INVITRO STUDY OF INHIBITORY EFFECT OF LEAVES OF*AEGLE MARMELOS* ON STRUVITE CRYSTAL URINARY STONES

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Abstract

Many people suffers urinary stone problems. Urinary stones contain calcium phosphate, calcium oxalate, uric acid and magnesium ammonium phosphate.. Struvite commonly known as Ammonium Magnesium Phosphate Hexahydrate (AMPH) and Magnesium Hydrogen Phosphate.Struvite calculi, found in 15–20% of urinary calculi are mostly connected to urinary tract infections. They are present in women and in persons older than 50 years. Herbals presently available for the treatment of kidney stones are leaves of *Aegle marmelos.*With an increase in the concentration of methanol extract of leaves of *Aegle marmelos.* The formed crystals weight were gradually reduced . The ranges were 2.86 g to 0.43 g in struvite crystals, respectively. This research provides a multidisciplinary approach in characterizing urinary stone struvite crystals grown *in vitro* to help, formulate prevention or dissolution strategies in controlling calcium urinary stone growth. Therefore, the purpose of this research is to investigate the beneficial effects of leaves of *Aegle marmelos* at a different dose and single compound for the prevention of kidney stone formation.In the result of the study it was concluded that the test drug *Aegle marmelos* has shown inhibitory activity on struvite crystals growth.

Key Words: *Aegle marmelos,* Kidney stone, Struvite crystals.

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Introduction:

Kidney stone (called urinary organ calculi) are solid concretions (crystal aggregations) designed within the kidneys from dissolved urinary minerals. Usually, urinary organ stones kind once the excretion becomes targeted to an excellent extent. These ends up in minerals, along side alternative substances to create into crystals, that happens within the inner surface of the kidneys. (Saeed R. Khan *et al*.,2017).

Struvite stones develop once associate infection among the systema urogenitale. acid stones kind once there's an excessive amount of acid within the excretion. Metabolic disorders that cause improper action of the excretion

principally finish within the formation of acid stones. Excessive accumulation of amino acid because of improper dietary factors may end up in Cystinuria, that ultimately winds up within the stone formation. *A. marmelos* are used for hundreds of years in Bharat not just for its dietary functions, however conjointly for its numerous healthful properties. (Sudharaneshwari, K.R 2007).

Hence, it's typically thought of safe and few studies are dole out with relation to its toxicity. withal, binary compound extract of A. marmelos fruit has been reportable to be non agent to S. typhimurium strain tantalum a hundred within the Ames assay.(Kruawan K, Kangsadalampai K 2006). Additionally, acute toxicity studies have reportable that a hydro alcoholic extract of *A. marmelos* fruit is non-toxic up to a dose of six g/kg bodyweight in mice. (Jagetia G, *et al*,. 2004). Medical specialty studies on animal models involving recurrent doses of *A. marmelos* fruit extract over a amount of up to thirty days haven't reportable any adverse result up to a most dose of 250 mg/kg bodyweight. (Jagetia gigacycle per second, *et al*,. 2004).

There have been no exceptional changes noticed in histopathological studies once fifty mg/kg bodyweight of the extracts of *A. marmelos* once administered intraperitoneally for fourteen days in turn. Pathologically, neither gross abnormalities nor histopathological changes were discovered. once calculation of LD (50) values victimization graphical ways, we tend to found a broad therapeutic window and a high therapeutic index worth for *A. marmelos* extracts. Extraperitoneal administration of the extract of the leaves of *A. marmelos* at doses of fifty to ninety and a hundred mg/kg bodyweight for fourteen consecutive days to male and feminine Wistar rats didn't index any short term toxicity. jointly, these information demonstrate that the extracts of the leaves of A. marmelos have a high amount of drug safety. (Veerappan A. *et al*,. 2007).

Hence, it is generally considered safe and few studies have been carried out with respect to its toxicity. Nevertheless aqueous extract of *A. marmelos*fruit has been reported to be non mutagenic toS. typhimuriumstrain TA 100 in the Ames assay.(Kruawan K, Kangsadalampai K 2006). In addition, acute toxicity studies have reported that a hydroalcoholic extract of*A. marmelos*fruit is non toxic up to a dose of 6 g/kg body weight in mice. (Jagetia G, *et al*,. 2004).

Pharmacological studies on animal models involving repeated doses of*A. marmelos*fruit extract over a period of up to 30 days have not reported any adverse effect up to a maximum dose of 250 mg/kg body weight. (Jagetia GC, *et al*,. 2004).

MATERIALS AND METHODS

Analytical grade of anhydrous methanol, ammonium dihydrogen ortho phosphate, magnesium acetate, sodium metasilicate, orthophosphoric acid 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.

Collection of plant material

The leaves of *Aegle marmelos* were collected in the month of December from the Kolli Hills, Trichy, Tamil Nadu, India. The plant sample was identified and confirmed by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli,Tamil Nadu. The voucher specimen number PP001 dated 18.12.2018.

Preparation of methanol extracts

The rhizomes of *Aegle marmelos* were washed in running water, cut into small pieces and then shade dried for a week at 35-40ºC, after that it was grinded to a uniform powder (Doughari *et al*., 2006). The methanol extracts were prepared by soaking 100 g of the dried powder plant materials in 1 L of methanol by using a soxhlet extractor for 10 hr continuously. The extracts were filtered by using whatmann filter paper No. 42 (125mm). The filtered extract was concentrated and dried by using a rotary evaporator under reduced pressure (Table 1).

