

GROWTH CHARACTERIZATION OF CALCIUM OXALATE MONOHYDRATE CRYSTALS INFLUENCED BY *COSTUS IGNEUS* AQUEOUS STEM EXTRACTK. MANJULA^a, K. PAZHANICHAMY^a, **S. KUMARAN^a***, T. EEVERA^b, C. DALE KEEFE^c AND **K. RAJENDRAN^a**^aDepartment of Biotechnology, Periyar Maniammai University, Thanjavur, Tamilnadu, India, ^bDryland Agricultural Research Station, Tamil Nadu Agriculture University, Chettinad, Tamil Nadu, India, ^cDepartment of Chemistry, Cape Breton University, Sydney, Nova Scotia, B1P 6L2, Canada. Email: nkraj64@yahoo.co.uk

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ABSTRACT

Calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) are frequently found in urinary calculi (stones). COM crystals were grown using both gel and liquid growth methods, and the inhibitory effect of aqueous *Costus igneus* stem extract on the growth of COM crystals was studied. The results indicate that with an increase in the concentration of aqueous *C. igneus* extract, the mass of the formed crystals was gradually reduced from 2.16 g to 0.08 g and from 2.89 g to 0.035 g for the gel and liquid methods, respectively. The crystals were characterized by Fourier transform infrared spectroscopy (FTIR) to confirm the functional groups and powder X-ray diffraction (XRD) analyses to confirm the phases of the COM and COD crystals. Scanning electron microscopy (SEM) confirmed that the morphology of the crystals changed from hexagonal to bipyramidal, which is characteristic of a change from COM to COD. The average size of the crystals from the gel method was reduced from 1874.1 x 857.8 μm to 1075.5 x 990.5 μm from 0.524 μm to 0.291 μm using the liquid method. Lupeol and the Stigmasterol are the active compound were identified and isolated by High performance thin layer chromatography (HPTLC) techniques. This study confirms that the use of aqueous *C. igneus* extracts can promote the formation of COD crystals and reduce the nucleation rate of COM crystals, a major component of oxalate urinary stones.

Keywords: Nucleation, Characterization, Crystal Morphology, X-Ray Diffraction, Calcium Oxalate Monohydrate, *Costus igneus***INTRODUCTION**

Many people suffer from problems resulting from urinary stones. Calcium-containing stones are the most common variety of urinary stone, and they comprise about 75% of all urinary calculi, which are found in the form of pure calcium oxalate (50%), calcium phosphate (5%) or a mixture of both (45%)¹⁻⁵. Calcium oxalate (CaOxa) is a major component of urinary stones that is generally found in two different varieties, calcium oxalate monohydrate (COM), or Whewellite, and calcium oxalate dihydrate (COD), or Weddellite. It is difficult to form urinary stones from COD because COD crystals are unstable and are easily excreted in the urine of both humans and animals⁶⁻¹¹. The formation of COD crystals actually protects against stone disease because of their reduced capacity to form stable aggregates or strong adhesion contacts to renal epithelial cells¹² due to the single micron-sized crystals. Urinary stones are characterized by high recurrence rates and would therefore benefit from a preventive treatment using medicinal plants¹³⁻¹⁵. *Costus igneus*, also known as Fiery Costus, Spiral Flag or Insulin Plant, belongs to the Costaceae family. It contains a range of phytochemicals¹⁶, such as flavonoids, alkaloids and terpenoids, and is traditionally used in India to control diabetes¹⁷. Administration of the aqueous extract of *C. spiralis* to rats with experimentally induced urolithiasis has been found to reduce the growth of urinary stones¹⁸. Additionally, the blood glucose levels of alloxan-induced diabetic rats were controlled after the administration of ethanolic extracts of *C. igneus* leaves¹⁹. However, it has been cautioned that the constant use of *C. pictus* increases the LDL to HDL cholesterol ratio¹⁷ due to higher levels (24.51% in leaves, 28.30% in the stem and 25.26% in the rhizome) of hexadecanoic acid found in diethyl ether extractions²⁰. In the present study, the effects of aqueous *C. igneus* stem extracts on the nucleation and growth of CaOxa crystals, using both gel and liquid growth methods, are reported for the first time. Lupeol and the Stigmasterol were identified and isolated by HPTLC techniques. This study incorporates a multidisciplinary approach for the characterization of COM crystals grown *in vitro* to facilitate the development of prevention and dissolution strategies aimed at managing urinary stone growth.

MATERIALS AND METHODS**Materials and instruments**

Anhydrous ethanol, calcium chloride, sodium oxalate, magnesium acetate, oxalic acid and sodium metasilicate were purchased from Sigma-Aldrich (New Delhi, India) and were labeled analytical grade. All reagents and double distilled water were used without further purification. 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 diffractometer using CuKα radiation. Scanning Electron Microscopy (SEM) with an accelerating voltage of 20 kV was utilized to analyze our products.

