 ml of 0.75% of ethanol extract of leaves of *Memecylon edule* separately
VII(1.00% methanol extract)	5 ml of 1 M calcium chloride+5 ml of 1.00% of ethanol extract of leaves of *Memecylon edule* separately

The nomenclature of different additive solution on the growth of CHPD crystals

An attempt was made to investigate the putative activity of the plant extracts as inhibitors of CHPD crystal formation in gel method. The supernatant solutions as given in (table 1) were added to the set gels and the results were noted. The experiments were repeated four times. To study the effect of the ethanol extract of leaves of *Memecylon edule* on the growth of CHPD crystals, a series of five different concentrations of 0.15, 0.25, 0.50, 0.75 and 1.00% of these plant extracts were added in equal amounts in supernatant solution and the average weight of the grown crystal were measured.

Statistical analysis

The masses of the crystals (gm) are presented as the mean ±standard deviation for the control and treatment samples. One-way analysis of variance (ANOVA) followed by tukey's test for multiple comparisons were made between groups. Values of $p<0.05$ was considered to be significant.

Results

Effect of *Memecylon edule* on CHPD crystals

The effect of the ethanol extract of the leaves of *Memecylon edule* on nucleation and crystallization characteristics of CHPD crystals is determined by measuring the weight of the formed crystals. The control using pure calcium chloride led

to the nucleation of crystal growth within 24 h of adding the supernatant solutions. The liesegang ring was observed after 48 h of pouring the supernatant solution. The formation of liesegang (5-10 rings) rings which have promoted crystals growth as observed in the present study (fig. 1a). However, at the same time the first few liesegang rings started diffusion. The distance between two consecutive liesegang rings was found to be increased towards bottom of the test tubes. The elongated broad needle shaped crystals were grown within the liesegang ring as observed after 96 h. In the presence of ethanol extract of leaves of *Memecylon edule*, nucleation was delayed and reduced masses of the crystals were observed after adding the supernatant solutions (fig. 1b-g). The liesegang rings formation was reduced after the addition of ethanol *Memecylon edule* extracts. Moreover, supernatant solutions (ethanol leaves of *Memecylon edule*) exhibited an inhibitive effect compared to control (pure calcium chloride), and a minimum apparent length of growing crystals was observed. CHPD growth habit was observed during and after harvesting crystals from the gel systems. Morphology of the harvested CHPD crystals as shown in (fig. 2). The largest single CHPD crystals having dimensions of 2.7 cm and 2.4 cm as observed in (fig. 3a). The sizes of the CHPD crystals were reduced from 2.7 cm to 1.7 cm and 1.4 cm at 0.15% extract, 1.5 cm and 1 cm at 0.25%, 0.9 cm and 0.7 cm at 0.50%, 0.6 cm and 0.6 cm at 0.75% and 0.5 cm and 0.3 cm at 1.00% observed in (figs. 3b-g). With an increase in the concentration of ethanol extracts of *Memecylon edule* from 0.15% to 1.00% (w/v), the weight of the formed crystals were gradually reduced from 2.55 g to 0.13 g (leaves) respectively. The ANOVA statistical analysis was performed for masses of CHPD crystals have been evaluated, and $p<0.05$ has suggested that the correlation is significant as shown in (table. 2).In the present work, CHPD crystals growth were reduced due to the inhibitory effect of ethanol extracts of *Memecylon edule* under *in vitro* conditions.

Table 2: ANOVA statistical analysis for harvested CHPD crystals

Groups and Treatments	Mean weight of the CHPD crystals (gm)±S.D
	Leaves
I (Control)	2.55±0.057
II (Distilled water)	2.34±0.081
III (0.15% ethanol extracts)	$1.28\pm0.014^{a,b}$
IV (0.25% ethanol extracts)	$0.59\pm0.014^{a,b,c}$
V (0.50% ethanol extracts)	$0.38\pm0.014^{a,b,c,d}$
VI (0.75% ethanol extracts)	$0.25\pm0.014^{a,b,c,d}$
VII (1.00% ethanol extracts)	$0.13\pm0.014^{a,b,c,d,e}$

Values represent mean (gm) ± S.D (n=4) Comparisons between means are as follows. a: I *vs* II-VII, b: II *vs* III-VII, c: III *vs* IV-VII, d: IV *vs* V-VII, e: V *vs* VI-VII, f: VI *vs* VII. Statistical significance were considered to be $^{a}pe<0.05$, $^{bp}<0.05$, $^{c}p<0.05$, $^{DP<}0.05$, $^{e}p<0.05$.

