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Evaluation of Anti- Urolithiasis Potential of Indian Medicinal Herbs *Ficus hispida, Morinda tinctoria* and *Sapindus emarginatus* by Struvite Crystal Growth Inhibition Assay

D.Sivaraman*

Centre for Laboratory Animal Technology and Research, Col.Dr.Jeppiaar Research Park, Sathyabama University, Jeppiaar Nagar, Rajiv Gandhi road, Chennai – 600 119, Tamil Nadu, India

Abstract : Renal calculi is a form of solid concentrates formed with in the collecting tubule of kidney. Renal stone normally composed of Calcium oxalate dehydrate, calcium oxalate monohydrate, uric acid, calcium phosphate cystine. In India, 12% of the population is expected to have urinary stone complication. Presently various treatment strategy has been followed for treating nephrolithiasis condition such as hydration, alkali treatment and shock wave lithotripsy and surgery. All the current line treatment for kidney stone offers only symptomatic relief. The major crisis faced by the patients with renal kidney stone is recurrence. It was estimated that average recurrence period could be within 5 to 10 years. Hence the society is in high need of discovering an alternate complimentary medicine that can halts the progression of stone formation. In the present investigation anti-urolithiasis potential of the three significant medicinal herbs such as *Ficus hispida*, *Morinda tinctoria* and *Sapindus emarginatus* were screened using single diffusion gel growth technique at two dose level of 0.5% and 1%. The efficacy of all the herbs was evaluated by comparing the crystal size of treated medium with that of the control. The average size of the crystal in the control medium ranging from 1.3 ± 0.15 cm to 1.56 ± 0.17 cm. The crystal size was significantly decreased in medium contains 0.5% and 1% of ethanolic extract of *Ficu shispida* (EEFH) with the size of 0.92 ± 0.13 and 0.7 ± 0.12cm. Similar type of results were achieved in medium contains 0.5% and 1% of ethanolic extract of *Morinda tinctoria*(EEMT) with the size of 1.02 ± 0.13 and 0.64 ± 0.16cm. Ethanolic extract of *Sapindus emarginatus*(EESE) at the dose of 0.5 and 1 % reveals significant decrease in crystal size of 1.08± 0.16 and 0.72± 0.08cm. From the result of the Study, it was concluded that the all the three extracts has shown promising anti-urolithiasis property in the tested medium.
Keywords : Renal calculi, *Ficus hispida ,Morinda tinctoria , Sapindus emarginatus,* crystal size, Anti-urolithiasis, Gel diffusion.

1.Introduction

Incidence of urolithiasis has steadily increasing as it prevails 10% in men and 5% I women's. It seems men's are at higher risk rather than women's[1]. As per the recent literature survey it was suggested that patients with nephrolithiasis may even leads to end-stage renal disease (ESRD) at a younger age [2].

There are several potential mechanisms for kidney stones leading to chronic kidney disease. Extension of calcifications of the renal interstitium and tubular basement membrane into the tubular lumen can lead to scarring and a decrease in renal function[3].

Struvite kidney stones attains larger size because of series nucleation and aggregation further continuous inter crystal attraction and plaque formation which often requires surgical attention and it may even

leads to kidney function if not properly treated [4].Endouroscopic procedures as well as extracorporeal shockwave lithotripsy procedure offers grater relief in kidney stone cases when compare to other conventional treatment methods but the problem relies in such modern treatment modalities involves direct renal injury via vasoconstriction [5,6].

Herbs become the integral part of the mankind since several centuries. India is considered to be one of the most significant zones for cultivation and export of the medicinal plant. According to the literature nearly 60–80% of the world's population still relies on traditional medicine [7]. Indian system of traditional medicine such Ayurveda, siddha and unani formulation comprises greater of herbs or herbomineral combinations. As per the report of The ethnobotanical survey for India it was estimated that about 20,000 medicinal plants were recorded and only 7500 plants were utilized by the traditional practitioners for drug development, and about 25,000 herb-based formulations were prepared and provided in traditional Indian medicines for curing various disorders[8]. Plants become a useful remedy because of its bioactive components such as alkaloids, flavonoids, tannins, saponins, phenols etc.

Plant extracts are unique blend of mixtures which consist of numerous bioactive compounds which can be extracted using various solvents and several extraction techniques. Different in screening models such as such as plant bioassays, tissue or cell culture, receptor enzyme and bio-chromatography were used for instigation biological activities of secondary metabolites from plants[9].