Collection and extraction of plant materials

The medicinal plant *C. igneus* was collected from the herbarium of the Periyar Maniammai University, Vallam, Thanjavur and identified at the Rapinat Herbarium, St. Joseph College, Tiruchirappalli, Tamil Nadu, India. The *C. igneus* aqueous extract (A.E) was prepared by boiling 25 g of *C. igneus* stems in 100 mL distilled water for 30 minutes and then filtering through Whatman filter paper twice³. The filtrate was condensed using a rotary evaporator and the obtained residue (1.4 g) was used to prepare the series of aqueous supernatant concentrations for *in vitro* studies shown in Table 1.

Growth and characterization of COM crystals with gel methods

Glass test tubes were used as a crystallization apparatus, and the single diffusion reaction technique was employed. A solution of Na₂SiO₃•9H₂O (density of 1.04 g/cm³ at pH 9.4) was mixed with 1M (COOH)₂•2H₂O so that the pH of the mixture was maintained at 5, and it was left undisturbed for 2-3 days. After gelation occurred, a supernatant solution of 1 M CaCl₂ and 1 M MgCH₃COO₂•4H₂O 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 nomenclature of different additive solutions on the growth of COM crystals with the gel method

An attempt was made to investigate the putative activity of the plant extracts as inhibitors of COM crystal formation in the gel method. The supernatant solutions shown in Table 1 were added to the set gels and

the results were noted. The experiments were repeated four times. To study the effects of the aqueous extract of *C. igneus* on the growth of COM crystals, five different concentrations of the plant extracts (see Table 1) were added to equal amounts of the supernatant solution, and the average weights of the grown crystals were measured.

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

Supernatant Solutions (Groups and Treatments)	Compositions
I (CONTROL)	10 mL of 1M CaCl ₂ + 10 mL of 1M (CH ₃ COO ₂)Mg.4H ₂ O
II (Dist.H ₂ O)	5 mL of 1M CaCl ₂ + 5 mL of 1M (CH ₃ COO ₂)Mg.4H ₂ O + 10 mL of distilled water
III (0.15% A.E)	5 mL of 1M CaCl ₂ + 5 mL of 1M (CH ₃ COO ₂)Mg.4H ₂ O + 10 mL of 0.15% aqueous extract of stem of <i>Costus igneus</i>
IV (0.25% A.E)	5 mL of 1M CaCl ₂ + 5 mL of 1M (CH ₃ COO ₂)Mg.4H ₂ O + 10 mL of 0.25% aqueous extract of stem of <i>Costus igneus</i>
V (0.50% A.E)	5 mL of 1M CaCl ₂ + 5 mL of 1M (CH ₃ COO ₂)Mg.4H ₂ O + 10 mL of 0.50% aqueous extract of stem of <i>Costus igneus</i>
VI (0.75% A.E)	5 mL of 1M CaCl ₂ + 5 mL of 1M (CH ₃ COO ₂)Mg.4H ₂ O + 10 mL of 0.75% aqueous extract of stem of <i>Costus igneus</i>
VII (1.00% A.E)	5 mL of 1M CaCl ₂ + 5 mL of 1M (CH ₃ COO ₂)Mg.4H ₂ O + 10 mL of 1.0% aqueous extract of stem of <i>Costus igneus</i>

Growth and characterization of COM crystals with liquid methods

In the liquid method, 15 mL of 5 mmol/L CaCl₂ solution was added to 30 mL of 0.15, 0.25, 0.50, 0.75 and 1.00% (w/v) aqueous *C. igneus* extract and was thoroughly mixed. The solution was combined with 15 mL of 5 mmol/L Na₂C₂O₄ solution and was thoroughly mixed again. A corresponding control experiment was carried out by reacting CaCl₂ with Na₂C₂O₄ under the same conditions, without the addition of any additives. The mixtures were covered with Parafilm and stored at 10°C for 3 days. The products were then carefully extracted from the aqueous solution, washed three times with ethanol and double distilled water, and dried for further characterization using FTIR, powder XRD and SEM technique¹⁹.

Isolation and Identification of active compounds

Sample preparation

All the chemicals, including solvents, were of analytical grade from E. Merck, India. The HPTLC plates Si 60F₂₅₄(20cmX10cm) were purchased from E. Merck (India). Standards of Lupeol(97% purity), Stigmasterol (99% purity) were purchased from Sigma (New Delhi, India). 100mg/ml of ethanolic extracts of stem of *Costus igneus* was taken for analysis. The extracts were filtered and vacuum dried at 45°C. The dried extracts were separately redissolved in 1ml of ethanol and sample of varying concentration (1-3 µl) for Lupeol and (5-30 µl) for Stigmasterol –were spotted for quantification. 1mg of standard 1 (Lupeol) and Standard 2 (Stigmasterol) were prepared in 1ml of chloroform, and different amounts of (5000-10000 ng) Lupeol and (1000-6000 ng) Stigmasterol were loaded onto a TLC plate to get the calibration curve²³.

