

# Medicinal Implication of Syzygium Cumini Nut on the Growth of Brushite Crystals

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Received: 17 December 2014/Revised: 28 September 2016/Accepted: 23 May 2017/Published online: 29 March 2018  
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**Abstract** Brushite crystal formation and deposition is the central cause for recurrent kidney stone disease among the global population. The present study was aimed to rationalize the use of syzygium cumini nut on the brushite crystal formation invitro and to investigate the mechanism of action. Brushite crystals were grown at room temperature by single diffusion gel growth technique and the growth influencing study of these crystals in the presence of the natural product of syzygium cumini nut were carried out. The number of nucleated crystals was found to be less for the syzygium cumini nut extract added system when compared to the control system. The scanning electron micrograph images shows plate like morphology for the grown crystals. The thermal analysis shows that there is a decrease in the stability for the crystals grown in the presence of the syzygium cumini nut extract. By carefully observing the morphology and size of the crystals obtained, it is concluded that the syzygium cumini nut extract partially inhibits the growth of brushite crystals, particularly on its size. The reduction in stability may be due to the interaction of the host molecule on the surface.

**Keywords** Calcium phosphate · Crystallization · XRD · FTIR · Scanning electron microscope · Thermal analysis

## 1 Introduction

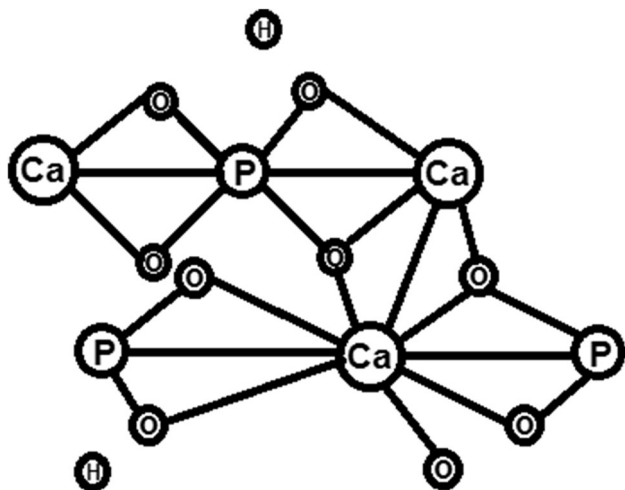
The pathological mineral deposit effects results in the cause for crystal deposition diseases which is associated with the presence of micro crystals contributing to the tissue damage [1] and cause pain and suffering to human population. In humans, the presence of kidney stones has become a substantial burden to the health service and is one of the major causes of morbidity [2]. Of the various biological crystals formed in the renal system, calcium containing calculi is more predominant and has become a serious threat to human life and affects a large population world-wide. Brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) stones are an unstable form of calcium phosphate [3] which affects almost 1–2% of the population and have a tendency to recur frequently if appropriate stone prevention measures had not been taken. The molecular structure of brushite is shown in Fig. 1. These calculi are exceptionally hard, resistant to shock wave lithotripsy and difficult to remove surgically [4, 5]. In view of this, a success in finding a possible way to prevent the brushite calculi formation will be of immense help to mankind. The challenge lies in the discovery and development of better drugs to fight against these diseases. The over use of synthetic drugs, which results in higher incidence of adverse drug reactions, has motivated humans to return to nature for safe remedies [6]. The use of medicinal plants as a rich source of therapeutic agents for the treatment of various disorders dates back to several centuries. In recent years, popularity of complementary medicine has increased because of their efficacy,

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**Fig. 1** Molecular structure of brushite

lesser side effects and low cost [7]. Many investigators have demonstrated that studies of herbal plant used in traditional medicine as diuretic have increased recent years [8] and might be a useful tool in the treatment of urolithiasis. Traditional medicine has large potential to treat various ailments among large communities. Hence, further investigation in this area for detecting a suitable compound for inhibiting kidney stone growth and preventing recurrence is encouraged.

In the present work, the *in vitro* growth of brushite crystals using single diffusion gel growth technique and the effect of the natural product of *syzygium cumini* nut (SCN) has been investigated. This could be used as an incipient for the synthesis of new drugs with improved pharmacological properties which can shed some light on this matter thereby causing a soothing effect on the patient.

