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Effect of *Euphorbia hirta* root extract on struvite crystals

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Abstract
To investigate the inhibitory effect of ethanolic extract of root of *Euphorbia hirta* on the growth of struvite crystals. Struvite crystals were grown by the single diffusion gel growth technique and the inhibitory effect of ethanolic extract of root of *Euphorbia hirta* on the growth of struvite crystals has been studied. The crystals were characterized by Fourier Transform Infrared Spectroscopy (FTIR) to confirm the functional groups and Powder X-Ray Diffraction technique (XRD) analysis to confirm the crystalline phases of the struvite crystals. The ethanolic extract of root of *Euphorbia hirta* gradually reduce the weight of the formed struvite crystals reduced from 0.77 gm to 0.23gm Results obtained are indicated that root of *Euphorbia hirta* have the potential to inhibit the formation of struvite crystals. This study confirms that using ethanolic extract of root of *Euphorbia hirta* can reduce the nucleation rate of struvite crystals, a major component of triple phosphate urinary stone.

Keywords: Struvite, *Euphorbia hirta*, phosphate urinary stone, powder x-ray diffraction (XRD)

Introduction
A large number of people are suffering from urinary stones problems [1]. Urinary stones have been found to contain calcium phosphate, calcium oxalate, uric acid and magnesium ammonium phosphate or struvite crystals [2-4]. Among the magnesium phosphates namely Ammonium Magnesium Phosphate Hexahydrate (AMPH) commonly known as Struvite. Magnesium Hydrogen Phosphate Trihydrate have also been reported to occur as constituents in renal calculi [5–8] not only in adults but also in children [9, 10]. Struvite calculi, found in 15–20% of urinary calculi [11, 12], are mostly related to urinary tract infections with ureolithic microorganisms in humans and animals [5, 13, 14]. Struvite is also known as triple phosphate stone, infection stone or urase stone. They are found more frequently in women and in persons older than 50 years [15, 16, 17]. Urinary stones are characterized by high recurrence rate therefore requiring a preventive treatment by using the medicinal plants [18, 19].
Euphorbia hirta Linn. is a small herb, belongs to family Euphorbiaceae, distributed throughout the hotter part of India, often found in waste places along the roadsides [20,21]. The plant possesses gastro intestinal disorders (diarrhea, dysentery, intestinal parasitosis, bowel complaints, digestive problems), respiratory diseases (cough, cold, asthma, bronchitis, hay fever, emphysema), urinary apparatus (diuretic, kidney stones), genital apparatus (metrorrhagic, agalactosis, gonorrhoea, urethritis), various ocular ailments (conjunctivitis, corneal ulcer), skin and mucous membranes problems (guinea worm, scabies, tinea, trush, aphtha) and tumour [22-25]. The chemical constituents shows that the presence of quercitrin, quercitol and derivatives containing rhamnose, quercetinrhamnoside, a chlorophenolic acid, rutin, leucocyanidin, leucocyanidol, myricitrin, cyaniding 3,5-diglucoside, pelargonium 3,5-digucoside and camphol, flavonol glycoside xanthrhamnin, hentriacontane, myricyl alcohol, inositol, tetraxerol, friedelin, β-sitosterol, ellagic acid, kaempferol [26,27].
In the present investigation, the effects of ethanolic extract of root of *Euphorbia hirta* are used as additives to induce the nucleation and growth of struvite crystals by single diffusion gel growth technique and are reported for the first time. This study incorporated a multidisciplinary approach in characterizing struvite crystals grown *in vitro* to help formulate prevention or dissolution strategies in controlling urinary stone growth.

Materials and Methods
Materials and instruments
Analytical grade of anhydrous methanol, magnesium acetate, sodium metasilicate, ammonium dihydrogen phosphate were all purchased from sigma-aldrich, New Delhi, India. 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 CuKα radiation.

Collection of plant material

The root of *Euphorbia hirta* were collected from Ponmalaipatti road Trichy, Tamil Nadu, and India. The plant was identified and confirmed by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, and Tamil Nadu.

Preparation of aqueous extracts

The root of *Euphorbia hirta* were washed in running water, cut into small pieces and then shade dried for a week at 35-40ºC, after that it was grinded and sieved through 40 mesh size [6]. The ethanolic extract of root of *Euphorbia hirta* were prepared by soaking 100 g of the dried powder in 1 L of ethanol by using a 250 ml beaker for 10 hr. The extracts were filtered through Whatmann filter paper No. 42 (125mm). The filtered extract was concentrated and dried by using a rotary evaporator under reduced pressure. The obtained residue 60 ml (root) was used to prepare the series (1, 2, 3, 4 and 5 %) of aqueous supernatant concentrations for *in vitro* studies (table 1).