Thin layer chromatography

A Camag HPTLC systems A Camag HPTLC system equipped with an automatic TLC sampler ATS₄, TLC scanner 3 and integrated software WinCATS version 3, was used for the analysis. Samples were washed on a pre-coated silica gel HPTLC plates Si 60F₂₅₄(20cm x 10cm) plate of 200µm-layer thickness, for quantification of Lupeol and stigmasterol in stem of *Costus igneus*. The samples and standards were applied on the plate as 8mm wide bands with a constant application rate of 150nl s⁻¹, with an automatic TLC sampler (ATS₄) under a flow of N₂ gas, 15mm from the bottom, 15mm from the side, and the space between two spots was 6mm in the plate.

Detection and Estimation of Lupeol and Stigmasterol

The linear ascending development was carried out in a Camag twin through chamber (20cm x 10cm), which was pre-saturated with a 25ml mobile phase, with n-Hexane : Ethyl acetate (80:20v/v) for lupeol, Toluene: Acetone: Acetic acid (8:9: 0.9 : 0.2 v/v/v) for stigmasterol for –30 minutes, at room temperature (25°C±2°C) and 50±5% relative humidity. The length of the chromatogram run was up to 90 mm. Subsequent to the development; the TLC plate was dried in a current of air, with the help of air dryer, in a wooden chamber with adequate ventilation. The dried plate was dipped into freshly prepared Anisaldehyde sulphuric acid Reagents and subsequently in Libermann Burchard reagent. Quantitative estimation of the plate was performed in the absorption-reflection mode at 538nm, using a slit width 6.00 x 0.45 mm, with data resolution 100µm/step and scanning speed 20mm/sec. The source of radiation utilized was a tungsten lamp emitting continuous visible spectra of 366 nm. Determination of Lupeol and Stigmasterol in extracts of *Costus igneus* was performed by the external standard method, using pure standards. Each was carried out in triplicate.

Calibration Curve and Linearity

The calibration were performed by analysis of working standard solutions of Lupeol (5000 to 10000ng for *Costus igneus*), Stigmasterol (1000 to 6000ng for *Costus igneus*) were spotted on precoated TLC plate, using semiautomatic spotter under nitrogen stream. The TLC plates were developed, dried by hot air and photometrically analyzed as described earlier. The calibration curves were prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

Statistical Analysis

The masses of the crystals are presented as the mean ± SD for the control and treatment samples. One-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons, was made between the groups shown in Table 2. Values of p <0.05 were considered significant.

RESULTS AND DISCUSSION

Effect of *C. igneus* extract on COM crystals

The effect of the aqueous *C. igneus* stem extract on the nucleation and crystallization characteristics of COM was determined by measuring the mass of the formed crystals. We found that the aqueous extract favored the formation of the dihydrate (metastable) form of calcium oxalate crystals in both the gel and liquid methods. In both the gel and liquid methods, the control solutions led to the maximum nucleation of crystal growth within 24 h of adding the supernatant solutions (Fig. 1).

In the presence of the aqueous *C. igneus* stem extract, nucleation was delayed and reduced crystal masses were observed 96 h following addition of the supernatant solutions. As the concentration of aqueous *C. igneus* extract increased from 0.15% to 1.00% (w/v), the average weights of the formed crystals gradually decreased from 2.16 g to 0.08 g and from 2.89 g to 0.035 g for the gel and liquid methods, respectively. ANOVA was performed for the crystal masses, and a $p < 0.05$ suggested that the correlation was significant as shown in Table 2. The crystal masses from Group III to VII, treated with various concentrations of aqueous *C. igneus* extract ranging from 0.15% to 1%, were significantly different at $p < 0.05$ when compared to Group I, the untreated control. However, Group II, which was treated with distilled water, was not significantly different at $p < 0.05$ compared to Group I.

This result indicates that distilled water did not show any inhibitory activity with regard to crystal growth, whereas the aqueous extract of *C. igneus* possessed inhibitory activity due to the presence of bioorganic molecules¹⁶. Group VI and VII, treated with 0.75% and 1% aqueous extracts, respectively, were not significantly different. In general, an increase in aqueous extract concentrations resulted in a decrease in the crystal mass due to a reduction in the sizes of the crystals during treatments. Morphology of the harvested crystals changed from hexagonal (COM) to tetragonal (COD) as shown in (Fig. 2). In the present study, COM crystal growth was reduced, and the morphology of the crystals was altered due to the inhibitory effect of aqueous *C. igneus* extracts under *in vitro* conditions by the gel and liquid methods, as has previously been reported^{1-3,11,26}.