Ficus hispida Linn belongs to the family Moraceae is a widely distributed and most commonly found in the interior and coastal regions distributed throughout India, Srilanka, Myanmar, and Southern region of the Republic china .A mixture of honey and the juice of these fruit is a good antihemorrhagic[10] but the barks and leaves are of particular interest from a medicinal point of view as an antidiarrhoeal[11], Antidiabetic[12] and as cardio protective [13] among others.

Morinda tinctoria belongs to the family Rubiaceae grows wildly and distributed throughout Southeast Asia, commercially known as Nunaa, is indigenous to tropical countries and is considered as an important folklore medicine. In the traditional system of medicine, leaves and roots of MTR are used as astringent, deobstruent, emmenagogue and to relive pain in the gout [14] .It has been reported to have a broad range of therapeutic and nutritional values [15] .There is a greater demand for fruit extract of morinda species in treatment for different kinds of illness such as arthritis, cancer, gastric ulcer and other heart disease [16]. The major components have been identified in the Nunaa plant which includes octoanicacid, potassium, vitamin C, terpenoids, scopoletin, flavones glycosides, lineoleic acid, anthraquinones, morindone, rubiadin and alizarin [17-19].

The fruits of *Sapindus emarginatus* are commonly used for hair problems and also in preparation of shampoos. Traditionally it used as anti-inflammatory and antipyretic. The seed is intoxicant and the fruit rind has oxytropic action. Nut powder is used as Nasal Insufflations. Seeds of *Sapindus emarginatus* contain anti-inflammatory oil which is traditionally used to purify the blood. Historically it has been used in folk remedies as a mucolytic agent, emetic, paralysis of limbs, treatment of chlorosis. Soapnuts are also used as effective aid for the treatment of skin problems like eczema, itching and psoriasis. Its fruits are natural substitute for chemical soaps and hair dyes. Pericarp contains triterpene, saponins, commonly used as antifertility, antipruritic and anti-inflammatory agents in traditional Indian and Thai medicines. The roots are used as expectorant and demulcent and also are used for cure of hysteria and epilepsy [20-24].

The main aim of the present investigation is to evaluate the anti-urolithiasis potential of the three significant medicinal herbs such as *Ficu shispida*,*Morinda tinctoria* and *Sapindus emarginatus* were screened using single diffusion gel growth technique at two dose level of 0.5% and 1%.

2.Materials and Methods

2.1.Plant material

The fresh leaves of *Morinda tinctoria*(MT) and *Ficus hispida* (FH) were collected from (Peranakkavur Village is a Village in Uttiramerur Taluk in Kanchipuram District, Tamil Nadu, India). The fruits of *Sapindus emarginatus*(SE) were purchased from traditional drug suppliers, Parrys, Chennai, Tamil Nadu, India. The plant

materials were identified and authenticated by botanist one Dr. Sasikala Ethirajulu. Captain Srinivasa Murthy research Foundation, Chennai, Tamil Nadu, India. The specimen voucher was deposited in the Department of Pharmacology and toxicology, Sathyabama University, Chennai, Tamil Nadu, India.

2.2.Preparation of the Plant Extracts

The fresh leaf of MT, FH and SE was collected and washed with running water. It was shade dried at room temperature and 1kg of the dried leaf and fruit was made in to coarse powder. The powder was passed through a 60 No mesh sieve. Air dried Powdered drug was extracted with the solvent ethanol by using soxhlet extraction. Then the extracts obtained such as ethanolic extract of *Ficus hispida*(EEFH), ethanolic extract of *Morinda tinctoria*(EEMT) and ethanolic extract of *Sapindus emarginatus*(EESE) was filtered, concentrated by rotary vacuum pump to get the solid mass.

2.3.Test Drug concentration

EEFH,EEMT and EESE was prepared at two different concentrations of 0.5 and 1 % dispersed in 1.0 M magnesium acetate solution

2.4.Single diffusion gel growth technique

The growth of Struvite can be simulated in the laboratory by growing crystals in silica hydro gel medium. In the gel growth technique, the gel acts as a 'three dimensional crucible' which supports the crystals; at the same time yields to its growth without exerting major forces upon it. This relative freedom from constraint is believed to be an important factor in the achievement of high structural perfection.