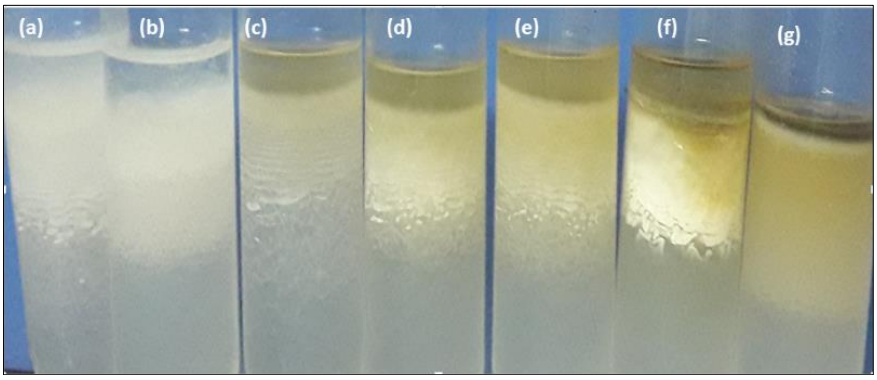

Fig 1: The effect of *Memecylon edule* on CHPD crystals in the gel method (a) without any additive (b) with the distilled water (c) with the 0.15% of ethanol extract (d) with the 0.25% of ethanol extract (e) with the 0.50% of ethanol extract (f) with the 0.75% of ethanol extract (g) with the 1.00% of ethanol extract after 7 days.

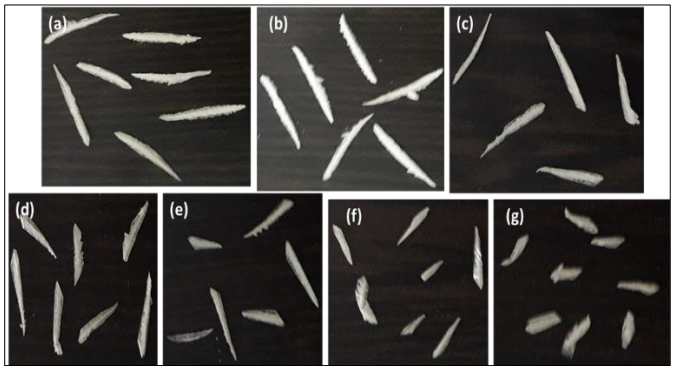

Fig 2: The harvested crystals of CHPD obtained from *Memecylon edule* in the gel method (a) without any additive (b) with the distilled water (c) with the 0.15% of ethanol extract (d) with the 0.25% of ethanol extract (e) with the 0.50% of ethanol extract (f) with the 0.75% of ethanol extract (g) with the 1.00% of ethanol extract.

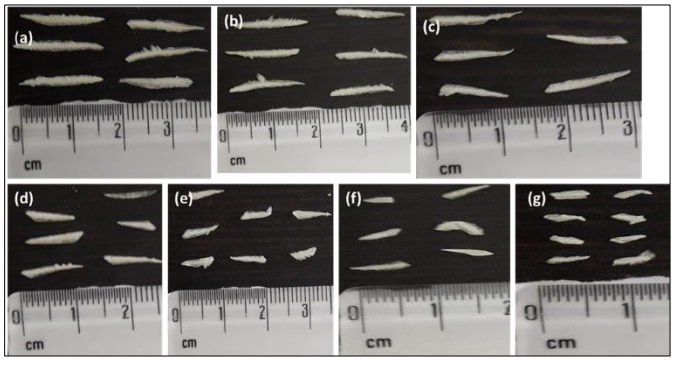

Fig 3: The measurement of CHPD obtained from *Memecylon edule* in the gel method (a) without any additive (b) with the distilled water (c) with the 0.15% of ethanol extract (d) with the 0.25% of ethanol extract (e) with the 0.50% of ethanol extract (f) with the 0.75% of ethanol extract (g) with the 1.00% of ethanol extract.