Table 2: ANOVA statistical analysis for harvested crystals

Groups	Treatments	Mean (g)±S.D Gel	Liquid
I	Control	2.16±0.05	2.89±0.04
II	Distilled water	2.07±0.09 ^{a,ns}	2.8±0.08 ^{a,ns}
III	0.15% extract	1.6 ± 0.08 ^{ab}	1.07±0.01 ^{ab}
IV	0.25% extract	0.6 ± 0.08 ^{abc}	0.42±0.05 ^{abc}
V	0.50% extract	0.31±0.01 ^{abcd}	0.19±0.02 ^{abcd}
VI	0.75% extract	0.11± 0.008 ^{abcd}	0.07±0.008 ^{abcd}
VII	1.00% extract	0.08± 0.006 ^{abcd,ef,ns}	0.035±0.006 ^{abcd,ef,ns}

Values represent mean (g) ± 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.

a-ns, f-ns were not significantly different. Statistical significance were considered to be ^a $p < 0.05$, ^b $p < 0.05$, ^c $p < 0.05$, ^d $p < 0.05$, ^e $p < 0.05$.

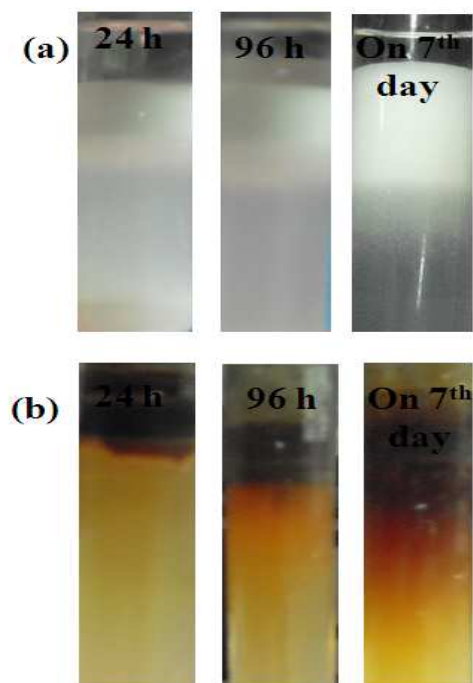


Fig. 1: Effect of *Costus igneus* extract on COM crystals (a) without any additive after 24hrs, 48hrs and 7th days (b) with the *Costus igneus* extract after 24hrs, 48hrs and 7th days

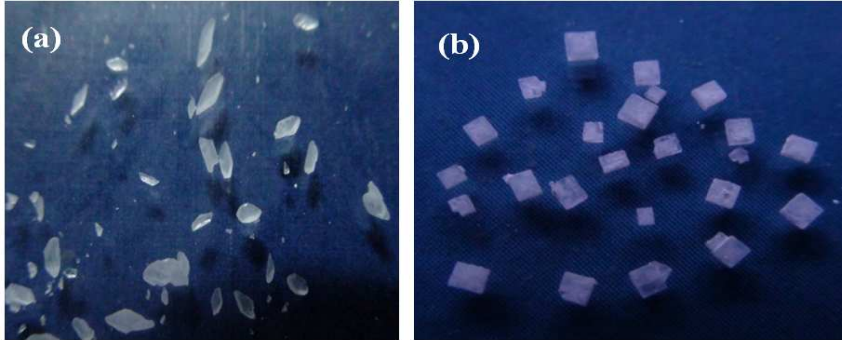


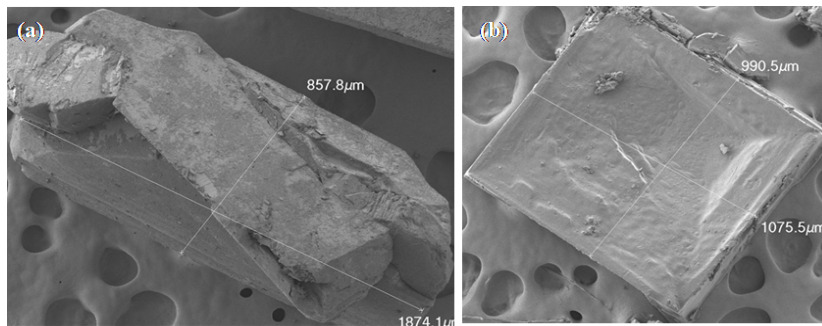
Fig. 2: Morphology of the harvested COM crystals (a) without any additives, (b) with the 0.15% (w/v) *Costus igneus* extract obtained using the gel methods

Characterization of harvested crystals

The changes in the morphology of CaOxa crystals obtained from SEM studies in the absence and presence of the *C. igneus* stem extract using both the gel and liquid methods (Fig.3). In the gel method, the CaOxa crystals that were formed in the absence of any additive were elongated hexagonal (Fig. 3(a)) with an average size of 1874.1 x 857.8 μm. In the presence of the *C. igneus* stem extract, the CaOxa crystals were elongated tetragonal bipyramidal (Fig. 3(b)) with an average size of 1075.5 x 990.5 μm. In the liquid method (Fig. 3(c)), the CaOxa

crystals formed in the absence of any additive were elongated hexagonal with an average size of 0.524 μm and 0.563 μm. In the presence of the *C. igneus* stem extract, the CaOxa crystals were elongated tetragonal bipyramidal with an average size of 0.291 x 0.239 μm and 0.295 x 0.272 μm (Fig. 3(d)).