Phytochemical screening

Phytochemical screenings were performed using standard procedures (Yadav M *et al*., 2014).

Test for terpenoids (Salkowski test)

Equal volume of extract,chloroform and concentrated sulphuric acid were added. Terpenoid was confirmed by the presence of reddish brown coloration in the interface (Mallikarjuna *et al.*, 2014)

Test for flavonoids

A few drops of 10% Lead acetate solution were added to1 ml of extract. A yellow coloration indicates the presence of flavonoidsn (Mallikarjuna *et al.*, 2014).

Test for saponins(Foam Test)

To 5 ml of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after

which it was observed for the formation of an emulsion.

Test for tannins (Braymer's Test)

2 ml of extract was added to 2 ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate. Green precipitate was regarded as positive for the presence of tannins.

Test for alkaloids (Hager's Test)

To 2 ml of extract, few drops of Hager's reagent were added and shaken gently to extract the alkaloidal base. Yellow precipitate was regarded as positive for the presence of alkaloids.

Test for Steroids (Salkowski Test)

To 2 ml of extract, 2 ml of chloroform and few drops of concentrated sulphuric acid were added. Reddish brown ring was regarded as positive for the presence of Steroids (Mallikarjuna *et al.*, 2014)

Test for Glycosides (Liebermann's Test)

To 2 ml of extract, 2 ml of chloroform and 2 ml of acetic acid were added. Violet to blue to green color was regarded as positive for the presence of Glycosides.

Test for Phlobactin(Precipitate Test)

To 2 ml of extract,2 ml of 1% Hydrochloric acid was added and boiled. Red precipitate was regarded as positive for the presence of Phlobactin.

Test for Proteins (Xanthoprotein Test)

To 1 ml of extract, 1 ml of concentrated sulphuric acid was added and boiled. White precipitate was regarded as positive for the presence of proteins.

Test for Coumarins

To 2 ml of extract, 3 ml of 10% Sodium hydroxide was added. Yellow color was regarded as positive for the presence of Coumarins.

Test for Emodin

To 2 ml of extract, 2 ml of ammonium hydroxide and 3 ml of benzene was added. Red color was regarded as positive for the presence of emodin.

Test for Anthraquinone

To 2 ml of extract, 1 ml of benzene and 2 ml of ammonia solution was added. Pink, violet or red coloration was regarded as positive for the presence of anthraquinones.

Test for Anthocyanins

To 2 ml of extract, 2 ml of hydrochloric acid and 1 ml of ammonia solution was added. Pinkish red to bluish violet coloration was regarded as positive for the presence of anthocyanins.

Test for Carbohydrate

To 2 ml of extract, 2 ml of distilled water, 2 drops of ethanolic alpha napthol and 2 ml of concentrated sulphuric acid

was added. Reddish violet ring at the junction was regarded as positive for the presence of carbohydrate

Test for Leucoanthocyanin

To 1ml of extract, 1ml of iso amyl alcohol was added. Observation of Organic layer into red was regarded as positive for the presence of lecoanthocyanin.

Test for Cardiac glycosides

To 1 ml of extract, 2ml of glacial acetic acid, 1ml of Ferric chloride, 1 drop of Con. Sulphuric acid was added. Formation of violet or brown ring was regarded as the presence of cardiac glycosides.

Test for Xanthoproteins

To 1ml of extract, 4 drops of Ferric chloride was added. Blue black coloration was regarded as the presence of xanthoproteins.

Test for Phenols

To 1ml of extract, few drops of Ammonia solution was added. Reddish orange precipitate formation was regarded as the presence of phenols.

QUANTITATIVE ANALYSIS OF LEAVES OF *AEGLE MARMELOS*

Preparation of fat free sample

2gram of the sample were defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 hours.

Determination of total phenols by spectrophotometric method:

The fat free plant sample was boiled with the combination 50ml of ether for the phenolic component extraction for 15 min. 5 ml of the plant extract was taken into a 50ml flask by using pipette, then 10ml of distilled water was added into the flask. 2ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added into the same flask. The samples were kept for 30 min for colour development and measured at 505nm (Mohd Yaseen *et al*., 2017).

Alkaloid determination using Harborne (1973) method:

5 g of the sample was taken into a 250 ml beaker and also 200 ml of 10% acetic acid in ethanol was added into the beaker. The beaker was covered and kept for 4 hours. The sample was filtered and the extract was concentrated and allowed to one-quarter of the original volume on a waterbath. Concentrated ammonium hydroxide was taken drop wise to the extract until the precipitation was complete. The total amount of solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Amin *et a*l., 2013)

Tannin determination by Van-Burden and Robinson (1981) method:

500 mg of the sample was taken into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. The sample was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was taken into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes.

Saponin determination:

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were taken into a conical flask and 100 cm 3 of 20% aqueous ethanol were taken. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. Then the mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml at 90°C in a water bath. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was taken and shaken vigorously.The aqueous layer was recovered while the ether layer was discarded. The process of purification was repeated. 60 ml of n-butanol was taken. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. The samples were dried in the oven to a constant weight after evaporation; the saponin content was calculated as percentage (Chukwuma et al., 2016).