Characterization of CHPD crystals

The FTIR spectra of CHPD crystals obtained in the presence and absence of the ethanol extract of leaves of *Memecylon edule* are shown in (fig. 4). In Fig. 4a, the absorptions at 3490 cm^{-1} are due to intermolecular and weakly H bonded OH because of water of crystallization. The weak absorption at 2378 cm^{-1} is due to HPO_4^{2-}. The H-O-H bending gives rise to absorption at 1650 cm^{-1}. The absorption at 1217 and 1133 cm^{-1} are due to P=O associated stretching vibrations. Whereas, the absorption at 1064 cm^{-1} is due to P=O stretching vibrations. The P-O-P asymmetric stretching vibrations give rise to absorption at 990, 872 cm^{-1}. The absorption at 666 cm^{-1} is due to (H-O-) P=O. However, the strong absorption at 576 and 526 cm^{-1} are again due to acid phosphate. In (fig. 4b), the absorptions at 3485 cm^{-1} are due to intermolecular and weakly H bonded OH because of water of crystallization. The week absorption at 2385 cm^{-1} is due to HPO_4^{2-}. The H-O-H bending give rise to absorption at 1647 cm^{-1}. The absorption at 1132 cm^{-1} is due to P=O associated stretching vibrations. Whereas, the absorption at 1065 cm^{-1} is due to P=O stretching vibrations. The P-O-P asymmetric stretching vibrations give rise to absorption at 990, 872 and 790 cm^{-1}. The absorption at 667 cm^{-1} is due to (H-O-) P=O. However, the strong absorption at 575 and 526 cm^{-1} are again due to acid phosphate. At higher concentration of ethanolic extract of leaves of *Memecylon edule* (1.00%) shifting from brushite crystals band at 1064 cm^{-1} to hydroxyapatite crystals band at 1012 cm^{-1}. The shifting further supports that the leaves of *Memecylon edule* favour the nucleation and or transformation of brushite into hydroxyapatite crystals.

The XRD patterns of CHPD crystals obtained in the presence and absence of the ethanol extract of leaves of *Memecylon edule* are shown in (fig. 5). The diffraction peaks obtained were well correlated to the (hkl) indices of CHPD phase (JCPDS card number 09-0077) and the hydroxyapatite phase (JCPDS card number 9-432). It is inferred from the above results that the leaves extract of *Memecylon edule* effected the nucleation and growth of hydroxyapatite crystals.

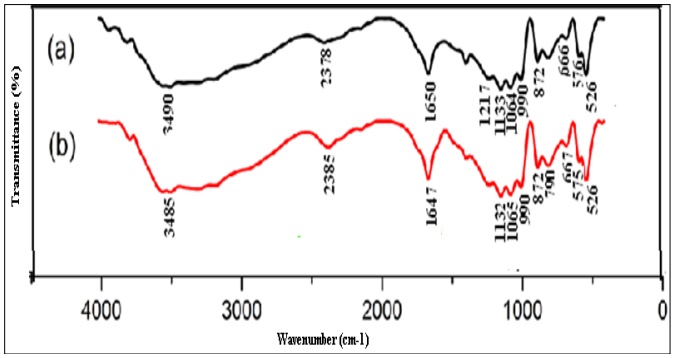

Fig 4: The FTIR spectra of CHPD in the gel method (a) without any additive (b) with the 1.00% of ethanol extract of leaves of *Memecylon edule*.

