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Prevention of Kidney Stone and Antimicrobial Potential of Aqueous Extracts of *Linum usitatissimum*

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ABSTRACT

Globally, brushite and struvite kidney stones are one of the aged known diseases in both men and women causing critical urinary disarray. So, the present study accomplished the effect of aqueous extracts of *Linum usitatissimum* preventing stone. *In vitro* study brushite and struvite stones were gel formed and treated with extracts of *L. usitatissimum* at different concentrations. The crystal formation was analyzed by the FTIR and XRD method. The maximum inhibition of crystals using *L. usitatissimum* extracts ranged from 1.68 to 0.30 g for brushite crystal and 1.74 to 0.12 g for struvite crystal. Additionally, the better inhibition of bacterial and fungal activity was observed at the concentration ranging from 60-100 µg/ml. In conclusion, good antibacterial, antifungal and antiurolithiatic activity was recorded for *L. usitatissimum* extract.

Key words: Calcium chloride, struvite crystals, magnesium acetate, anticrystal, antibacterial

INTRODUCTION

Urolithiasis and nephrolithiasis are two major kidney stones formed as solid hard material that accumulates in the urinary tract. Calculi are one of the most well-known and frequent urinary tract illnesses. Traditional herb with anti-urolithiasis activities is commonly used for the treatment of kidney stone related problems(Kaleeswaran *et al.,* 2019). Several human studies have suggested that eating a diet rich in vegetables and fruits can help prevent kidney stones. In addition to these edible plants, antioxidant phyto-phenols such as catechin, epicatechin, epigallocatechin-3-gallate, diosmin, rutin, quercetin, hyperoside and curcumin have been proven to be useful in the reduction of urolithiasis (Nirumand *et al.*, 2018). According to epidemiological data, kidney stones afflict 12% of the world's population, with 47-60% of women and 70-80% of males.Saturation, nucleation, development, agglomeration and detention within renal tubules are the first steps in the creation of kidney stones (Arya *et al.,* 2017). Urinary stones are made up of calcium oxalate, uric acid, calcium phosphate and magnesium ammonium phosphate which can easily be dissolved by water extract of medicinal plant (Ram *et al.*, 2016). The production of calculi is aided by calcium phosphate and struvite stone (Panigrahi *et al.,* 2016). Brushite crystal development is similar to the ailment of renal tubular acidosis and hyperparathyroidism. Struvite stone or infectious stone occurs increasingly in women compared to men (Zhang *et al.,* 2021). Advanced therapeutic treatments are available for the prevention of kidney stone formation in humans with side effects (Prabhu *et al.,* 2016). The most common component of kidney stones is calcium oxalate. Plants and their phytomolecules were being reported to influence cure, prevention and reduction of reappearance of kidney stones by acting on several different parts of renal stone (Hardik *et al.*, 2016). Plant leaf extract can successfully reduce the relapse of stone formation in its initial phases as well as reduce UTI pathogens (Pratiwi and Elin-Yulinah, 2016). The World Health Organization (WHO) evaluated 80% of the population following a medicinal way of preventing kidney stone formation. So, the increasing rate of urinary stone treatment using medicinal plant is much focused on research.

MATERIALS AND METHODS

The seeds of *Linum usitatissimum* were procured from the field of Trichy, Tamil Nadu, India. It was confirmed and authenticated by Dr. S. John Britto, Director, Rapinat Herbarium, St.

Joseph College, Tiruchirappalli, Tamil Nadu. Ammonium dihydrogen orthophosphate, magnesium acetate, orthophosphoric acid, calcium chloride, methanol, orthophosphoric acid, distilled water, sodium metasilicate and other chemicals were bought from Sigma Aldrich, New Delhi, India. Fourier Transform Infrared (FTIR) was absorbed with a resolution of 4/cm and a wave number ranged from 400-4000/cm using KBr pellet technique. X-ray Diffraction (XRD) was done with a PW1710 dependent using CuKα radiation.

The aqueous seed extracts of *L. usitatissimum* were cleaned with water and dried for a week at 35-40°C, later these were grinded into a powder. In a 250 ml beaker, 100 g of dried powder was mixed with 1 l of aqueous solution and allowed for extraction by Soxhlet extractor for 10 h. Then, it was filtered with Whatman filter paper No. 42. By rotatory evaporator, the filtered extract was dried. The series of 20,40,60,80 and 100 µg/ml of aqueous supernatant solution was prepared using obtained 60 ml (flower) remnant for *in vitro* studies.