Flavonoid determination by the method of Bohm and KocipaiAbyazan (1994):

10 gram of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature.

The total amount of solution was filtered by using whatman filter paper No 42 (125 mm). The filtrate wastransferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Amin Mir *et al*.,2016).

Terpenoid determination

10 gram of the plant sample was soaked in 50ml of 70% ethanol for 24 hours. Then it was extracted repeatedly with 40 ml of petroleum ether at room temperature. The total amount of solution was filtered by using whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Sanjay R *et al*., 2016)

Growth and characterization of Struvite crystals

Glass test tubes were used as a crystallization apparatus and the single diffusion reaction technique was employed (Chandrabhan seniya et al., 2011). One of the reactants, 0.5 M ammonium dihydrogen phosphate (ADP), was mixed with sodium metasilicate solution the density of 1.04g/cm3 at pH 9.4, so that the the mixture pH was maintained at 6 and kept for 2-3 days. After gelation took place, the supernatant solution of 1 M Magnesium acetate was gentlyadded onto the set gel in various test tubes. After the test tubes were capped by using airtight stopples. The experiments were conducted at room temperature (37C). The grown struvite crystals were characterized by 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 (Pramila kori et al., 2014).

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

An attempt was made to study the effect of the methanol extract of Aegle marmelos leaves 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 recorded. The experiments were repeated 4 times. 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 for the study of the aqueous extract of 5 medicinal plants on the growth of struvite crystals and the average weight of the grown crystal were measured.

Calculation of the percentage of inhibition (1%) was based on the formula:

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

TSI represents the number of crystals without inhibitors and TAI the number of crystals after addition of inhibitors (M Beghalia *et al*., 2007).

ANTIBACTERIAL ACTIVITY:

The purpose of this study was to examine the antibacterial and antifungal activity of the crude methanolic extracts toward selected pathogens using disc diffusion method.

Collection of test organisms:

To examine the antibacterial activity of plant extract, three strains (Escherichia coli (MTCC 25922), Enterococcus aerogenes(MTCC29212),Staphylococcu saureus(MTCC 25923) were prepared as test organisms. All the strains were

obtained from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37ºC and maintained on nutrient agar (Difco, USA) slant at for 4ºC.

SCREENING OF ANTIBACTERIAL ACTIVITIES:

Antibacterial activity of crude aqueous extracts (Disc Diffusion method)

Antibacterial activity of crude aqueous extract was determined using the disc diffusion method.Muller Hinton Agar medium was prepared and inoculated with test organisms on the petri dishes. Sterile disc of six millimeter width were impregnated with 10 µl of crude aqueous extract at various concentrations of 20-100µl/mg respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minutes at room temperature for compound diffusion. Negative control was prepared using the aqueous solvent. The dishes were incubated for 24 hours at 37ºC and the zone of inhibition was recorded in millimeters and the experiment was repeated twice (Simone *et al*., 2007).

Determination of antifungal activity of methanolic extract of leaves of *Aegle marmelos:*

Antifungal activity of crude extracts was determined using the disc diffusion method. Sabouraud's dextrose agar (SDA) medium was prepared on the petridishes and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of crude extract at various concentrations of 20-100 µg/ml respectively. Prepared discs were placed onto the top layer of the agar plates and kept for 30 minutes at room temperature for compound diffusion and incubated for 24 hours at 37ºC. The zone of inhibition was measured in millimetres (Vivek et al., 2013).

Statistical analysis

The masses of the crystals (gm) are presented as the mean ±standard deviation for the control and treatment samples. One-way analysis of variance (ANOVA) followed by tukey's test for multiple comparisons were made between groups. Values of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSIONS

QualitativePhytochemicalAnalysis

The present study carried out on the leaves of *Aegle marmelos* revealed the presence of medicinal active constituents (Figure1). The phytochemical active compounds were qualitatively analyzed for leaves of *Aegle marmelos* of Phytochemical

compounds such as steroids, terpenoids, alkaloids, phlobatanin, flavonoids, saponins, tannins, coumarins, proteins, carbohydrates and glycosides were screened in methanol extracts of leaves of *Aegle marmelos.* Among these compounds alkaloids, phlobatanin, flavonoids, saponins and tannins are important secondary metabolites and are responsible principles for medicinal values of the respective plant.

These twelve compounds were present in methanol extracts of leaves of *Aegle marmelos* (table 2). Alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic properties. The steroids and saponins were responsible for central nervous system activities (Gracelin *et al*., 2013).

QUANTITATIVE ANALYSIS OF COMPOUNDS FROM THE LEAVES OF *AEGLE MARMELOS*

Quantitative test revealed that the presence of important phytochemical constitution in the leaves of *Aegle marmelos* present in different amounts in the medicinal plants. The phytochemical constitution with the highest quantity was terpenoid followed by alkaloid, saponin, tannin, flavonoid, and phenol in that order, as shown in (Table 2). The highest constitution of terpenoids (0.006mg/g), alkaloid (0.023mg/g), saponin (0.012mg/g), tannin (0.053mg/g), flavonoid (0.012mg/g), phenol (0.004mg/g) (Fig.2).