## 2 Method of Analysis

### 2.1 Natural Product Extraction

The SCN used in the present study was obtained from the fruits grown in the vellimalai hills of southern part of Tamil Nadu, India. The *syzygium cumini* nuts (SCN) were thoroughly washed using distilled water, dried in sunlight and powdered well using milling technique. This crude material was used as such without further refinement to perform the experiment. The aqueous extract of SCN was prepared by boiling 20 g of nut powder in 100 cc of distilled water to get a concentrated solution. This solution was then filtered using Whatman filter paper to remove the undissolved components if any and was used as the extract. It was then precipitated out using acetone and was collected and dried. This dried form of precipitate was further used to examine

the effect of SCN on the growth of the crystals. To investigate the effect of SCN, the solution was prepared at various concentration varying from 1 to 5% W/V concentration. Among the various concentration, the optimized concentration obtained was 5% and the results are presented according to this concentration.

### 2.2 Growth of Brushite Crystals

Sodium metasilicate ( $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ ), orthophosphoric acid, calcium chloride and calcium acetate were purchased from Merck. Sodium meta silicate ( $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ ) solution of specific gravity 1.03 was used to prepare the gel. The pH of the medium was adjusted to 6.0 using orthophosphoric acid. Glass test tubes (25 mm  $\times$  140 mm) were employed as the crystallization vessels. The vessels were covered with rubber cork and kept undisturbed for proper gel setting. After gelation, equal volume (15 ml) of 1.5 M of calcium chloride and calcium acetate were used as the supernatant solution for the control setup. To investigate the effect of SCN, the prepared SCN extract at an optimized concentration of 5% W/V concentration was separately added along with the 15 ml of supernatant solution and then transferred over the set gel in different crystallization vessels in order to observe its effect on the growth of crystals in all the vessels. This procedure was done simultaneously with the control setups in order to compare the growth and morphology of the crystals. Thus the growth of brushite crystals in the control system and in the system containing SCN extract was observed simultaneously under identical conditions. Proper care should be taken so that the gel surface will not be disturbed while adding the supernatant solution which in turn permits the free diffusion of the  $\text{Ca}^{2+}$  ions into the gel, where reaction with  $\text{PO}_4^{3-}$  results in the appearance of precipitate instantaneously at the interface between the gel and the supernatant solution. Tiny plate like crystals appeared within the gel column for the control system and tiny star like crystal for the SCN extract added system. The grown crystals were harvested after 22 days. In order to ensure the repeatability, the same procedure was carried out thrice. The harvested crystals were subjected to various characterization techniques such as XRD, FTIR, SEM-EDX and TGA-DTA analysis.

### 2.3 Instrumentation

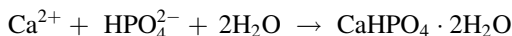
The cell parameters of the grown crystals were determined using the Bruker Enraf-Nonius CAD 4/MACH 3 single crystal diffractometer, with Mo-K $\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ) at 293 K. The powder XRD pattern of the samples were performed using an automated X-ray diffractometer operated at 40 kV and 30 mA. The radiation used in the X-ray

diffraction pattern in  $\text{CuK}\alpha$  whose wave length is given by  $1.5405600 \text{ \AA}$ . The samples are scanned over  $2\theta$  ranges of  $0^\circ$  to  $70^\circ$  at  $2^\circ \text{ min}^{-1}$ . The Fourier transform infrared spectroscopy (FT-IR) spectra were recorded at room temperature using Perkin-Elmer Spectrophotometer using KBr pellet technique in the wave number range between  $400$  and  $4000 \text{ cm}^{-1}$  to analyze the sample qualitatively. The resolution of the instrument is down to  $10$  microns. The FTIR spectrophotometer was used to explore the vibrational signatures of different organic and inorganic compounds present in the sample. The topographical and morphological features of all the samples grown have been observed using JEOL Model JSM—6390LV SEM with a resolution of ( $\sim 15 \text{ nm}$ ). For SEM analysis, well powdered samples were taken. The TGA–DTA analysis was carried out using Perkin Elmer, Diamond TG/DTA instrument under nitrogen atmosphere. The temperature range selected for the present study was from ambient to  $1000 \text{ }^\circ\text{C}$  at a heating rate of  $10 \text{ }^\circ\text{C min}^{-1}$ .