Single diffusion method was performed for the growth of CHPD and struvite crystals. For CHPD crystal, clean glass tubes were taken, then 1 M orthophosphoric acid and sodium metasilicate was mixed with a density of 1.05 g/cm^3 at pH 9.2. This solution was allowed for gel growth for 2-3 days. After gelation, 1 M calcium chloride was flooded onto the gel and gelation took place at the end. For struvite crystal, 0.5 M ammonium dihydrogen phosphate (ADP) and sodium metasilicate were added and growth took place in 2-3 days. Then 1 M magnesium acetate was added onto the gel for support. Finally, both the settled gels were closed with stopples. The both experiments were done at room temperature 37°C. XRD and FTIR technique were done using Hitachi 570 FTIR to find the occurrence of crystals.

The aqueous extract of *L. usitatissimum*on the growth of crystal studies was made with little failure (Bindhu *et al.,* 2015). On a set of gels, the supernatant solutions were added and the outcome was noticed (Table 1). For struvite crystals, magnesium acetate was added instead of using $CaCl_2$ with aqueous extracts of *L. usitatissimum*. The effect of aqueous extract of *L. usitatissimum* on the crystal growth was studied by repeating the experiment four times. For both CHPD and struvite crystals, the series concentrations of aqueous extracts (1, 2, 3, 4 and 5%) were added in a supernatant solution and the crystal growth weight was observed. The inhibitory concentration was calculated as follows:

$$1\% = [(TSI-TAI)]/TSI \times 100\ TSI$$

Where, TSI–Crystal number without inhibitors and TAI–Number of crystals after addition of inhibitors.

To scrutinize the antimicrobial activity of aqueous extracts of seeds of *L. usitatissimum,* the bacterial and fungal cultures were acquired from Microbial Type Culture and collected from Chandigarh, India. Disc diffusion method was performed for the observation of antimicrobial activity of aqueous extracts of *L. usitatissimum*. In clean Petri dishes, the medium of Muller Hinton Agar was prepared for bacteria culture and Sabouraud's dextrose agar (SDA) for fungal culture. For the test, bacterial and fungal organisms were inoculated. Six mm sterile discs were filled with 10 µl of aqueous extract at various concentrations (20-100 µg/ml). Then, the loaded disc was placed on the agar dishes and for compound diffusion, it was allowed for 30 min. Appropriate solvent served as negative control. Finally, all the plates were incubated for 24 h and the region of growth suppression was observed in millimeters and the experiment was sustained for two times.

Table1. Test tube gels treatment for CPD crystals

Treatment group	Compositions
I (Control)	10 ml of 1M $CaCl_2$
II (Control+distilled water)	5 ml of 1M $CaCl_2$+5 ml of distilled water
III (1% aqueous extract)	5 ml of 1M $CaCl_2$+5 ml of 1% *L. usitatissimum*
IV (2% aqueous extract)	5 ml of 1M $CaCl_2$+5 ml of 2% *L. usitatissimum*
V (3% aqueous extract)	5 ml of 1M $CaCl_2$+5 ml of 3% *L. usitatissimum*
VI (4% aqueous extract)	5 ml of 1M $CaCl_2$+5 ml of 4% *L. usitatissimum*
VII (5% aqueous extract)	5 ml of 1M $CaCl_2$+5 ml of 5% *L. usitatissimum*

RESULTS AND DISCUSSION

By quantifying the quantity of the produced crystals, the effect of aqueous *L. usitatissimum* on nucleation and crystal development features of CHPD and struvite crystals was revealed. Calcium chloride (CHPD crystals) and Mg $CH_3COO_2.4H_2O$ (struvite crystals) were introduced to the crystal nucleation test for a control, and the development of bands was noticed after 24 h of adding up the supernatant solution. After 96 h, the expanded expansive needle-shaped stones had grown up within the precipitant ring. CHPD crystal development reached a maximum size of 3.1 cm, while struvite crystals reached a maximum size of 2.5 cm. CHPD crystals shrank from 3.1 to 0.5 cm, whereas struvite crystals shrank from 2.5 to 0.6 cm. The burden of the fashioned CHPD crystals was gradually reduced from 1.68 to 0.30 g by changing the amount of extracts from 1 to 5% (w/v), and the extracts from 1 to 5% (v/v) for struvite crystals were reduced from 1.74 to 0.12 g. The percentage of inhibition of CHPD and struvite crystals using aqueous extracts is predicted in Table 2. In a previous study, inhibition of growth of crystals using herbal plants was tried and reported by many workers (Karumaran *et al.,* 2016). In the current work, the seed extracts of *L. usitatissimum* showed better activity in the CPHD inhibition of crystals recorded as 54, 63, 68, 75 and 82%. Inhibitory effect of aqueous plant extracts on urinary calcium hydrogen phosphate dehydrates crystals was well documented on many medicinal plants.