Earlier study was proposed that the ethanol and n-hexane extracts revealed the presence of alkaloids. Flavonoids, glycosides and saponins were present in only the ethanol extract whereas tannins were present in the n-hexane extract (Esther *et al*., 2014).

EFFECT OF LEAVES OF *AEGLE MARMELOS* ON STRUVITE CRYSTALS

The effect of leaves of *Aegle marmelos* 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 hours of adding the supernatant solutions Fig.3 (4a). In the presence of leaves of *Aegle marmelos* nucleation was delayed and reduced masses of the crystals were observed 96 hours after adding the supernatant solutions Fig.3 (4b-g). Morphology of the harvested crystals after addition of leaves of *Aegle marmelos* as shown in Fig.4. The largest single struvite crystals having

dimensions of 2.5 cm as observed in (S.P.Anand *et al.*, 2017)(Fig.5a).

The struvite crystal sizes were reduced from 2.2 cm to 1.7 cm at 1% concentration of extracts was observed in (Fig.6).With an increase in the concentration of leaves of *Aegle marmelos* from 0.15% to 1% (v/v), the weight of the formed crystals was gradually reduced from 2.86 g to 0.43 g at 5% concentration of extracts respectively. The percentage of inhibition of Struvite crystals by medicinal plants are shown in (Table3).

Recently, growth inhibition studies of Struvite crystals in the presence of some of the herbal extracts. (Chauhan *et al*., 2009; Chauhan and Joshi, 2008) were attempted in literature. In the present work, Struvite crystals growth was reduced due to the inhibitory effect of leaves of *Aegle marmelos* under in vitro conditions. This result indicates that distilled water did not show any inhibitory activity with regard to crystal growth, whereas the methanol extract of leaves of *Aegle marmelos* possessed inhibitory activity due to the presence of bioorganic molecules (alkaloids, glycosides, mudarine. Stembark: β-calotropeol, -amyrin, giganteol). In the present work, Struvite crystals growth was reduced due to the inhibitory effect of leaves of *Aegle marmelos* under in vitro conditions.

CHARACTERIZATION OF STRUVITE CRYSTALS

The FTIR spectra of Struvite crystals obtained in the presence and absence of the plant samples are shown in Figure 6.

In Figure 6(a), the band at 2358 cm-1 is due to the antisymmetric and symmetric stretching vibration of NH4 units. The peak at 1633 cm-1 is due to HOH deformation of water and the peak at 1440 cm-1 is due to the HNH deformation modes of NH4 units. The band at 1004 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 PO4 units.

In Figure 6(b), the band at 2358 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 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 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.

1004cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1633cm^{-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 V3 antisymmetric stretching vibration and the peak at 768 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 6(d), the band at 2352 $cm^{-1}$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 568cm^{-1} is due to the V4 bending modes of the PO_4 units.

In Figure 6(e), the band at 2364 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 V3 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 V4 bending modes of the PO_4 units.

In Fig. 6(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 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 V4 bending modes of the PO_4 units. In the presence of 5% orange juice

Figure 6(g), the band at 2362 cm^{-1} is due to the antisymmetric and symmetric stretching vibration of NH_4 units. The peak at 1625 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 1004 cm^{-1} is due to V3 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 V4 bending modes of the PO_4 units.

Figure 6(h), the band at 2374cm^{-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 1004 cm^{-1}1 is due to V3 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 V4 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.

ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *AEGLE MARMELOS* BY DISC DIFFUSION ASSAY METHOD

The leaves of *Aegle marmelos* showed growth inhibitory activity against *Escherichia coli* (20 mm), *Staphylococcus aureus* (19 mm) at concentration of 100µg/ml.The extracts exhibited the antibacterial activity all the five bacteria, but was more susceptible against *Escherichia coli* (18 mm), *Pseudomonas aeruginosa* and *Proteus vulgaris* (17 mm) at concentration of 80 µg/m. However, the extracts indicated better inhibitory actions against pathogens at a concentration of 60, 80 and 100 µg/ml than at lower concentration (fig 2). As the concentration of extracts increased from 20-100 µg/ml, the inhibitory actions of the plant extracts increased towards all the strains used in this study. Previous study suggested that the quercetin inhibited *S. aureus, P. aeruginosa* at concentration 20 mcg/mL while *P. vulgaris* and *E. coli* were inhibited at concentration 300 mcg/mL and 400 mcg/mL respectively (Renu Narendra *et al*., 2017)

ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *AEGLE MARMELOS*

The antifungal susceptibility test of the different concentration of methanolic extract of leaves of *Aegle marmelos* against the test organisms (Table 3). From the result, the leaves of *Aegle marmelos* were the most effective and the highest activity was demonstrated against *Aspergillus flavus* and *Candida albicans* (10 mm zone of inhibition) at 100 µg/ml, followed by the highest activity against *Aspergillus niger*, *Candida vulgaris* and *Candida*

tropicalis (9 mm zone of inhibition) at 100 µg/ml(fig 3). At concentration 80 µg/ml, the extracts exhibited the antifungal activity all the five bacteria, but was more susceptible against *Candida albicans, Candida vulgaris* and *Aspergillus flavus* (9 mm). However, the methanolic extract of leaves of *Aegle marmelos* showed better inhibitory actions against pathogens at a concentration 60, 80 and 100 µg/ml than at lower concentration. As the concentration of extracts increased from 20-100 µg/ml, the inhibitory actions of

the leaves of *Aegle marmelos* increased towards all the strains used in this study.