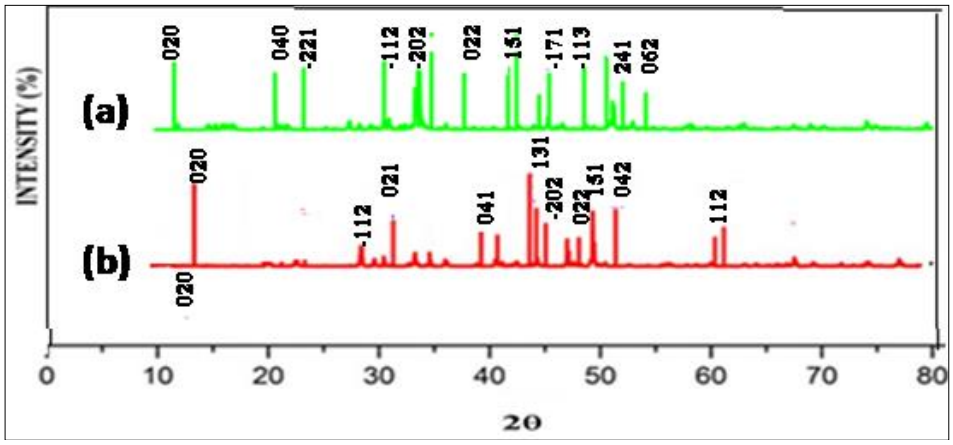

Fig 5: The XRD pattern of CHPD in the gel method (a) without any additive (b) with the 1.00% of ethanol extract of leaves of *Memecylon edule*.

Discussion

The single diffusion gel growth technique has found to be promising method to grow CHPD crystals. This technique provides much simplified method to understand the growth of urinary crystal *in vitro*. It can be seen from the above results that the ethanol extracts of leaves of *Memecylon edule* inhibit the nucleation and growth of CHPD crystals. The reduction of the length of crystals and the number of liesegang rings are due to the presence of inhibitive solution containing *Memecylon edule* extracts. This reduction in the average apparent length is minimum in case of the supernatant solution containing 0.75% and 1.00% extracts of *Memecylon edule* followed by 0.25% and 0.50% extracts of *Memecylon edule.* The formation of liesegang rings was observed in the present study. The effect of various parameters such as, the gel pH, the concentration of reactants and the formation of liesegang rings were previously reported (Joshi *et al*., 2005; Joseph and Joshi, 2002; Henisch *et al*., 1986) [28-30]. With an increase in the concentration of aqueous extracts of *Memecylon edule* from 0.15% to 1.00% (w/v), the weight of the formed crystals were gradually reduced as shown in (tables 2). The ANOVA statistical analysis was performed for masses of CHPD crystals have been evaluated, and $p<0.05$ has suggested that the correlation is significant. Group III to VII (treated with aqueous *Memecylon edule* extract at various concentration 0.15% to 1%) of crystal masses were significantly different at $p<0.05$ when compared to Group I (untreated control), whereas Group II (treated with distilled water) was not significantly different at $p<0.05$ compared to Group I.

This Group II indicates that distilled water has not contained any inhibitory activity on crystal growth whereas ethanol extract of *Memecylon edule* has inhibitory activity due to the presence of natural substances such as Furfural, 2-Cyclopenten-1-one, 2-hydroxy-, 1-Benzoyl-3-amino-4-cyano-3-pyrroline, 2(3H)-Furanone, 3-acetyldihydro-, Phentermin-propionyl, cis-1,2-Dihydrocatechol, 1,2-Butanediol, 1-phenyl-, Hydrouracil, 1-methyl-, Methyl 2-furoate, Levoglucosenone,