Table 2. Growth value of CHPD and struvite crystals

S. No.	Group	Harvested crystals		% of inhibition	
		CHPD	Struvite	CHPD	Struvite
1.	I	1.68	1.74	0	0
2.	II	0.93	0.88	44	49
3.	III	0.77	0.46	54	73
4.	IV	0.61	0.38	63	78
5.	V	0.53	0.29	68	83
6.	VI	0.42	0.23	75	86
7.	VII	0.30	0.12	82	93

FTIR spectra for both CHPD (Fig. 1) and struvite crystals (Fig. 2) were measured by the presence and absence of the aqueous extracts of *L. usitatissimum*.The FTIR spectrum results of band formation of harvested crystals using various concentrations of aqueous extracts of plant were evaluated. In CHPD crystals, the peak shifted from 3016 to 3017/cm and from 2023 to 2029/cm for P=O stretching and for struvite crystals, band ranged from 2368 to 2379/cm and 1638 to 1600/cm for deformation modes of NH_4 units. The shifting support that the grape seeds could induce the formation of brushite and ammonium magnesium phosphate hexahydrate crystals and decreased the development of nucleation rate of both crystals.

XRD patterns for crystals acquired by the appearance and absence of aqueous extracts of *L. usitatissimum* were found to have variation on spectral data. In CHPD crystals (Fig. 3), the peak for diffraction pattern attained as well correlated to hkl indices of CHPD phase (JCPDS card number 09-0077) and the hydroxyapatite phase (JCPDS card number 9-432). For

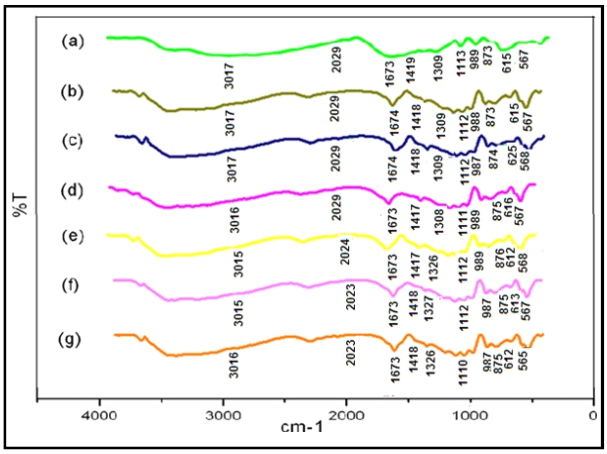

Fig. 1. FTIR spectra band for CHPD crystals.

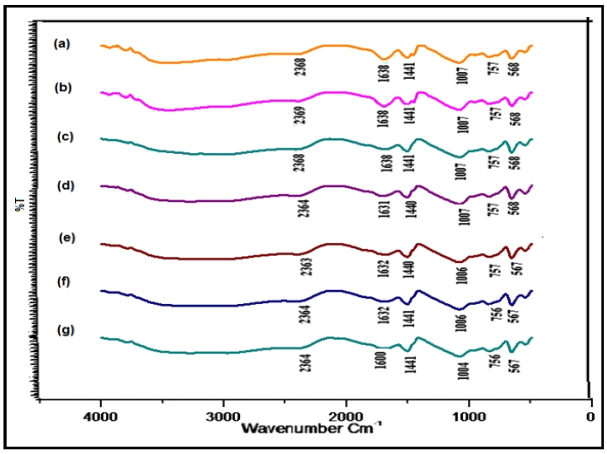

Fig. 2. FTIR spectra for struvite crystals.

(a) no additives, (b) with water, (c) with 1% extracts, (d) 2% extracts, (e) 3% extracts, (f) 4% extracts and (g) 5% extracts.

struvite crystals (Fig. 4) peaks interrelated to the directions of struvite phase (JCPDS card number 04-010-2894). From the results, this experiment revealed the better effect of *L. usitatissimum* on the nucleation and growth of hydroxyapatite and struvite crystals.

The results of antibacterial activity of seed extracts of *L.usitatissimum* were tested in opposition of bacteria. The aqueous extracts showed higher growth inhibitory activity against *Escherichia coli* (20 mm), *Staphylococcus aureus* (19 mm), *Enterococcus aerogenes* (17 mm), *Pseudomonas aeruginosa* (18 mm) and *Proteus vulgaris* (18 mm) at the concentration of 100 µg/ml. As the concentration of extracts from 20-100 µg/ml increased, the inhibitory action against pathogens also increased based on concentration. In a previous study, the extracts of herbs showed better antibacterial activity in opposition to gram-positive and gram-negative bacteria associated with UTI (Josephine *et al.,* 2017). Antifungal activity of a series of concentrations of aqueous extracts of *L. usitatissimum* against fungi was performed. From the results, the experiment revealed the most effective and highest activity of aqueous extracts against *Aspergillus flavus* (10 mm), *Candida vulgaris* (9 mm), *Aspergillus niger* (9 mm) and *Candida tropicalis* (9 mm) at the concentration of 100 µg/ml. Fadzir *et al.* (2018) also reported that *L. usitatissimum* was a potent antimicrobial phytomedicine. Bongoni *et al.* (2016) found significant antibacterial and antifungal activities of Linum seeds against various pathogens.