### 3 Results and Discussions

#### 3.1 Influence of SCN on Brushite Crystal Formation

The reaction that takes place in the gel medium is as follows:



Initially the crystals were needle like and as days proceeded, secondary arms were developed. The appearance of the grown crystals seems to be elongated and platy like. The number of nucleated crystals in the SCN added system is found to be less when compared with the control system. As far as the morphology is concerned, the control system has saw tooth shaped sharp edges whereas for the SCN extract added system tiny star like crystals are observed (Fig. 2). The most important feature noticed in these two systems of crystallization is: in the

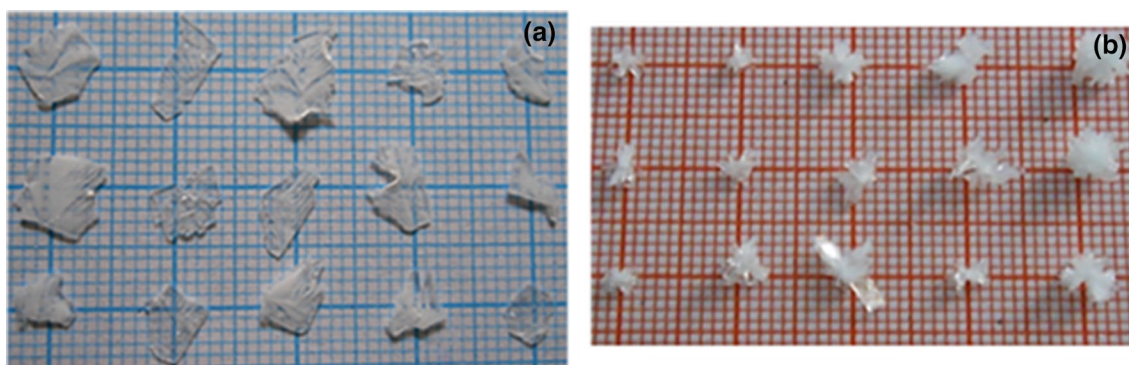
control system, the size of the crystal is five times more than that of the crystals grown in the treated medium. This clearly shows that the nut extract thus played a crucial role in controlling the growth of the brushite crystals. Hence the medicinal value of the syzygium cumini nut is understood.

#### 3.2 Characterization

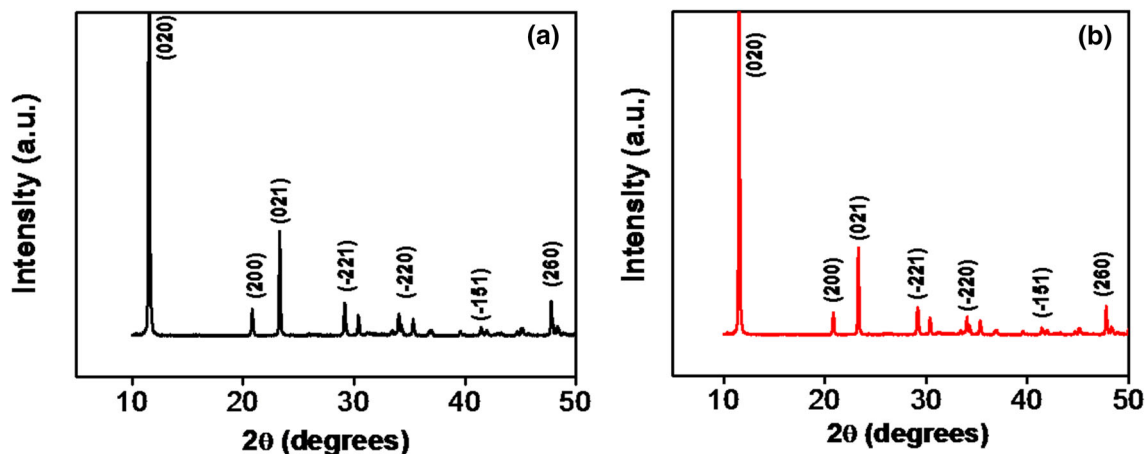
The single crystal XRD analysis showed no marked difference in the unit cell parameters of the crystals grown in the control system and the obtained values are,  $a = 5.780 \text{ \AA}$ ,  $b = 6.210 \text{ \AA}$ ,  $c = 15.178 \text{ \AA}$ ,  $\beta = 116.30^\circ$ ,  $V = 489.90 \text{ \AA}^3$  and the number of molecules per unit cell is  $Z = 4$ , thus confirming monoclinic system of the grown crystal [9–11]. The powder XRD patterns (Fig. 3) of the samples are recorded over the  $2\theta$  ranges of  $0^\circ$  to  $70^\circ$  at  $2^\circ \text{ min}^{-1}$ . Table 1 shows the X-ray powder diffraction data for pure brushite. There is no significant change in the diffraction pattern of SCN added brushite.