Growth and characterization of Struvite crystals

Glass test tubes were used as a crystallization apparatus and the single diffusion reaction technique was employed [28, 29]. One of the reactants, 0.5 M ammonium dihydrogen phosphate (ADP), was mixed with sodium metasilicate solution the density of 1.04g/cm^3 at pH 9.4, so that the pH of the mixture was maintained at 6 and left undisturbed for 2-3 days. After gelation took place, the supernatant solution of 1 M Magnesium acetate was gently poured onto the set gel in various test tubes. After pouring on each supernatant solution, the test tubes were capped with airtight stopples. The experiments were conducted at room temperature (37°C). The grown Struvite crystals were characterized using FTIR to verify the compound and structure of the grown crystal. FTIR was performed by Hitachi 570 FT-IR spectrophotometer technique to verify the proper formation of crystal and their purity [30].

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

Supernatant Solutions (SS) (Groups and Treatments)	Compositions
I (Control)	10 ml of 1 M magnesium acetate
II (Distilled water)	5 ml of 1 M magnesium acetate +5 ml of distilled water
III (0.15% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 1% of ethanolic extract of root of *Euphorbia hirta*
IV (0.25% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 2% of ethanolic extract of root of *Euphorbia hirta*
V(0.50% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 3% of ethanolic extract of root of *Euphorbia hirta*
VI(0.75% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 4% of ethanolic extract of root of *Euphorbia hirta*
VII(1.00% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 5% of ethanolic extract of root of *Euphorbia hirta*

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

An attempt was made to study the effect of ethanolic extract of root of *Euphorbia hirta* on the growth of struvite crystals 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 aqueous extract of five medicinal plants on the growth of Struvite crystals, a series of five different concentrations of 1,2,3,4 and 5% of these each plant extracts were added in equal amounts in supernatant solution and the average weight of the grown crystal were measured.

Results and Discussions

Effect of ethanolic extract of root of *Euphorbia hirta* on struvite crystals

The effect of ethanolic extract of root of *Euphorbia hirta* on nucleation and crystallization characteristics of struvite crystals is determined by measuring the weight of the formed crystals. In the gel method, the control using pure Mg CH_3COO_2•$4H_2O$ led to the maximum nucleation of crystals growth within 24 h of adding the supernatant solutions Fig. 1 (1a). In the presence of root of *Euphorbia hirta*, nucleation was delayed and reduced masses of the crystals were observed 96 h after adding the supernatant solutions Fig. 1 (1b-g). Morphology of the crystals after treated with an ethanolic extract of root of *Euphorbia hirta* as shown in Fig. 2. The largest struvite crystals dimensions were 2.5 cm shown in Fig. 3a). The size of the struvite crystals were reduced from 2.5 cm to 0.6 cm at 1% concentration of extracts was given in (Fig. 3c-g).With an increase in the concentration of extracts from 1 to 5% (v/v), the weight of the for11med crystals was gradually reduced from 0.77 gm to 0.235 gm.

Recently, growth inhibition studies of Struvite crystals in the presence of some of the herbal extracts [17, 18, 31] were attempted in literature. In the present work, Struvite crystals growth was reduced due to the inhibitory effect of root of *Euphorbia hirta* under *in vitro* conditions. This result indicates that distilled water did not show any inhibitory activity with regard to crystal growth, whereas the of ethanolic extract of root of *Euphorbia hirta* possessed inhibitory activity due to the presence of bioorganic molecules volatile oil, chiefly sesquiterpene, hydrocarbons, sesquiterpene alcohols, gingerole., starch, tannins flavonoids like galangin [24-27].