2.5.Methodology

An aqueous solution of 0.5M Ammonium dihydrogen phosphate was admixed with the sodium metasilicate solution of specific gravity 1.05 in the appropriate amount using magnetic stirrer so that the pH value 7.0 .pH of the reaction was ensured by using pH probe meter. The gel solution of 10 mL was transferred into the test tubes of 140 mm length and 25 mm diameter. After the gelation took place, 5 mL of supernatant solutions of 0.5 and 1% concentration of EEFH, EEMT and EESE in 1.0 M magnesium acetate were gently poured on the set gels in test tubes to enumerate the growth inhibition of Struvite crystals. About 5 ml of 1.0 M magnesium acetate without test drug were added as supernatant to control tubes which serve as crystal control group. All the procedures were done in the aseptic medium in laminar flow hood to avoid microbial contaminations. All test tubes and other glassware were autoclaved at 120°C for 15 min. After pouring supernatant solution, the test tubes were capped with airtight stopples. The experiment was conducted at the room temperature. Study on the growth of crystal was carried out for five consecutive days [25].

Figure 1 A: Control Gel medium Figure 1 B: Medium + 0.5 % EEFH Figure 1 C: Medium + 1 % EEFH

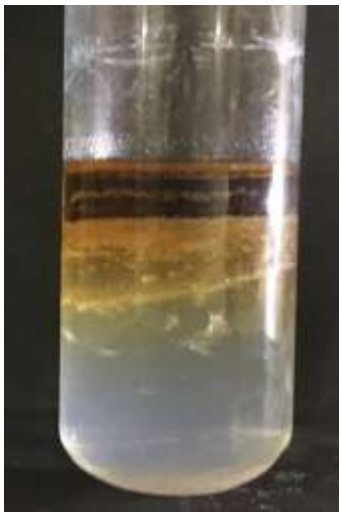

Figure 2 A: Medium + 0.5 % EEMT **Figure 2 B: Medium + 1 % EEMT**

Figure 3 A: Medium + 0.5 % EESE **Figure 3 B: Medium + 1 % EESE**

3.Results

3.1.Effect of EEFH on Size variation of Struvite crystals

The average size of the crystal was higher in the control medium with the length of 1.3 ± 0.15and similarly the average size of the crystal was significantly decreased in medium contains 0.5% of EEFH with the average length of 0.92 ± 0.13 cm. The average size of the crystal was even much reduced in medium contains 1 % of test drug EEFH with the Avg length of 0.7 ± 0.12 cm. As shown in figure 4 and table 1.

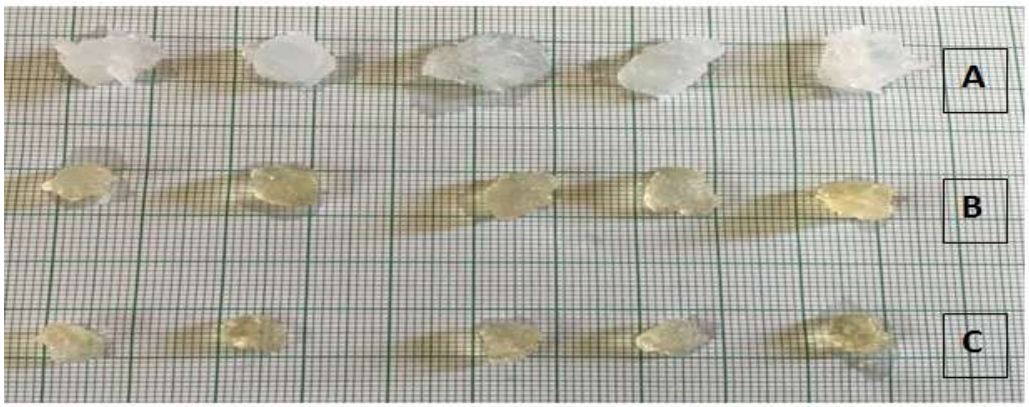

Figure 4: Size variation of Struvite crystals in control and EEFH treated Medium
A - Size variation ofStruvite crystals in Control Gel medium
B- Size variation ofStruvite crystals in Gel medium with 0.5 % of *EEFH*
C- Size variation ofStruvite crystals in Gel medium with 1 % of *EEFH*

3.2.Effect of EEMT on Size variation of Struvite crystals

The average size of the crystal was higher in the control medium with the length of 1.56 ± 0.17and similarly the average size of the crystal was significantly decreased in medium contains 0.5% of EEMT with the average length of 1.02 ± 0.13 cm. The average size of the crystal was even much reduced in medium contains 1 % of test drug EEMT with the Avg length of 0.64 ± 0.16 cm. As shown in figure 5 and table 2.