The XRD pattern reveals the identity of the grown brushite crystal. The miller indices of the grown crystals are identified using JCPDS File No. 98. The sharp peak in the pattern confirms the crystalline nature of the materials. The spectrum shows peaks corresponding mainly to the (020), (021), ( $-221$ ), ( $-220$ ), (260) planes respectively.

The FTIR spectra of brushite crystals grown in the control system and in the presence of the treated system are shown in Fig. 4. The FTIR spectrum of brushite crystals obtained in the present study is essentially identical to the published one [12, 13]. The bands at  $3839$ ,  $3753$ ,  $3537$ ,  $3270$ ,  $3481$  and  $3165 \text{ cm}^{-1}$ , was caused by asymmetric and symmetric stretching vibration of free water molecules. From the spectra, it is observed that there is an intense doublet occurring at  $3537$  and  $3481 \text{ cm}^{-1}$  and also another weak doublet at  $3266$  and  $3160 \text{ cm}^{-1}$ . The presence of these doublets is attributed to the existence of two different types of water molecules in the unit cell of brushite. The very sharp and intense band present at  $1646 \text{ cm}^{-1}$  is assigned to the in-plane bending of water. A strong band



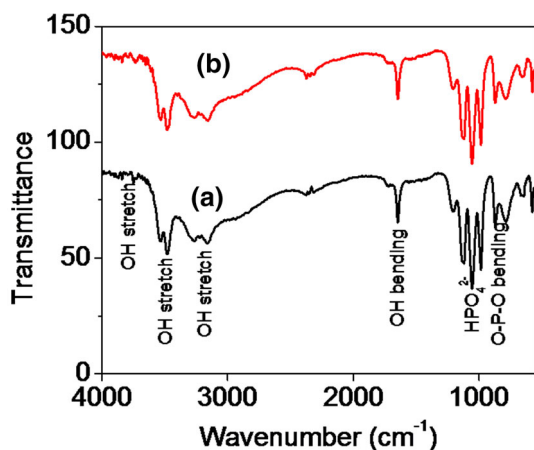
**Fig. 2** **a** Brushite crystals grown in the control system, **b** brushite crystals grown in the presence of SCN (scaling is identical)



**Fig. 3** Powder XRD pattern of **a** crystals grown in the control system and **b** crystals grown in the presence of SCN

**Table 1** X-ray powder diffraction data for pure brushite

Assigned (hkl) planes	From JCPDS File (98) 2θ°	From present work 2θ°
020	11.680	11.585
021	20.934	23.251
– 221	30.505	29.140
– 220	34.155	34.091
– 151	41.543	39.564
260	48.430	47.938



**Fig. 4** FTIR spectra for **a** crystals grown in the control system, **b** crystals grown in the presence of SCN

occurring at  $985\text{ cm}^{-1}$  is assigned to symmetric P–O stretching mode and bands at  $1055$ ,  $1120$  and  $1206\text{ cm}^{-1}$  are assigned to P–O antisymmetric stretching mode. The bands at  $577$ ,  $646$ ,  $786$ ,  $871\text{ cm}^{-1}$  are assigned to O–P–O bending mode.