The COM crystals grown displayed many different morphologies, but the most prominent faces were (100), (001), (101), (021). COD crystals usually appeared as bipyramids with (101) being the dominant face as was previously reported^{21,22}.



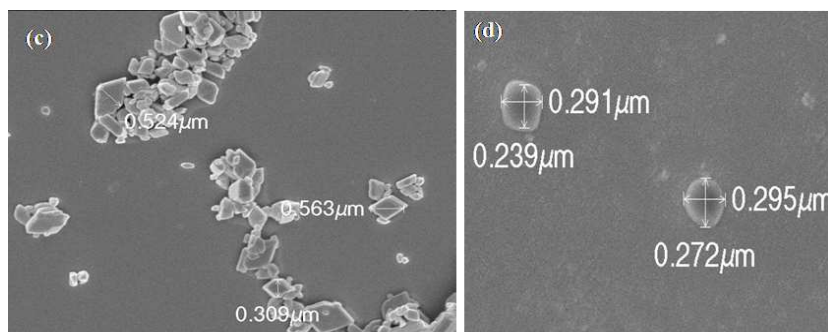


Fig. 3: SEM images of CaOxa crystals (a) and (c) without any additives, (b) and (d) with the 0.15% (w/v) *Costus igneus* extract obtained using the gel and liquid methods

The FTIR spectra of CaOxa crystals obtained in the presence and absence of the *C. igneus* extract, using both the gel and liquid methods (Fig. 4 and 5). In Fig. 4(a), the peaks at 1642 and 1330 cm⁻¹ are the main antisymmetric carbonyl stretching bands, and the band at 1031 cm⁻¹ shows C-O stretching. The band at 886 cm⁻¹ is due to C-C stretching, which reveals the presence of two carboxylate anions. This confirms the existence of the oxalate group in COM crystals. In Fig. 4(b), shifts from 1642 cm⁻¹ for COM to 1635 cm⁻¹ for COD as well as from 1330 cm⁻¹ for COM to 1386 cm⁻¹ for COD were observed, and the band at 1007 cm⁻¹ is due to C-O stretching. The band at 877 cm⁻¹ demonstrates a C-C stretch, which reveals the existence of an oxalate group in the COD crystals. In Fig. 5(a), the peaks at 1636 cm⁻¹ and 1326 cm⁻¹ were the main antisymmetric carbonyl stretching bands, and the band at 1024 cm⁻¹ is due to the C-O stretching. This result indicates the presence of a carboxylate anion in COM crystals. In Fig. 5(b), shifts from 1636 cm⁻¹ for COM to 1639 cm⁻¹ for COD as well as from 1326 cm⁻¹ for COM to 1366 cm⁻¹ for COD were observed. In

addition, two bands located at 925 cm⁻¹ and 654 cm⁻¹ were also assigned to COD. Figs. 4(c) and 5(c) display the FTIR spectra of the raw stem powder of *C. igneus*, which is rich in protein, iron, antioxidant components and phytochemicals (i.e., flavonoids, alkaloids, terpenoids, steroids, saponins and phenolics). Figs. 4(d) and 5(d) show the FTIR spectra of the aqueous extract powder of *C. igneus* containing water-soluble phytochemicals, which were separated according to the polarity of the solvent¹⁶. There were no impurities found in the *C. igneus*-treated COD crystals (Figs. 2(b) and 3(b)).

The peaks at 1618 cm⁻¹ and 1318 cm⁻¹ are the main antisymmetric carbonyl stretching bands specific to the oxalate family and the metal carboxylate stretch for COM, respectively. The peaks shifted from 1618 cm⁻¹ to 1652 cm⁻¹ or 1647 cm⁻¹ and from 1318 cm⁻¹ to 1328 cm⁻¹ or 1327 cm⁻¹ for COD, as previously reported^{7,10,11,21}. The shifting further supports the hypothesis that *C. igneus* stem extracts favor the nucleation and or transformation of COM into COD.