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

Supernatant Solutions (SS) (Groups and Treatments)	**Compositions**
A (Control)	10 ml of 1 M magnesium acetate
B (Distilled water)	5 ml of 1 M magnesium acetate +5 ml of distilled water
C (0.15% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 0.15% of methanol extract of leaves of *Aegle marmelos* separately
D (0.25% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 0.25% of methanol extract of leaves of *Aegle marmelos* separately
E (0.50% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 0.50% of methanol extract of leaves of *Aegle marmelos* separately
F (0.75% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 0.75% of methanol extract of leaves of *Aegle marmelos* separately
G (1.00% methanol extract)	5 ml of 1 M magnesium acetate +5 ml of 1.00% of methanol extract of leaves of *Aegle marmelos* separately

Table1.Qualitativephytochemicalanalysisofmethanolextractofleavesof*Aegle marmelos*

S. No	**Phytochemical constituents**	**Methanol extracts of leaves of *Aegle marmelos***

1	Tannin	+++
2	Flavonoid	++
3	Terpenoid	++
4	Saponin	+++
5	Phlobatanin	_
6	Steroid	+++
7	Carbohydrate	+
8	Glycoside	+++
9	Coumarin	++
10	Alkaloid	++
11	Protein	_
12	Emodin	+
13	Anthoquinone	_
14	Anthocyanine	+
15	Cardiac glycosides	+++
16	Leucoanthocyanin	++
17	Phenol	+++
18	Xanthoprotein	_

Table 2: Quantitative analysis of compound from the leaves of *Aegle marmelos*

S.NO	**Phytochemical Constitution**	***Aegle marmelos*(LEAVES) (mg/g)**
1.	FLAVONOID	0.012
2.	TANNIN	0.053
3.	SAPONIN	0.012
4.	ALKALOID	0.023
5.	PHENOL	0.004
6.	TERPENOID	0.006

Table- 3: ANOVA statistical analysis for harvested leaves of *Aegle marmelos* struvite crystals

Crystals	Group	Treatents	HarvestedCrystals (Gram)	Percentage of Inhibition
	A	Control	0.744	0%
	B	Control+Distilled water	0.280	62.36%
	C	Control + Methanol	0.271	63.57%
	D	Control+1% extracts	0.260	65.05%
struvite	E	Control+2% extracts	0.167	77.55%
	F	Control+3% extracts	0.140	81.18%
	G	Control+4% extracts	0.132	82.25%
	H	Control+5% extracts	0.023	96.90%

Table 4: Antibacterial activity of methanolic extract of leaves of *Aegle marmelos*

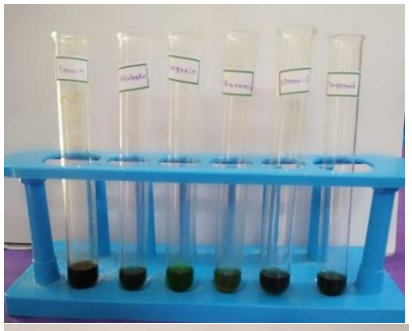
Plant extracts	**Concentrations (µg/ml)**	**Organisms/Zone of inhibition (mm)**	
		methanolic extract of leaves of *Aegle marmelos*	
		Escherichia Coli	***Staphylococcus aureus***
Extracts	20	0	2
	40	0	3
	60	2	4
	80	3	5
	100	4	6
Methanol	10 µl/disc	0	0

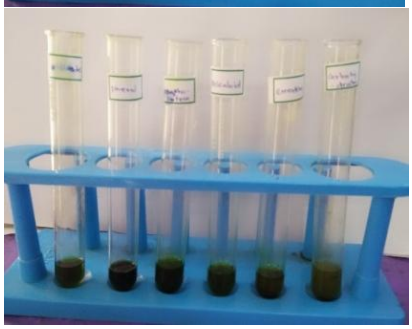
Table 5 Antifungal activity of methanolic extract of leavesof *Aegle marmelos*

Plant ext Racts	**Concentration (µg/ml)**	**Organisms/Zone of inhibition (mm)**
		Methanolic extract of leaves of *Aegle marmelos*

		Candida albicans	***Candida tropicalis***
Extracts	20 40 60 80 100	2 3 4 5 6	0 0 0 0 2
Methanol	10 µl/disc	0	0

