ANTIUROLITHIATIC AND ANTIMICROBIAL ACTIVITY OF SYNTHESIZED ZINC NANOPARTICLES USING VILVAM MEDICINAL PLANTS ON URINARY TRACT INFECTION CAUSING STRUVITE URINARY STONES

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1. ABSTRACT

Urinary tract infections (UTIs) represent a significant health concern globally, often accompanied by the formation of urinary stones, particularly struvite stones. In this study, we synthesized zinc nanoparticles (ZnNPs) using extracts from Vilvam (Aegle marmelos), a medicinal plant renowned for its antimicrobial properties. We evaluated the potential of these nanoparticles as antiurolithiatic and antimicrobial agents against struvite urinary stones associated with UTIs. The green synthesis of ZnNPs was achieved through the reduction of zinc salts using Vilvam leaf extract. Characterization of the synthesized nanoparticles was conducted using UV-,and Fourier-transform infrared spectroscopy (FTIR). Antiurolithiatic activity was assessed by determining the ability of ZnNPs to inhibit the formation and growth of struvite crystals in simulated urine. Results demonstrated significant inhibition of struvite crystal formation in the presence of ZnNPs compared to control groups. In conclusion, the synthesized ZnNPs using Vilvam extracts show promising antiurolithiatic and antimicrobial activities against struvite urinary stones and UTI-causing pathogens.

Keywords: Urinary tract infections (UTIs), urinary stones, Vilvam (Aegle marmelos), synthesis of ZnNPs , antimicrobial activity, Antiurolithiatic activity , struvite crystals.

Figure 1: Aegle marmelos

2.INTRODUCTION

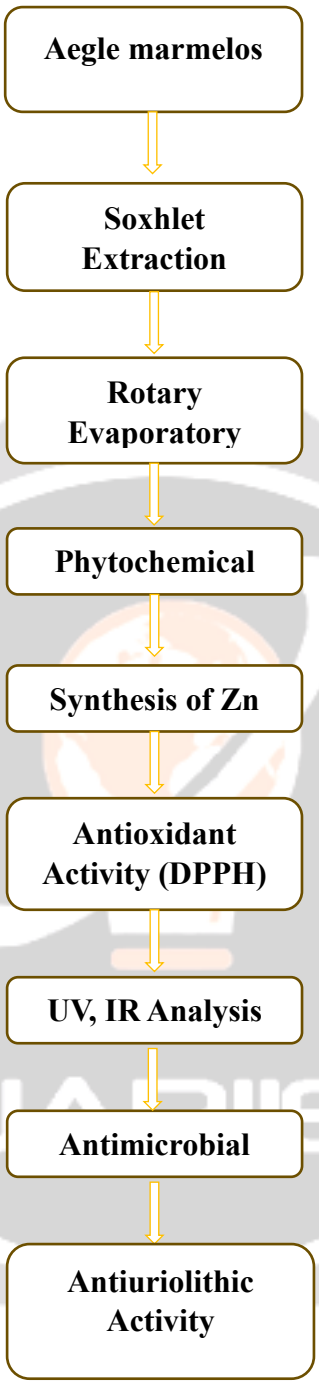
Aegle marmelos leaves, commonly known as bael leaves, are the foliage of the Aegle marmelos tree, native to the Indian subcontinent and Southeast Asia. These leaves have been traditionally revered for their medicinal properties and are widely used in various traditional systems of medicine, including Ayurveda, Siddha, and Unani. Aegle marmelos leaves are rich in bioactive compounds such as flavonoids, alkaloids, tannins, essential oils, and vitamins. They possess antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties, making them valuable in the treatment of various ailments.

In addition to their medicinal uses, Aegle marmelos leaves have also gained attention for their potential in other applications. Researchers are exploring their use in industries such as cosmetics, pharmaceuticals, and materials science. One such application is the extraction of zinc oxide from Aegle marmelos leaves, which offers a sustainable alternative to conventional methods. This process not only yields high-quality zinc oxide but also reduces the environmental impact associated with traditional extraction methods. Their rich phytochemical composition and versatile properties continue to inspire research and innovation in multiple fields.

This study aims to explore the antiurolithiatic and antimicrobial activities of zinc nanoparticles synthesized using Aegle marmelos plants against UTIs associated with struvite urinary stones. The utilization of Aegle marmelos in nanoparticle synthesis offers the advantage of harnessing its bioactive compounds to enhance the therapeutic efficacy of ZnNPs. By investigating the antimicrobial effects of ZnNPs against clinically relevant uropathogens and their ability to inhibit struvite crystal formation, this research seeks to provide insights into the potential of plant-based nanomedicine in UTI management**.**

Through comprehensive characterization and evaluation of the synthesized nanoparticles, including physicochemical characterization, antimicrobial assays, anti-adhesive studies, and cytotoxicity assessments, this study aims to contribute to the growing body of evidence supporting the use of plant- mediated zinc nanoparticles as a novel therapeutic approach for UTIs and struvite urinary stones.