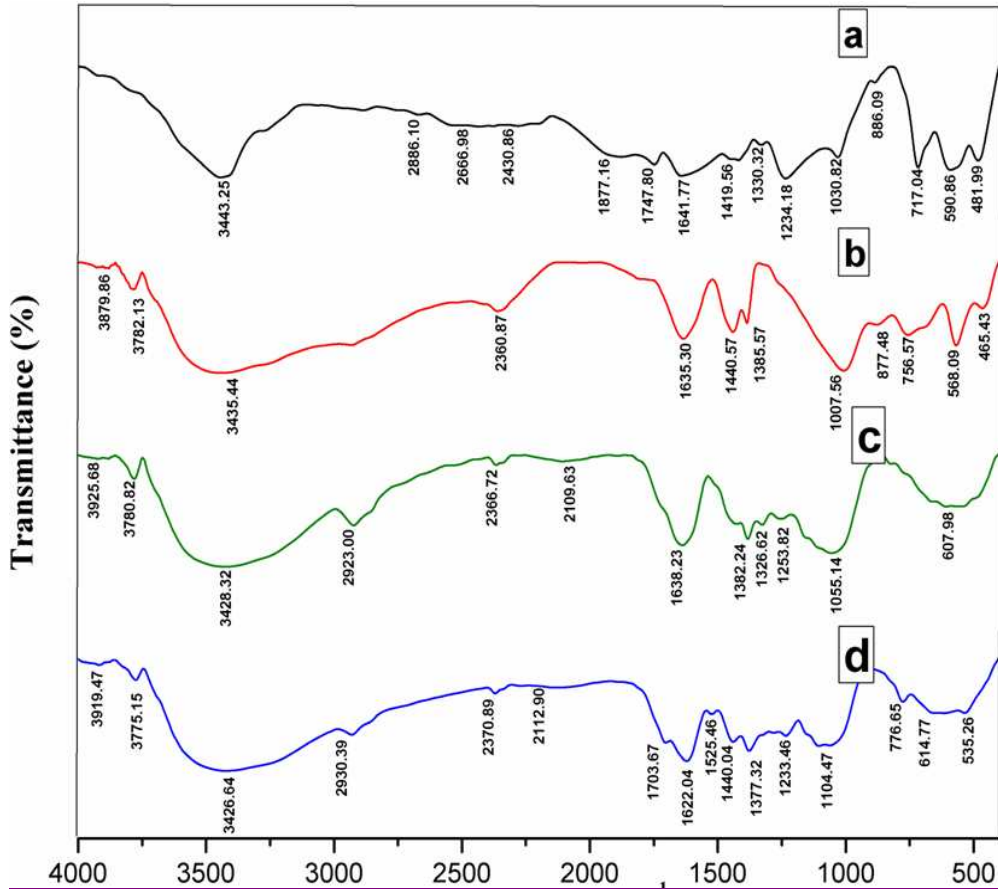


Fig. 4: FTIR spectra of CaOxa crystals obtained from the gel method (a) without any additives, (b) with 0.15% (w/v) *C. igneus* extract, (c) raw stem powder of *C. igneus* and (d) aqueous extract powder of *C. igneus* stem