Figure 1: Qualitative analysis of leaves of *Aegle marmelos.*





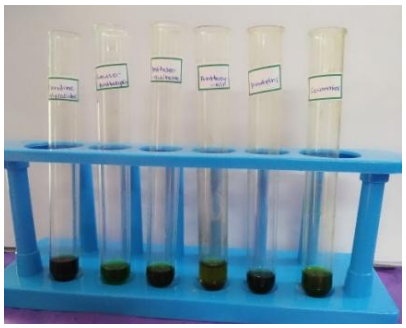


Figure 2: quantitative analysis of compound from leaves of *Aegle marmelos*

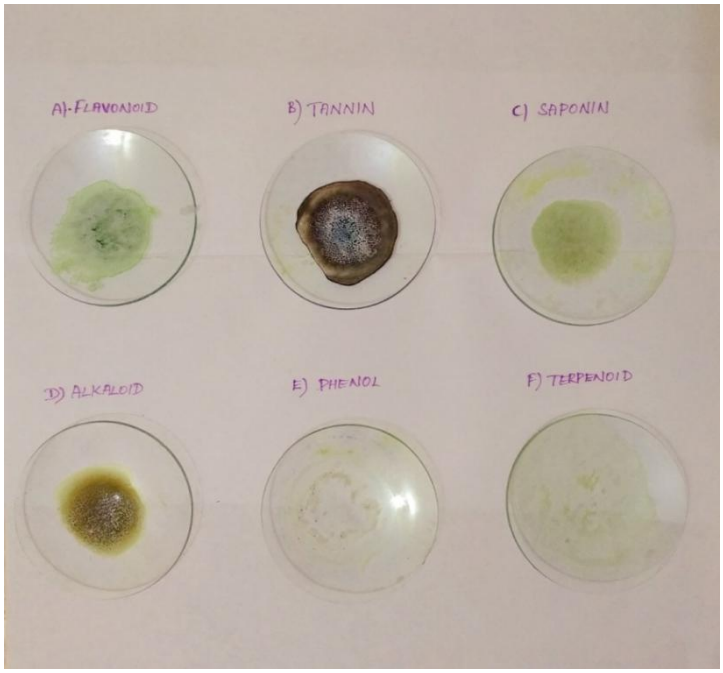


Figure 3: The effect of leaves of *Aegle marmelos* on struvite crystals in the gel method (a) without any additive (b) with the distilled water (c) with the methanol (d) with the 1%extract (e) with the 2% extract (f) with the 3% extract (g) with the 4% extract (h) wit the 5% extract after 7 day

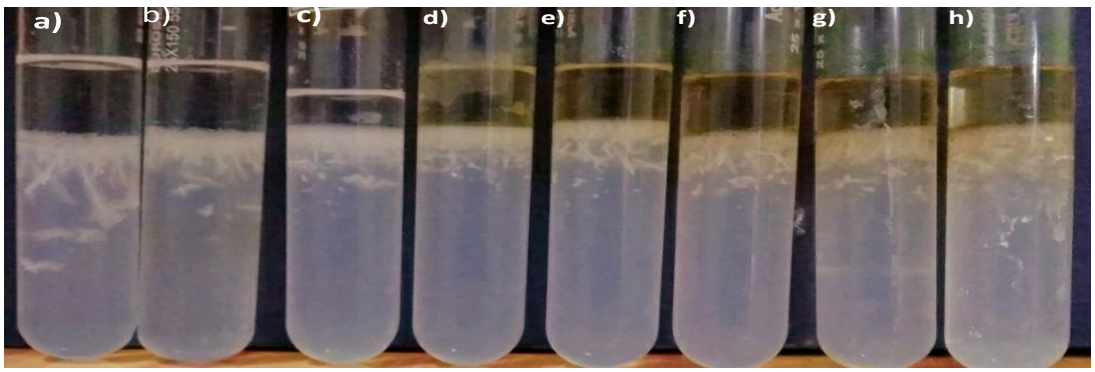


Figure 4: The harvested crystals of struvite obtained from leaves of *Aegle marmelos* in the gel method (a) without any additive (b) with the distilled water (c) with the methanol (d) with the 1% extract (e) with the 2% extract (f) with the 3% extract (g) with the 4% extract (h) with the 5% extract after 7 days.

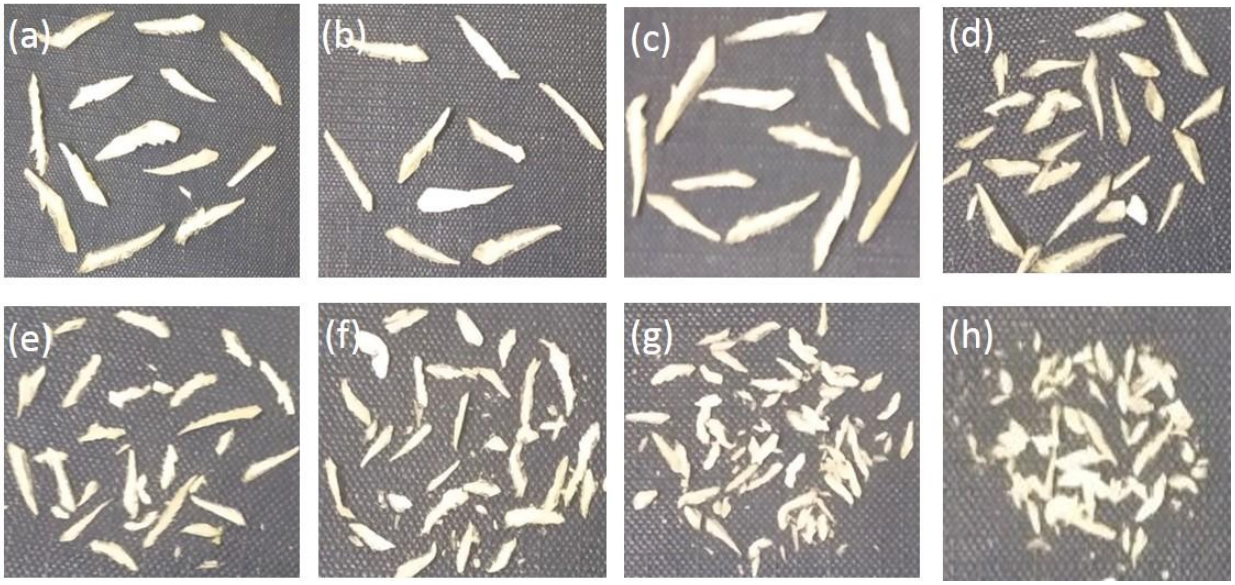


Figure 5: The measurement of struvite crystals obtained from leaves of *Aegle marmelos* in the gel method (a) without any additive (b) with the distilled water (c) with the methanol with the 1% extract (d) with the 2% extract (e) with the 3% extract (f) with the 4% extract (g) with the 5% extract after 7 days.