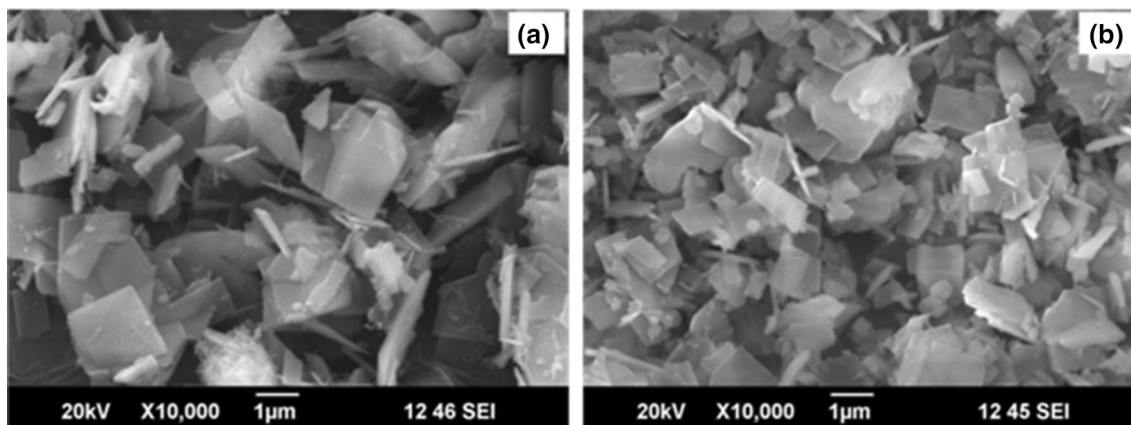
This shows that the grown crystal can be characterized as the brushite crystal. It was also observed from the

spectra that no major changes were noticed in the spectra of SCN extract added brushite.

The scanning electron micrograph images of magnification 10000X for the brushite crystals grown in the control system and SCN added system (Fig. 5) shows the fully crystallized, elongated and well defined platelet morphology which is the characteristic of brushite [12]. From the images, the average length and breadth of the crystal grains grown in the control system were found to be  $2.21$  and  $1.01\text{ }\mu\text{m}$  respectively. The SCN treated crystals has an average length and breadth of  $1.03$  and  $0.4\text{ }\mu\text{m}$  respectively. Based on the analysis of the SEM images, one can identify that small platelets were growing over larger ones, similar to mica sheets [14]. The growth took place at different centers in the form of platelets, were investigated by SEM. The SEM images of crystals grown in the presence of SCN showed a marked reduction in the grain size, thus reflects the inhibiting effect of syzygium cumini nut on the brushite crystal growth. The platelets are exhibiting the similar morphology as reported earlier [15]. The differences in platelet length and breadth can be attributed to the influence of SCN onto the brushite crystal formation altering the growth kinetics of the platelets. This clearly indicates a marked influence of SCN which inhibited the size of the crystal.

The elemental composition of the sample is identified using Energy Dispersive X-ray analysis. The EDX measurements were carried out for the brushite crystals grown in the control system as well as for the crystals grown in the presence of SCN, and the average atomic percentages of the individual elements are shown in Table 2. The Ca/P ratio of brushite crystals grown in the control system is  $1.02$ , which is closely associated with the actual value of  $1$  according to the chemical formula [16], whereas for the crystals grown in the presence of SCN the Ca/P ratio is  $1.32$ . This increase in Ca/P ratio for the crystals grown in





**Fig. 5** SEM images of **a** brushite crystals grown in the control system, **b** brushite crystals grown in the presence of SCN

**Table 2** EDX data of pure and SCN added brushite crystal

Element	Pure brushite (atm%)	SCN treated brushite (atm%)
C K	5.31	10.79
O K	19.24	27.06
P K	42.18	30.76
Ca K	33.28	31.39