Table 2: Showing the Percentage of inhibition of the harvested root of *Euphorbia hirta* struvite crystals Ethanol

Crystals	Group	Treatents	Harvested crystals of ethanol(gram)	Percentage of Inhibition
Struvite	A	Control	0.770	0%
	B	Control + Distilled water	0.615	20.1%
	C	Control + Ethanol	0.502	35.06%
	D	Control+1% extracts	0.460	40.25%
	E	Control+2% extracts	0.409	48.05%
	F	Control+3% extracts	0.390	49.35%
	G	Control+4% extracts	0.375	51.94%
	H	Control+5% extracts	0.235	70.1%

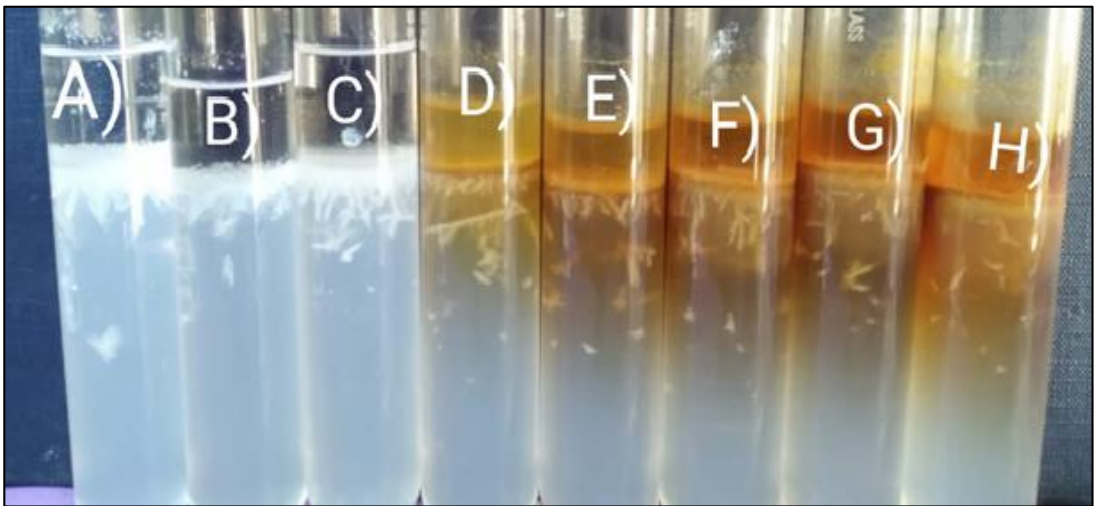

Fig 1: The effect of ethanolic extract of root of *Euphorbia hirta* on struvite crystals in single diffusion gel method (a) without any additive (b) with the distilled water (c) with the methanol (d) with the 1% extract (e) with the 2% extract (f) with the 3% extract (g) with the 4% extract (h) with the 5% extract.