1-Deoxy-d-altritol, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Benzoic acid, 2-hydroxy-, methyl ester, Hydroquinone, Methyl-à-d-ribofuranoside, 1,2,3-Benzenetriol, 1,3-Cyclohexanediol, 4,6-dimethyl-2-nitro-, diacetate (ester), (1à,2á,3à,4á,6á)-, Dodecanoic acid, D-Allose, Benzeneacetic acid, n-Hexadecanoic acid, cis-9-Hexadecenal (Elavazhagan *et al*., 2010; Nualkew *et al*., 2009) [20, 21]. Group VI and VII (treated with 0.75% and 1% extracts) were not significantly different. Recently, growth inhibition studies of CHPD crystals in the presence of some of the herbal extracts *Tribulus terrestris* and *Bergenia ligulata* (Murugesan *et al*., 2011) [26], *Terminalia arjuna* (Chaudhary *et al*., 2010) [31] citric acid and lemon juice along with human urine and artificial reference urine (Joshi and Joshi, 2003) [32], citric acid [14], tartaric acid and tamarind solution (Joseph and Parakh, 2010) [33] were attempted in literature. In the present work, CHPD crystals growth were reduced and the morphology of the crystals changed from hydroxyapatite in brushite crystals due to the inhibitory effect of ethanol extracts of leaves of *Memecylon edule* under *in vitro* conditions. Several researchers (Joshi and Joshi, 2003; Roop Kumar *et al*., 2001; Markovii *et al*., 2004) [34-36] have reported crystallization characterization of CHPD crystals using FTIR techniques. The formation of hydroxyapatite in brushite crystals due to leaves of *Memecylon edule* (fig.4). Further it has been reported for the CHPD crystals (Rajendran *et al*., 2010;Joshi and Joshi, 2003) [14, 34], the diffraction peaks 11.69, 21.0, 23.44, 29.32, 30.54, 34.18, 37.10, 41.6, 42.0, 45.28, 48.49 and 50.25 for brushite crystals and for the hydroxyapatite crystals, the diffraction peaks 16.87, 18.84, 21.75, 22.84, 25.86, 28.92, 32.18, 32.90, 34.04, 35.44, 39.79, 40.43, 43.84, 44.36, 45.29, 48.58, 49.46, 50.47, 51.25, 53.16, 54.43, 58.03 were attempted in the literature are well correlate in (fig. 5). Altogether, Crystal growth and inhibition in the presence of herbal extracts exhibits interesting results, *in vitro* study on the growth and inhibition of these CHPD crystals under the influence of herbal extracts *Memecylon edule* has been reported first time in the present study. The inhibition of Brushite crystals increases as the concentration of herbal extracts increases; consequently, the number of grown crystals and their average size decrease. The influence of the extracts of *Memecylon edule* on CHPD crystals by gel method showed that the leaves can promote the formation of hydroxyapatite crystals and reduce the nucleation rate of CHPD crystals. Although the stone formation process occurring in the human body is quite complex and takes place in a dynamic environment, the present study provided basic information, under laboratory conditions, which led us to identify new inhibiting herbal extracts for stone growth.

Conclusion

CHPD crystals were grown by single diffusion gel growth techniques and were characterized by FTIR and Powder XRD techniques for the experimental confirmations of the grown crystal. With an increase in the concentration of ethanol extract of *Memecylon edule*, the weight of the formed crystals were gradually reduced from 2.35 g to 0.13 g (leaves) for the CHPD crystals, respectively. The formation of hydroxyapatite was observed in brushite crystals due to inhibitory action by the ethanol extracts of leaves of *Memecylon edule* under *in-vitro* conditions. The leaves of *Memecylon edule* can reduce the nucleation rate of CHPD crystals. FTIR and Powder XRD techniques confirmed its functional groups and crystalline phases of CHPD crystals. One way ANOVA performed with treated and untreated crystal growth data obtained from CHPD crystals showed significant differences ($p<0.05$). This study confirmed that the leaves of *Memecylon edule* extracts can promote the formation of hydroxyapatite crystals and treat urinary stone by inhibiting the formation of CHPD crystals, a major component of calcium urinary stone. This study is focused to find new alternative medicine for the treatment of calcium oxalate urinary stone.