Figure 5: Size variation of Struvite crystals in control and EEMT treated Medium
A - Size variation ofStruvite crystals in Control Gel medium
B- Size variation ofStruvite crystals in Gel medium with 0.5 % of *EEMT*
C- Size variation ofStruvite crystals in Gel medium with 1 % of *EEMT*

3.3.Effect of EESE on Size variation of Struvite crystals

The average size of the crystal was higher in the control medium with the length of 1.34 ± 0.15and similarly the average size of the crystal was significantly decreased in medium contains 0.5% of EESE with the average length of 1.08 ± 0.16 cm. The averagesize of the crystal was even much reduced in medium contains 1 % of test drug EESE with the Avg length of 0.72 ± 0.08 cm. As shown in figure 6 and table 3.

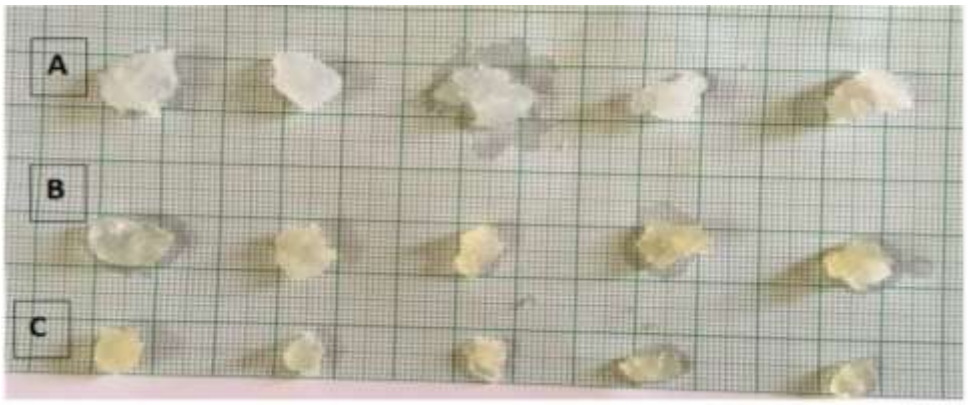

Figure 6: Size variation of Struvite crystals in control and EESE treated Medium
A - Size variation ofStruvite crystals in Control Gel medium
B- Size variation ofStruvite crystals in Gel medium with 0.5 % of *EESE*
C- Size variation ofStruvite crystals in Gel medium with 1 % of *EESE*

Microscopic observation of crystal belongs to control medium reveals the presence of large aggregate whereas treatment with 0.5% of the EEFH, EEMT and EESE reveals significant decrease in the aggregates resulting in projection of individual crystals similarly treatment with 1% of all three test extract shown fragmented crystals reveal the inhibition potential of the individual extracts when compared to that of the control medium crystals. As shown in the figure 7A to 9 C.