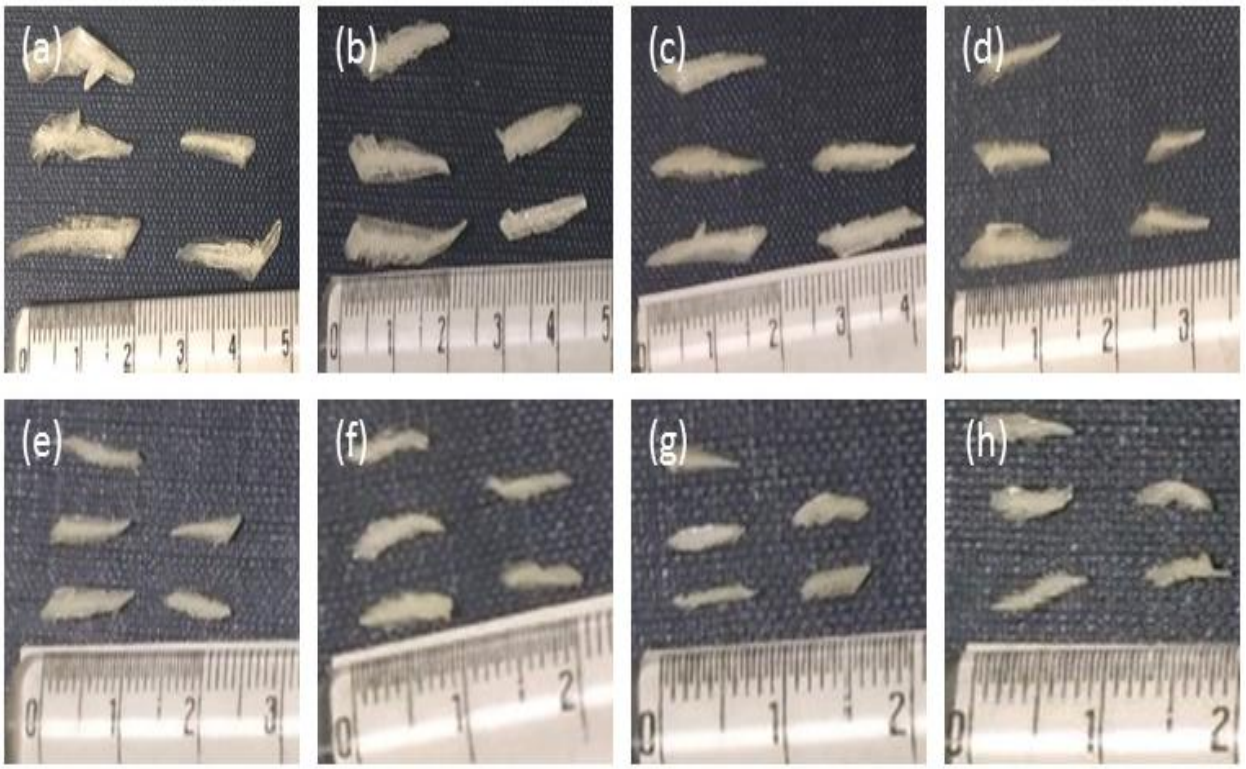


Fig 6:The FTIR spectra of Struvite crystals obtained in the presence and absence of the plant samples