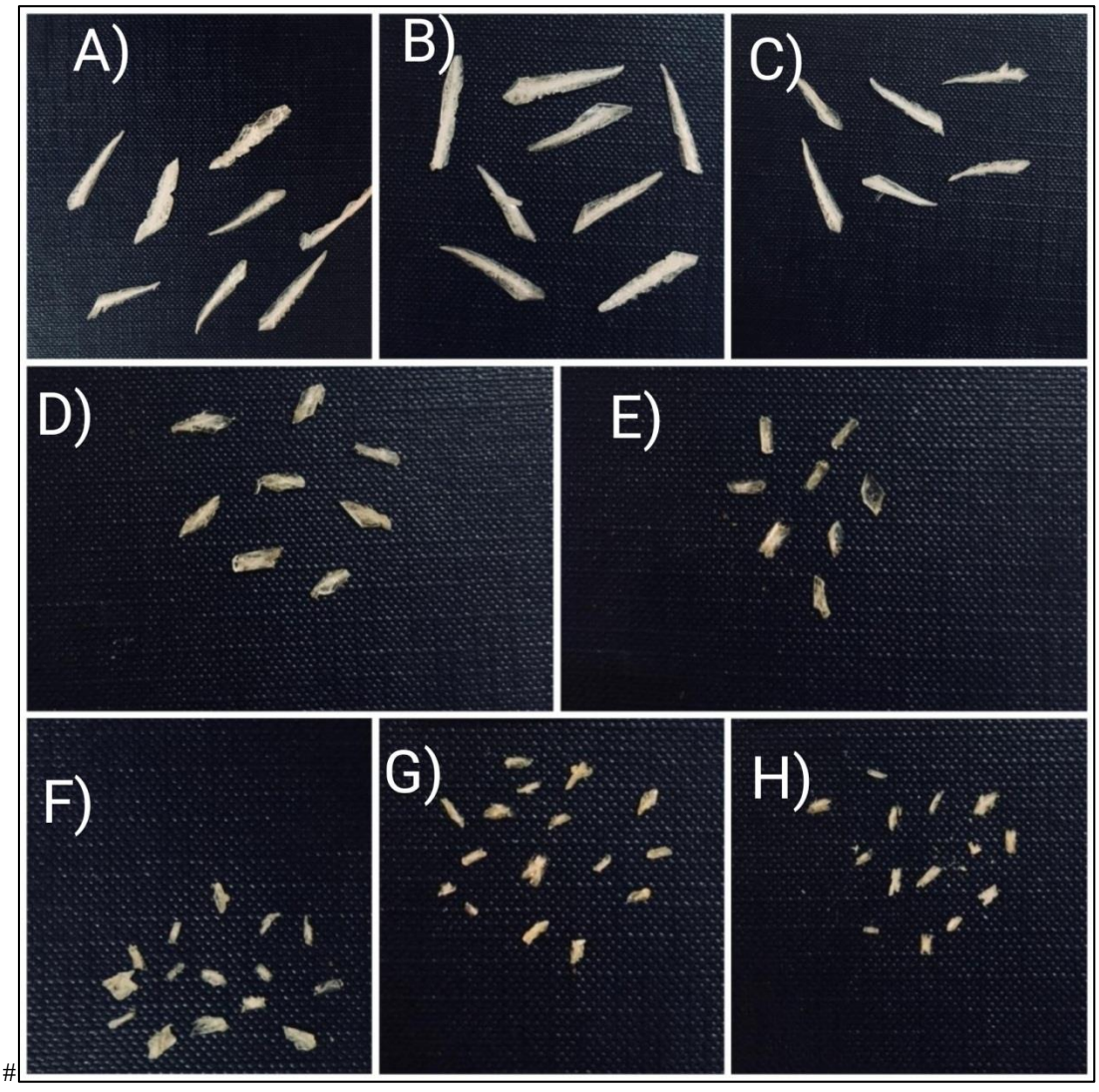

Fig 2: The harvested crystals of struvite obtained from the ethanolic extract of root of *Euphorbia hirta* in the gel method single diffusion gel method (a) without any additive (b) with the distilled water (c) with the methanol (d) with the 1% extract (e) with the 2% extract (f) with the 3% extract (g) with the 4% extract (h) with the 5% extract.