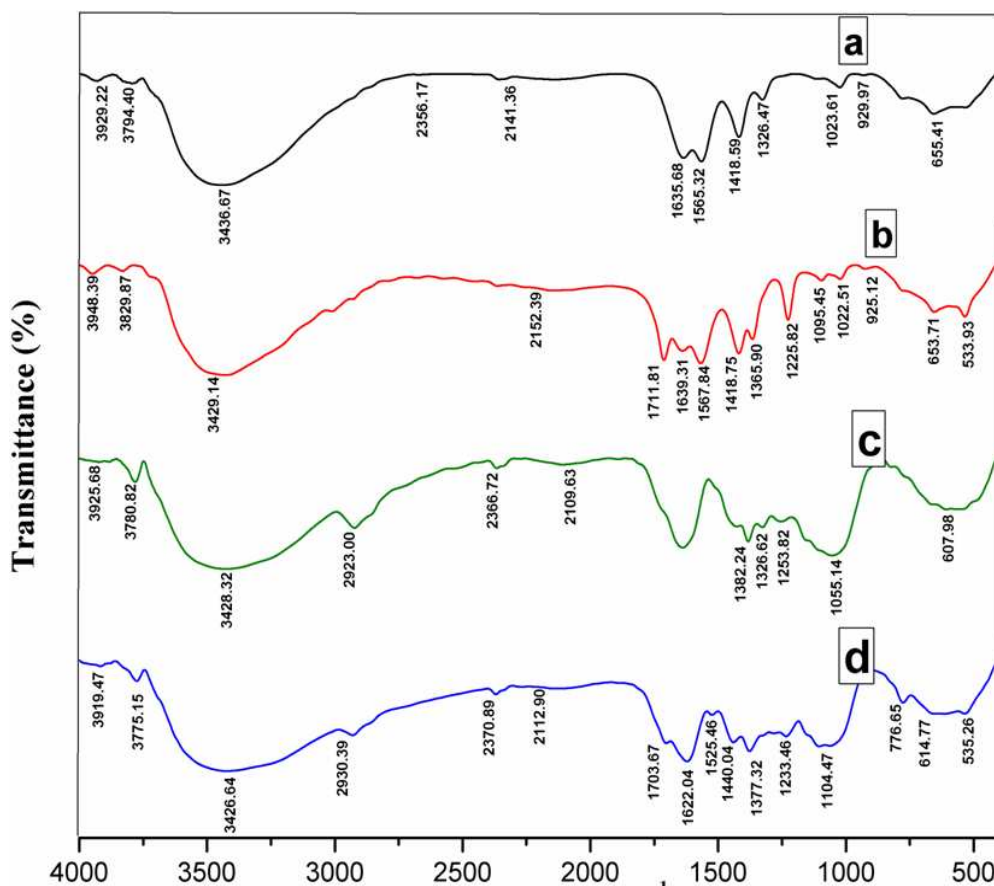


Fig. 5: FTIR spectra of CaOxa crystals obtained from the liquid method (a) without any additives, (b) with 0.15% (w/v) *C. igneus* extract, (c) raw stem powder of *C. igneus* and (d) aqueous extract powder of *C. igneus* stem

Fig. 6 shows the XRD patterns of CaOxa crystals obtained in the presence and absence of the *C. igneus* extract using both the gel (Fig. 6(a) and 6(b)) and liquid methods (Fig. 6(c) and 6(d)). The diffraction peaks obtained in both methods correlate well to the (hkl) indices of the COM phase (JCPDS card number 20-231) and the COD phase (JCPDS card number 17-541). As reported previously³¹, the diffraction peaks 14.95, 24.39, 30.12 and 33.13 for COM as well as 14.26, 20.01, 24.15, 32.17, 37.21 and 40.00 for COD were assigned. It is inferred from the above results that the *C. igneus* stem extract affected the nucleation and growth of COD crystals.

Quantitative determination of Lupeol and Stigmasterol in extracts of *Costus igneus* by HPTLC technique

HPTLC fingerprint patterns have been therefore evolved for extracts of *Costus igneus*. Lupeol standard was quantitated accurately using silica gel F₂₅₄ HPTLC pre-coated plates with the mobile phase for n-Hexane : Ethyl acetate (80:20v/v), the R_f value for Lupeol was about 0.55. The chromatographs of Lupeol and ethanol extract of *costus*

igneus are shown in (Fig. 7(a)). The R_f value of Lupeol was matched with the R_f value of *Costus igneus* extract was about 0.55. Stigmasterol standard was quantitated accurately using silica gel F₂₅₄ HPTLC pre-coated plates with the mobile phase Toluene: Acetic acid (8.9: 0.9 : 0.2 v/v/v), the R_f value was about 0.58. The chromatographs of stigmasterol and ethanol acetate of *Costus igneus* are shown in (Fig. 7(b)). The R_f value of stigmasterol was matched with the R_f value of extract was about 0.58. The amount of Lupeol (0.473 mg/100 mg) and Stigmasterol (1.913 mg/100 mg) in stem of *Costus igneus*.

A pentacyclic triterpenoid compound Lupeol and a steroid compound Stigmasterol were identified and isolated by HPTLC techniques for the first time. Previous study has reported that Lupeol, a triterpene compound has been isolated from *Crataeva nurvala* showed antioxaaluric and anticalciuric effects in rats against hydroxyproline-induced hyperoxaluria²⁴. The earlier investigators isolated lupeol from the methanol extract of stem bark of *Grewia titiaefolia* and evaluated the cytotoxic properties on *in vitro* cell lines²⁵.