CONCLUSION

The aqueous extracts of *Linum usitatissimum* showed better inhibitory action of antiurolithiatic and antimicrobial activity under *in vitro* condition. This study confirmed that the *L. usitatissimum* extracts inhibited the growth of crystals and UTI pathogens. This result showed the action of extracts on both the crystals. When compared to struvite crystals, the antiurolithiatic activity of CHPD was relatively high. This study will be useful for further *in vivo* studies for pharmaceutical production.

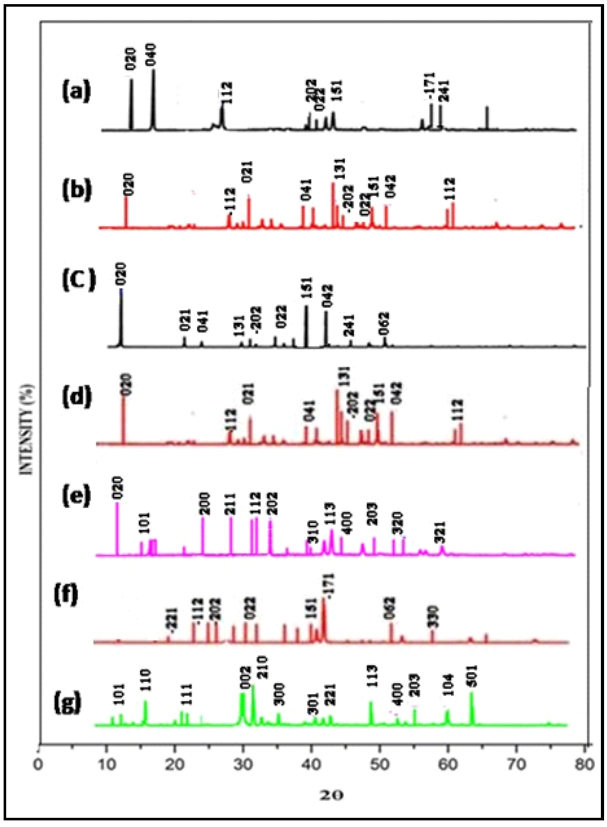

Fig. 3. XRD pattern for CHPD crystals.

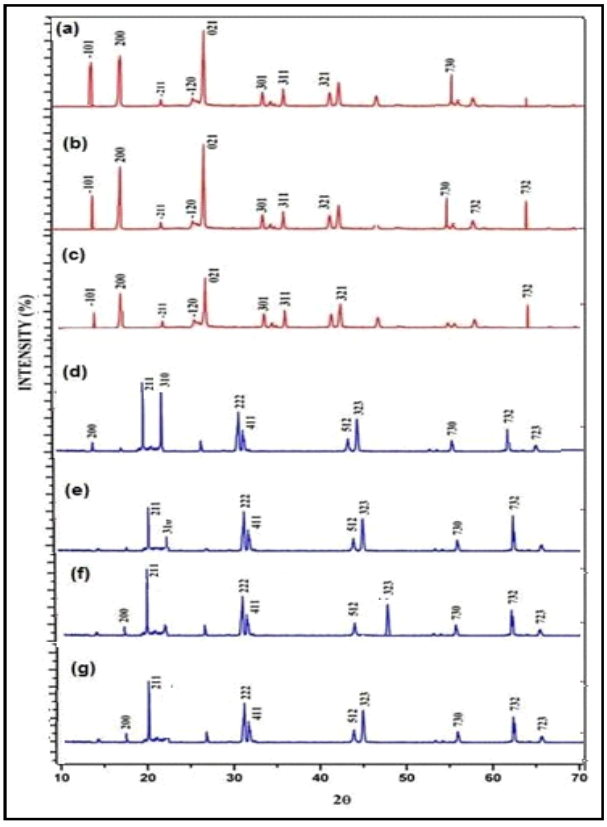

Fig. 4. XRD spectra for struvite crystals.

(a) no additives, (b) with water, (c) 1% extracts, (d) 2% extracts, (e) 3% extracts, (f) 4% extracts and (g) 5% extracts.

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