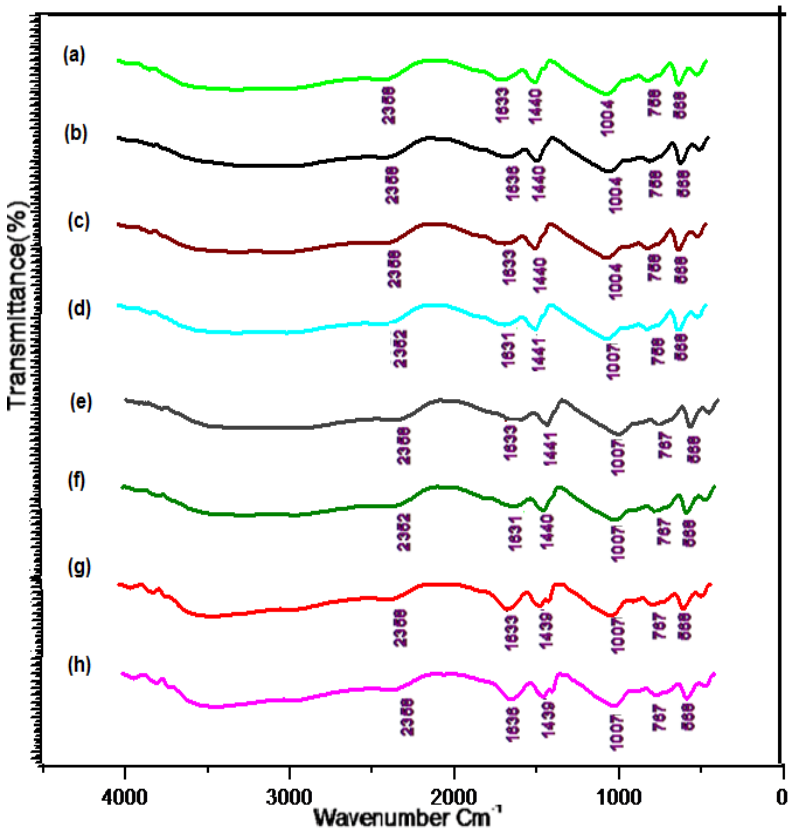
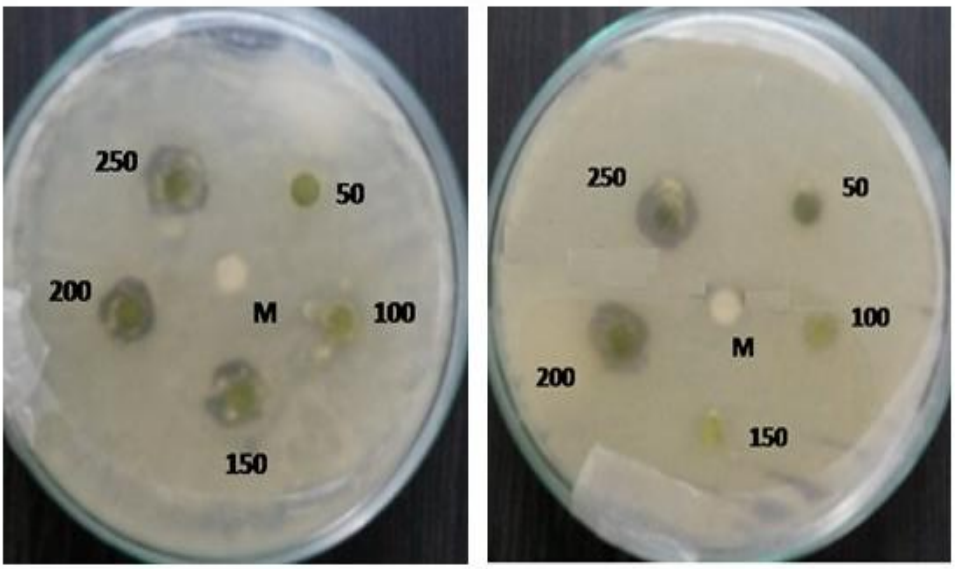


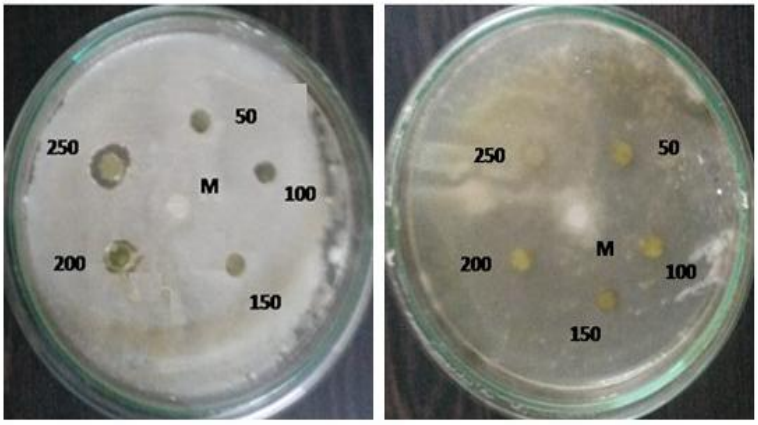
Fig: 7 Antibacterial activity ofmethanolic extract of leaves of *Aegle marmelos*



Activity of *Aegle marmelos* against *Escherchia coli*

Activity of *Aegle marmelos* against *Staphylococcus aureus*

Fig : 8 Antifungal activity of methanolic extract of leavesof *Aegle marmelos*



Activity of *Aegle marmelos* against *Candida albicans*

Activity of *Aegle marmelos* against *Candida tropicalis*

SUMMARY AND CONCLUSIONS

The most effective treatment of kidney stone is extracorporeal shock wave lithotripsy, the side effects of this method are grave and can lead to recurrence of kidney stones. Therefore alternative treatments are of high interest means by using medicinal plants or phytotherapy.

Single diffusion gel growth techniques were used to grown struvite crystals. It was characterized by FTIR techniques for the experimental confirmations of the grown Struvite crystal. With an increase in the concentration of methanol extract of leaves of *Aegal marmelos* the weight of the formed crystals were gradually reduced from 2.43 g to 0.08 g in struvite crystals, respectively. Struvite crystals showed significant differences ($p<0.05$) obtained by One way ANOVA performed with treated and untreated crystal growth data. This study confirmed that the leaves of *Aegle marmelos* extracts can promote the formation of hydroxyapatite crystals and treat urinary stone by inhibiting the formation of struvite crystals, a major component of calcium urinary stone.

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