Figure 7 A: Microscopic view of Struvite crystals in Control Gel medium

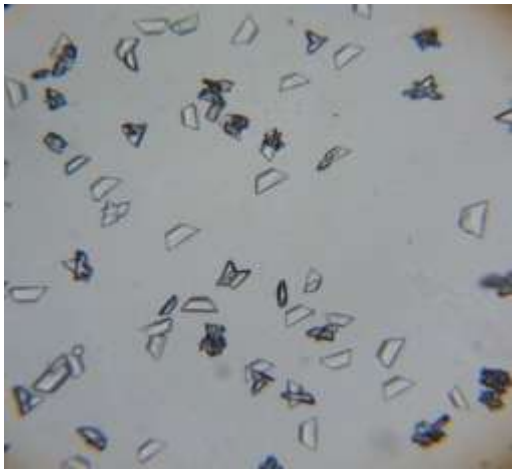

Figure 7 B: Microscopic view of Struvite crystals in 0.5% EEFH treated medium

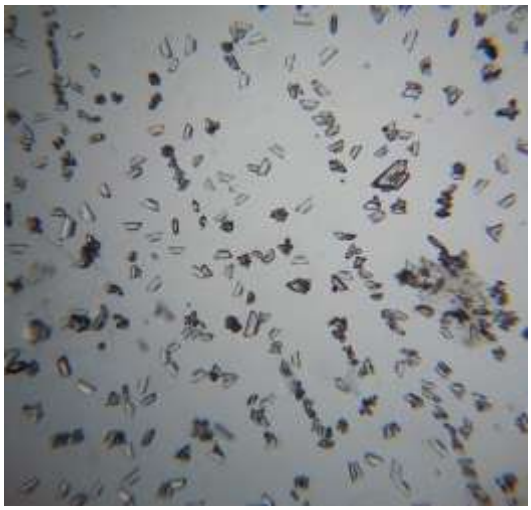

Figure 7 C: Microscopic view of Struvite crystals in 1% EEFH treated medium

Figure 8 A: Microscopic view of Struvite crystals in Control Gel medium

Figure 8 B: Microscopic view of Struvite crystals in 0.5% EEMT treated medium

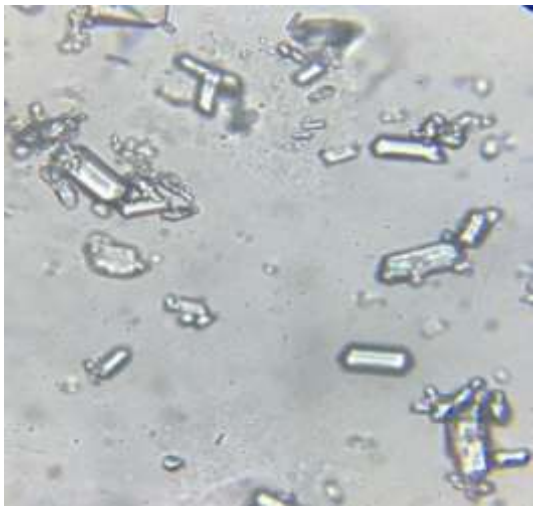

Figure 8 C: Microscopic view of Struvite crystals in 1% EEMT treated medium

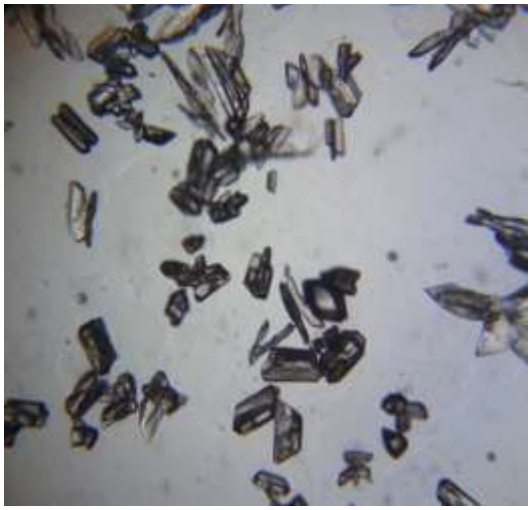

Figure 9 A: Microscopic view of Struvite crystals in Control Gel medium

Figure 9 B: Microscopic view of Struvite crystals in 0.5% EESE treated medium

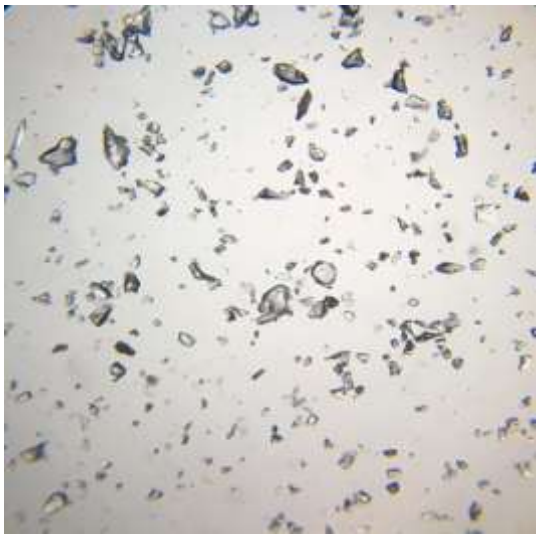

Figure 9 C: Microscopic view of Struvite crystals in 1% EESE treated medium

Discussion

The exact physiology involved in kidney stone formation is still seems to be mystery but through available literature it was suggested that liquid to solid transition of urine was achieved through the process of super saturation. Steady growth of nuclei present with in the crystal ultimately leads to formation of larger crystal particle. Intermolecular attraction of nearby crystal soon forms an aggregate thus further rise in size of the crystal [26]. In most of the cases the size of the crystals even seems larger than diameter of the collecting ducts and thus might be retained. Aggregate of crystals emerges in the form of plaques started interacts with the epithelial lining of the renal tubule [27]. This interaction brings in inflammatory cascade, thus further interferes with the tubular elasticity and disturbs the normal physiology of the kidney. All the above mentioned factors obstruct the flow of urine [28].