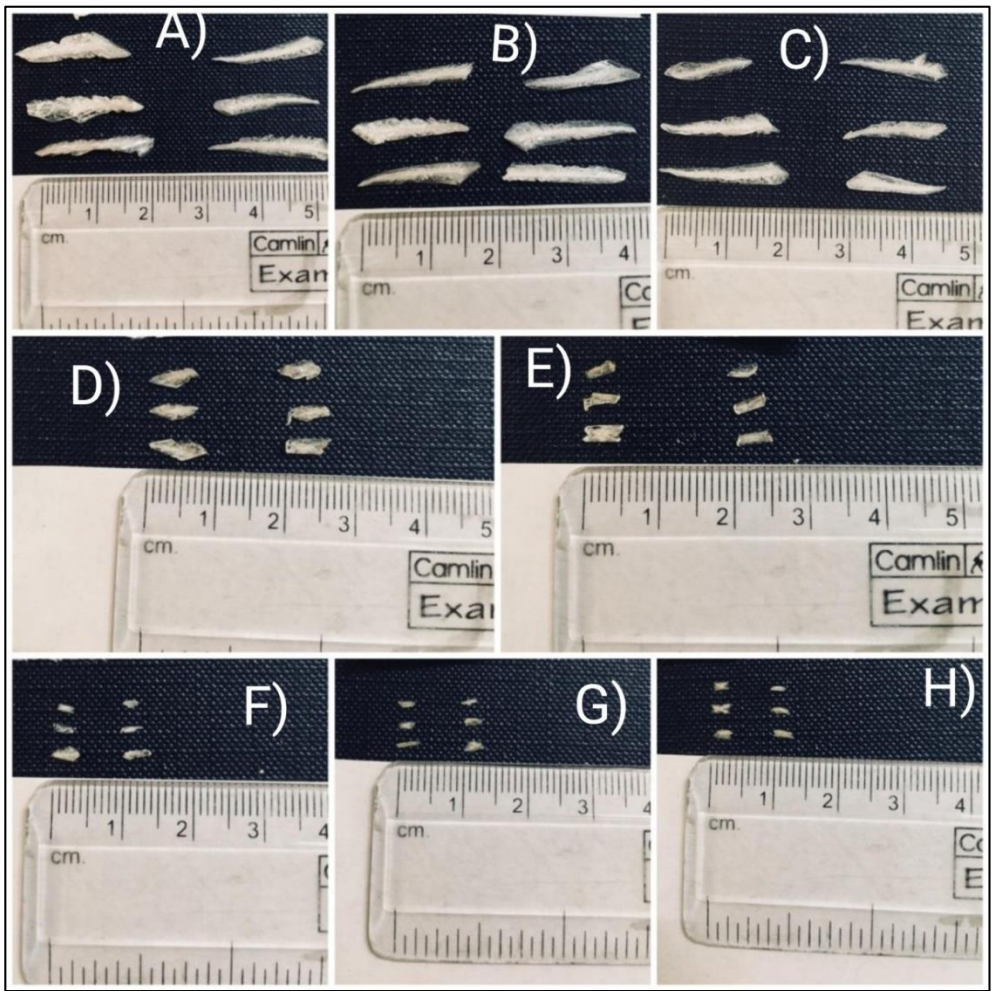

Fig 3: The measurement of struvite crystals after treatment with the ethanolic extract of root of *Euphorbia hirta* by the gel method single diffusion gel method (a) without any additive (b) with the distilled water (c) with the methanol (d) with the 1% extract (e) with the 2% extract (f) with the 3% extract (g) with the 4% extract (h) with the 5% extract.

Characterization of Struvite crystals

The FTIR spectra of struvite crystals obtained in the presence and absence of the plant samples are shown in Figure 7.

In Figure 4(a), the band at 2358 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1626 cm^{-1} is due to HOH deformation of water and the peak at 1441 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1007 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 757 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 568 cm^{-1} is due to the V_4 bending modes of the PO_4 units.

In Figure 4(b), the band at 2358 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1631 cm^{-1} is due to HOH deformation of water and the peak at 1441 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1007 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 757 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 568 cm^{-1} is due to the V_4 bending modes of the PO_4 units.

In Figure 4(c), the band at 2352 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1636 cm^{-1} is due to HOH deformation of water and the peak at 1441 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1007 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 767 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 568 cm^{-1} is due to the V_4 bending modes of the PO_4 units.

In Figure 4(d), the band at 2364 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1626 cm^{-1} is due to HOH deformation of water and the peak at 1440 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1004 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 757 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 568 cm^{-1} is due to the V_4 bending modes of the PO_4 units.

In Figure 4(e), the band at 2362 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1627 cm^{-1} is due to HOH deformation of water and the peak at 1440 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1004 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 757 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 568 cm^{-1} is due to the V_4 bending modes of the PO_4 units.

In Fig. 4(f), a band at 2364 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1631 cm^{-1} is due to HOH deformation of water and the peak at 1440 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1007 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 757 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 568 cm^{-1} is due to the V_4 bending modes of the PO_4 units. In the presence of 5% orange juice

Figure 4(g), the band at 2362 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1633 cm^{-1} is due to HOH deformation of water and the peak at 1439 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1007 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 757 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 568 cm^{-1} is due to the V_4 bending modes of the PO_4 units.

Figure 4(h), the band at 2374 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1600 cm^{-1} is due to HOH deformation of water and the peak at 1438 cm^{-1} is due to the HNH deformation modes of NH_4 units. The band at 1007 cm^{-1} is due to V_3 antisymmetric stretching vibration and the peak at 758 cm^{-1} is due to the water liberational and NH_4 rocking modes. The peak at 568 cm^{-1} is due to the V_4 bending modes of the PO_4 units.

Several researcher have reported crystallization characterization of Struvite crystals using FTIR techniques. The peaks shift from 2358 to 2374 cm^{-1} and from 1441 to 1438 cm^{-1} for HNH deformation modes of NH_4 units previously reported. The shifting further supports that the extract can promote the formation of ammonium magnesium phosphate hexahydrate crystals and reduce the nucleation rate of struvite crystals.