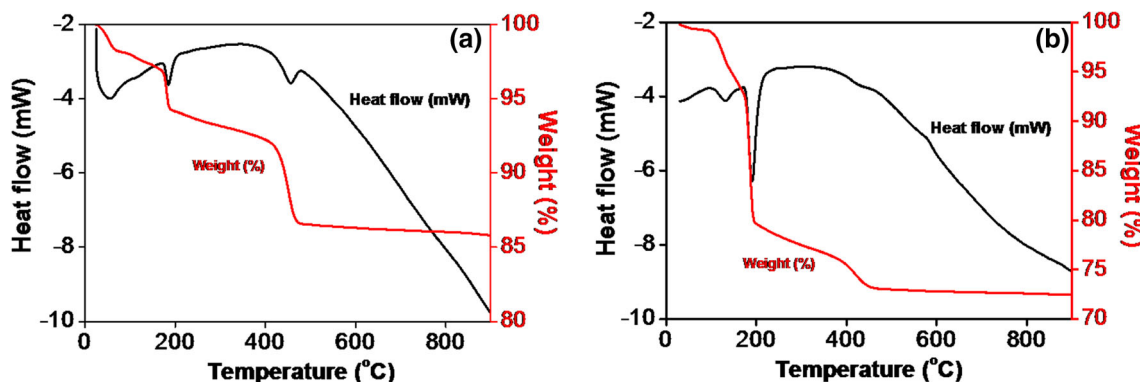
SCN syzygium cumini nut

the presence of SCN may be attributed to the surface phenomena where the phosphate ligands involved in coordination with calcium might have been replaced by the oxygen present in the host molecule, probably water soluble molecules present in the SCN, thereby leading to a reduction in phosphate content thus an enhancement in the Ca/P ratio. An increase in the carbon and oxygen content may also be due to the presence of host molecule. The influence of the host molecule on the surface phenomena can be attributed to the reduction in the size of the crystal.

The thermal analysis are the most familiar thermal technique employed to determine the thermal stability of the grown crystal and to identify the phase transitions. The thermal behaviour of the crystals grown in the control

system and the crystals grown in the presence of SCN measured from ambient temperature to 1000 °C at the constant heating rate of 10 °C min<sup>-1</sup> in nitrogen atmosphere is shown in Fig. 6.

From the thermal analysis of the grown brushite crystals, it was found that the weight loss of the samples happens at two stages [17]. The first stage of weight loss occurred below 200 °C for both the samples. However a drastic difference in the weight loss is noticed between the control sample and the sample treated with SCN. This can be due to the loss of lattice water molecule in the former system and loss of lattice water plus the surface host molecule (probably water soluble molecules present in the nut) involved in coordination in the latter as seen in the EDX. It is thus evident from the TGA curve the thermal stability of the sample decreases due to the addition of SCN in the crystal growth environment because of the surface influence of the host molecule. The second stage of weight loss occurred between 411 and 467 °C is due to the conversion of calcium hydrogen phosphate to calcium pyrophosphate. Therefore it remains stable up to the end of the analysis. The mass loss corresponds well with the DTA curve, which reveals the endothermic peak at 54, 190 and 454 °C



**Fig. 6** TGA–DTA images of **a** brushite crystals grown in the control system and **b** brushite crystals grown in the presence of SCN

respectively. A sharp endothermic peak at 190 °C indicates good crystallinity of the sample. This investigation paved a way for further exploration of brushite crystal formation in the presence of various natural products.

#### 4 Conclusions

The *syzygium cumini* nut influences the growth and morphology of brushite crystals. X-ray diffraction and FTIR spectra, confirmed that the grown crystals are brushite. The growth took place at different centers in the form of platelets, were investigated by SEM. The SEM images of crystals grown in the presence of SCN showed a marked reduction in the grain size, thus reflects the inhibiting effect of *syzygium cumini* nut on the brushite crystal growth. EDX confirmed the elemental composition. The reduction in the atomic percentages of phosphate and thus an enhancement in the Ca/P ratio for SCN treated samples by the EDX measurement is attributed to the involvement of the host molecule in the calcium co-ordination on the crystalline surface. The TGA measurement showed a reduced thermal stability for SCN treated brushite sample when compared with normal ones, which further supports the potential of *syzygium cumini* nut in inhibiting the brushite formation. This investigation paved a way for further exploration of brushite crystal formation in the presence of various natural products.

#### Compliance with Ethical Standards

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

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