Presently various treatment strategy has been followed for treating nephrolithiasis condition such as hydration, alkali treatment and shock wave lithotripsy and surgery [29]. All the current line treatment for kidney stone offers only symptomatic relief whereas nearly 50% of the patient reported with recurrence of stone after 5 to 10 years[30,31]. Hence it is of prior importance to identify a drug that offers selective inhibition of nucleation and aggregation of Calcium oxalate crystals[32-34].

As per WHO Traditional Medicine Strategy 2014–2023 integration of traditional and complementary medicine for universal healthcare is of prime importance. Further greater attention to be paid to ensure the quality, safety and effectiveness of such medicine[35].

The utmost reason for using herbal supplement for treating various disorder's relies on its safety but still the toxicological profiling of most of the mono and polyherbal formulations not be carried out. According to the WHO, herbs or herbal products are used by the large number of populations for basic healthcare needs [36,37].

The average size of the crystal was higher in the control medium with the length of 1.3 ± 0.15and similarly the average size of the crystal was significantly decreased in medium contains 0.5% of EEFH with the average length of 0.92 ± 0.13 cm. The average size of the crystal was even much reduced in medium contains 1 % of test drug EEFH with the Avg length of 0.7 ± 0.12 cm.

Struvite crystal growth techniques are considered to be most common methodology adopted by the researcher for the simulation of renal stone in-vitro worldwide. This technique was successfully adopted for evaluating the anti-urolithiasis potential of herbs and other traditional formulations[38-41].

The average size of the crystal was higher in the control medium with the length of 1.56 ± 0.17and similarly the average size of the crystal was significantly decreased in medium contains 0.5% of EEMT with the average length of 1.02 ± 0.13 cm. The average size of the crystal was even much reduced in medium contains 1 % of test drug EEMT with the Avg length of 0.64 ± 0.16 cm.

In India, nearly 70% of modern drug are derived from natural resources and number of other synthetic analogues have been prepared from prototype compounds isolated from plants[42-44]. It was reported that more than 60% of cancer drug available in market are based on natural products. Currently, about 80% of antimicrobial, immunosuppressive, cardiovascular, and anticancer drugs are derived from plant sources. More than 70% entities among 177 anticancer drugs approved are based on natural products or mimetic. About 25% prescription drug found globally are derived from plant sources, and nearly 121 such drugs entity are in use. Thirteen drugs of natural origin are approved in United States between 2005 and 2007, and clinical trials are going on more than 100 natural product-based drugs. It was also estimated that 11% of the total 252 drugs found in essential medicine list of WHO are exclusively of plant origin [45,46]. In Indian traditional medicine a large number of plants are used. It was estimated that Ayurveda uses 1200–1800 plants, Siddha medicine includes 500–900 plants, Unani utilize 400–700 medicinal plants and Amchi medicine uses nearly 300 plants while folk healers of India use more than 7500 medicinal plants in different medicine. Three classical Ayurvedic literature Charaka Samhita, Sushruta Samhita and Astanga Hridaya mentioned about 526,573 and 902 number of plants[47-49].

The average size of the crystal was higher in the control medium with the length of 1.34 ± 0.15and similarly the average size of the crystal was significantly decreased in medium contains 0.5% of EESE with the average length of 1.08 ± 0.16 cm. The average size of the crystal was even much reduced in medium contains 1 % of test drug EESE with the Avg length of 0.72 ± 0.08 cm.

Conclusion

In vitro models provide valuable evidence based results on exploring antiurolithiatic potential of most of the Indian medicinal plants. From the results of the present investigation it was clearly evident that all three extracts such as EEFH, EEMT and EESE possess promising crystal growth inhibition potential in the tested medium. Further, the mechanism by which these herbs arrest the growth of struvite crystal in the medium may be due to the presence of biologically significant phytocomponents within it, hence there are chances that these novel herbs may act as a potential drug lead for the clinical management of urolithiatic condition. In future the exact molecular mechanism underlying the crystal growth inhibition of these novel herbs has to be properly documented through standard in-vivo and molecular biology techniques.

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