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Conflicts of interests

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

References

1. Mohamed B, Said G, Hocine A, Aissa B. Effect of herbal extracts of *Tetraclinis articulata* and *Chamaerops humilis* on calcium oxalate crystals *in vitro*. Gomal J Med Sci. 2007; 5(2):55-8.
2. Tiwari A, Soni V, Londhe V, Brandarkar A, Bandawanw D, Nipate S. An overview on potent indigenous herbs for urinary tract infirmity: urolithiasis. Asian J Pharm Clin Res. 2012; 5(1):7-12.
3. Beghalia M, Ghalem S, Allali H, Belouatek A, Marouf A. Inhibition of calcium oxalate monohydrate crystal growth using Algerian medicinal plants. J Med Plants Res. 2008; 2(3):066-70
4. Aggarwal S, Tandon CD, Forouzanveh M, Simgla SK, Kiran R, Jethi RK. Role of biomolecules from human renal stone matrix on COM crystal growth. Mol Cell Biochem. 2000; 210:109-19.
5. Daudon M, Bader CA, Jungers P. Urinary calculi: review of classification methods and correlations with etiology. Scanning Microsc. 1993; 7(3):1081-106.
6. Joshi VS, Parekh BB, Joshi MJ, Vaidya AB. Herbal extract of *Tribulus terrestris* and *Bergenia ligulata* inhibit growth of calcium oxalate monohydrate crystals *in vitro*. J Cryst Growth. 2005; 275:1403-8.
7. El Shall H, Jeon JH, Abdel-Aal EA, Khan S, Gower L, Rabinovich Y. A study of primary nucleation of calcium oxalate monohydrate: II. Effect of urinary species. Cryst Res Technol. 2004; 39(7):577-85.
8. Bouropoulos C, Vagenas N, Klepetsanis P, Stavropoulos N, Bouropoulos N. Growth of calcium oxalate monohydrate on uric acid crystals at sustained supersaturation. Cryst Res Technol. 2004; 39(8):699-704.
9. Anjian X, Yuhua S, Dong M, Fangzhi H, Lingguang Q, Shikuo L *et al*. Growth of calcium oxalate crystals induced by complex films containing biomolecules. Cryst Res Technol. 2007; 42(7):667-72.
10. Monje PV, Baran EJ. Characterization of calcium oxalate generated as bio minerals in cacti. Plant Physiol. 2002; 128(2)**:**707-13.
11. Valarmathi D, Abraham L, Gunasekaran S. Growth of calcium oxalate monohydrate crystal by gel method and its spectroscopic analysis. Ind. J Pure Appl Phys. 2010; 48(8):36-8.