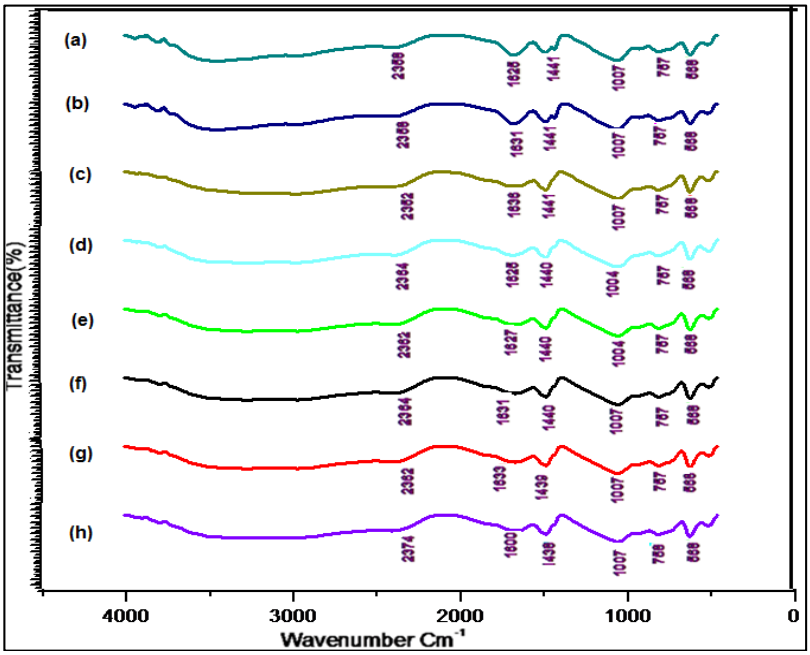

Fig. 4: The FTIR spectra of struvite obtained from ethanolic extract of root of *Euphorbia hirta* in the gel method (A) without any additive (B) with the distilled water (C) with the methanol (D) with the 1% methanol extract (E) with the 2% methanol extract (F) with the 3% methanol extract (G) with the 4% methanol extract (H) with the 5% methanol extract after 7 days.

The XRD patterns of struvite crystals obtained in the presence and absence of the ethanolic extract of root of *Euphorbia hirta* are shown in (fig. 5). The diffraction peaks obtained were well correlated to the (hkl) indices of struvite phase (JCPDS card number 04-010-2894). The effected root of *Euphorbia hirta* the nucleation and growth of struvite crystals crystals.

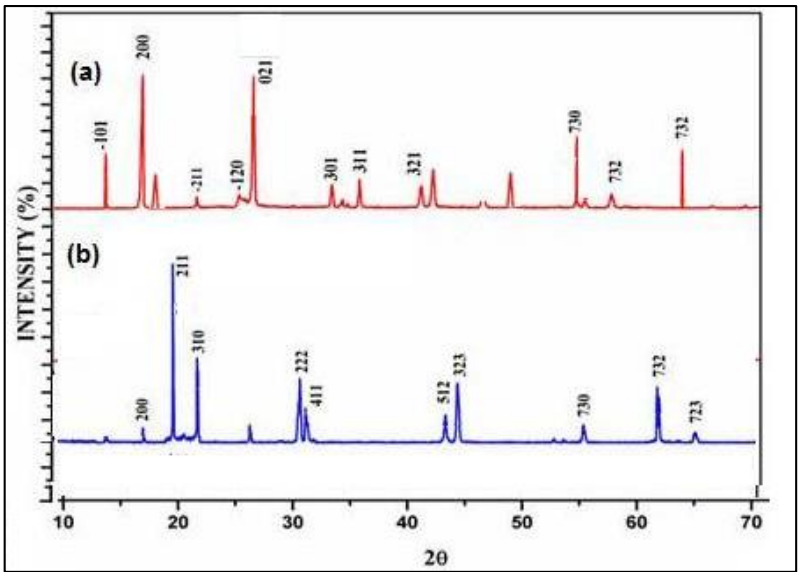

Fig. 5: The XRD pattern of struvite obtained from ethanolic extract of root of *Euphorbia hirta* in the gel method (a) without any additive (b) with the 5% aqueous extract after 7 days.

Conclusion

Struvite crystals were grown by single diffusion gel growth techniques and characterized by FTIR and Powder XRD techniques for the experimental confirmations of the grown crystal. With an increase in the concentration of ethanolic extract of root of *Euphorbia hirta* the weight of the formed crystals were gradually reduced from 0.77 g to 0.23 g in struvite crystals, respectively. FTIR and Powder XRD techniques confirmed its functional groups and crystalline phases of struvite crystals. This study confirmed that the root of *Euphorbia hirta* can promote the changes of ammonium magnesium phosphate hexahydrate crystals and treat urinary stone by inhibiting the formation of struvite crystals, a major component of triple phosphate 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.

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