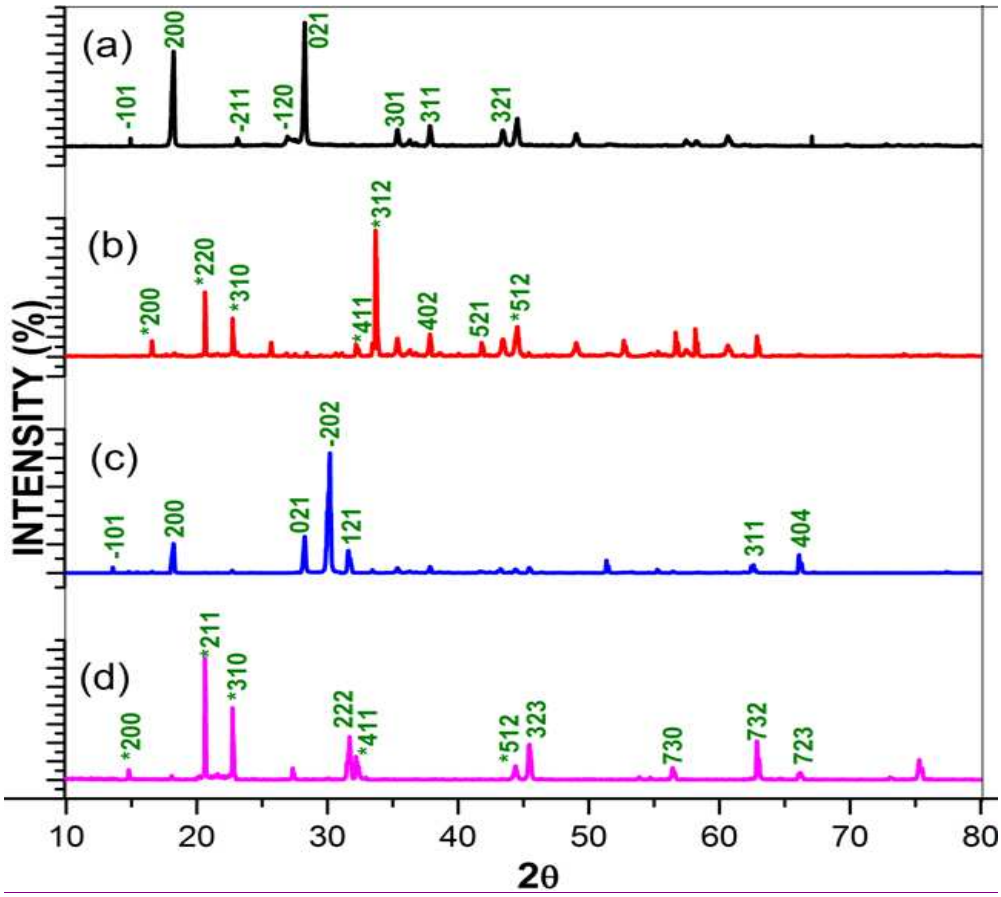


Fig. 6: XRD patterns of CaOxa crystals (a) and (c) without any additives, (b) and (d) with the 0.15% (w/v) *C. igneus* extract obtained using the gel and liquid methods. * denotes COD crystals

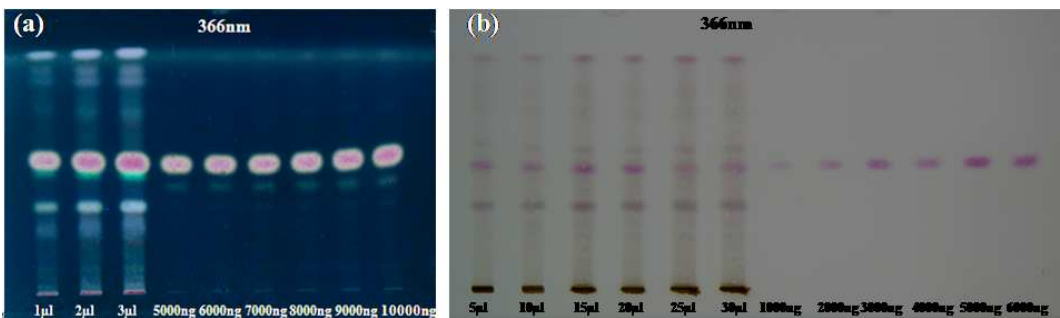


Fig. 7: (a) HPTLC chromatogram of Lupeol in *C. igneus* extract (b) HPTLC chromatogram of Stigmasterol in *C. igneus* extract

Calibration curve and Linearity

The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot. The regression equation and correlation curves for Lupeol in *Costus igneus* were, regression via height $y=149.076+32.745X$ and $r=0.99794$ $sdv=0.72$, regression via area $y=213.109+1731.406X$ and $r=0.99914$ $sdv=0.72$. Stigmasterol in *Costus igneus* were, regression via height $y=116.129+0.052X$ and $r=0.99956$ $sdv=1.78$, regression via area $y=1732.776+2.151X$ and $r=0.99999$ $sdv=0.08$.

CONCLUSION

COM crystals were grown using both gel and liquid growth methods and were characterized by SEM, FTIR and Powder XRD techniques. The COM crystal growth was reduced, and the morphology of the crystals changed from a hexagonal (COM) to a bipyramidal (COD) form due to the inhibitory action of the aqueous extracts of *C. igneus* under *in vitro* conditions. FTIR and Powder XRD techniques confirmed the functional groups and crystalline phases of the COM and COD crystals. SEM studies confirmed the morphology of the changed crystals. It also confirmed that the average size of the crystals was reduced from $1874.1 \times 857.8 \mu\text{m}$ to $1075.5 \times 990.5 \mu\text{m}$ and from $0.524 \mu\text{m}$ to $0.291 \mu\text{m}$ for the gel and liquid methods, respectively. One-way ANOVA performed with treated and untreated crystal growth data obtained from both the gel and liquid methods showed significant differences ($p < 0.05$). This inhibitory effect may be due to the presence of a terpenoid compound lupeol and a steroid compound stigmasterol from the stem of *Costus igneus* were identified by HPTLC techniques. This study confirmed that aqueous *C. igneus* extracts promote the formation of COD crystals and may possibly treat urinary stones by inhibiting the formation of COM crystals, which are a major component of urinary stones. This study was focused on finding a new alternative medicine for the treatment of calcium oxalate urinary stone.

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