12. Yongtai Z, Jiansheng T, Nianping F, Xiangdong H. Crystal growth of calcium oxalate induced by the extracts of *Semen plantaginis* and *Folium pyrrosiae*. Cryst Res Technol. 2008; 43:931-4.
13. Sheng X, Ward MD, Wesson JA. Crystal surface adhesion explains the pathological activity of calcium oxalate hydrates in kidney stone formation. J Am Soc. Nephrol. 2005; 16**:**1904-8.
14. Rajendran K, Dale keefe C. Growth and characterization of calcium hydrogen phosphate dihydrate crystals from single diffusion gel technique. Cryst Res Technol. 2010; 45:939-45.
15. Doddametikurke RB, Chandra SB, Anthony J, Browning Cartledge JJ. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. Eur Urol. 2007; 5:126-36.
16. Madhurambal G, Subha R, Mojumdar SC. Crystallization and thermal characterization of calcium hydrogen phosphate dihydrate crystals. J Therm Anal Calorim. 2009; 96:73-6.
17. Prasobh GR, Revikumar KG. Use of Musa AAB in kidney stone treatment and other diseases. Asian J Pharm Clin Res. 2011; 4(3):117-8.
18. Fouad A, Yamina S, Mostapha M, Mohammed A, Brahim H. Effect of aqueous extract from *Herniaria hirsute* L. on experimentally nephrolithiasic rats. J Ethnopharmacol. 2004; 95(l):87-93.
19. Bensatal A, Ouahrani MR. Inhibition of crystallization of calcium oxalate by the extraction of *Tamarix gallica* L. Urol Res. 2008; 36(6):283-7.
20. Elavazhagan T, Arunachalam KD. Phytochemical and antibacterial studies of seed extract of *Memecylon edule.* Int. J Eng. Sci. Tech. 2010; 5(4):498-503.
21. Nualkew, S, Rattanmanee K, Thongpraditchote A, Wongkrajang Y, Nahrstedt A. Anti-inflammatory, analgesic and wound healing activities of the leaves of *Memecylon edule* Roxb. J Ethnopharmacol. 2009; 121(2):278-281.
22. Harathi TR, Madhusudhan MC, Pradeep Kumar PM, Chandra Nayaka S, Prakash HS. Antimicrobial Potential of *Memecylon* L. species from Western Ghats against clinical isolates of pathogenic bacteria. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2015; 6(4):1280-1287.
23. Harathi TR, Madhusudhan MC, Pradeep Kumar PM, Chandra Nayaka S and Prakash HS. Antimicrobial Potential of *Memecylon* L. species from Western Ghats against clinical isolates of pathogenic bacteria. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2015; 6(4):1280-1287.
24. Manickam V S, Murugan C, Jothi GJ and Sunderesan V. *Memecylon courtallense* A new species (Melastomataceae) from Courtallam Hills of Tamil Nadu, India. Indian J For. 2007; 30(1):77-80.
25. Murugan C and Gopalan R. Four new additions to Indian *Memecylon* L. (Melastomataceae) from South India. Indian J For. 2006; 29(1):105-108.
26. Murugesan S, Vijayakumar R and Panneerselvam A. Evaluation of Phytochemical Constituents from the Leaves of *Memecylon umbellatum* Burm. f. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011; 2(4):1145-1152.
27. Rajendraprasad M, Prathapan SL, Pandurangan AG and Shaju T. *Memecylon royenii* Blume (Melastomataceae): A new record for India. Indian For. 2006; 132(2):229-32.
28. Joshi VS, Parekh BB, Joshi MJ, Vaidya ADB. Inhibition of growth of urinary calcium hydrogen phosphate dihydrate crystals with aqueous extracts of Tribulus *terrestris* and *Bergenia ligulata*. Urol Res. 2005; 33:80-6.
29. Joseph KC, Joshi MJ. The study of different parameters affecting liesegang ring formation during the growth of calcium hydrogen phosphate dihydrate crystals. Indian J Phys. 2002; 76:159.
30. Henisch HK, Garcia Ruiz JM. Crystal growth in gels and liesegang ring formation. J Cryst Growth. 1986; 75:195.
31. Chaudhary A, Singla SK, Tandon C. *In vitro* evaluation of *Terminalia Arjuna* on calcium phosphate and calcium oxalate crystallization. Indian J Pharm Sci. 2010; 72(3): 340-5.
32. Joshi VS, Joshi MJ. Influence of inhibition of citric acid and lemon juice to the growth of calcium hydrogen phosphate dihydrate urinary crystals. Indian J pure Appl Phys. 2003; 41:183-192.
33. Joseph KC, Parekh BB, Joshi MJ. Inhibition of growth of urinary type calcium hydrogen phosphate dihydrate crystals by tartaric acid and tamarind. Curr Sci. 2005; 88 8(l25):1232-8.
34. Joshi VS, Joshi MJ. FTIR spectroscopic, thermal and growth morphological studies of calcium hydrogen phosphate dihydrate crystals. Cryst Res Technol. 2003; 38:817-21.
35. Roop Kumar R, Wang M. Biomimetic deposition of hydroxyapatite on brushite single crystals grown by the gel technique. Mater Lett. 2001; 49:15-9.
36. Markovie M, Fowler BO, Tung MS. Preparation and comprehensive characterization of a calcium hydroxyapatite reference material. J Res Nat Inst Stand Technol. 2004; 109:553-68.