3. FLOW CHART



4. MATERIALS AND METHODS

4.1 Sample preparation – Aegle marmelos

The (***Aegle marmelos***) is collected and shear dried for 3 days. The leaf grind powder weighed 40 grams, added to 400 ml of methanol solution. Then it is extracted using hot percolation methods(Soxhlet) and the sample is condensed using rotary evaporation.

Figure 2: Vilvam sample

4.2 Phytochemical Test

The Phytochemical present in the sample are Alkaloids, Carbohydrates, Proteins and Phenolic compounds.

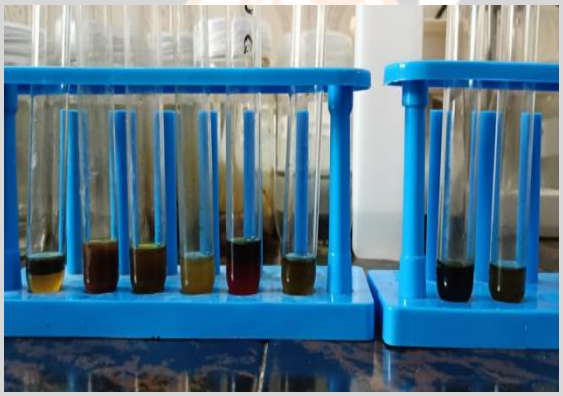


Figure 3: Phytochemical test

4.3 Preparation of Zno

A total of 0.00018 mg of Zno powder was dissolved in 150 ml of H2O and then homogenized.

4.4 Synthesis of Zinc Nanoparticles

1 mm of zinc oxide solution in double distilled water was the source of Zinc. Zinc oxide and vivam extract were mixed together in a ratio of 1:9. The reaction mixture was heated below the boiling point and continuously stirred at 800 rpm using magnetic stirrer. The mixture turned light green in color within 1 h. The obtained suspension of Ag/*T. grandis* was centrifuged at 15,000 rpm for 45 min. The precipitated nanoparticles were lyophilized. Lyophilized nanoparticles were stored in a cool, dry, and dark place and further their characterization was carried out.

4.4 Characterization of Zinc Nanoparticles

The compound obtained from the synthesis of Zinc nanoparticles using water extract of vivam leaf was then characterized on several parameters, namely organoleptic, the yield was observed visually for its color characteristics. The absorbance of the synthesis solution was observed over time to confirm the formation of Zinc nanoparticles.

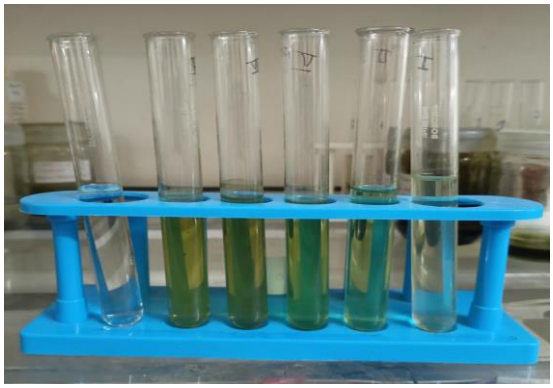


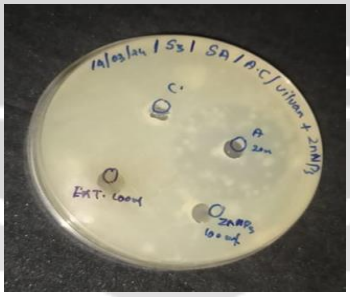
Figure 4: Characterization of Zinc Nanoparticles

4.5 Antioxidant Activity – DPPH Assay

The percentage of antioxidant activity of each substance was assessed by DPPH free radical assay. The samples were reacted with the stable DPPH radical in methanol solution. The reaction mixture consisted of adding 20 µL of sample, 980 µL of H2O and 300 µL of DPPH radical solution. Repeat it with different measurement. The control solution was prepared by mixing 1 ml of H2O and 300 µL of DPPH, . The scavenging activity percentage was determined according to % of inhibition = Control O.D – Sample O.D/Control O.D X 100.

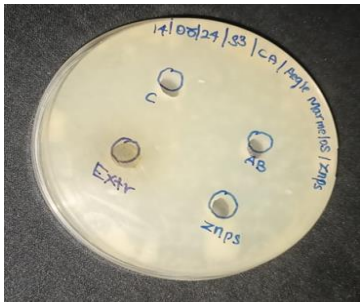
4.6 Antimicrobial Activity

The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C. MHA plates was cultured with standardized microbial culture broth. Each well was filled with varying concentrations from 100, 125, 150 µg/ml of the samples with positive control as streptomycin 25 mcg and negative/solvent control as DMSO, respectively. The plate was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of the tested samples.



(a)

(b)



(c)

Figure: 5

(a) Gram-positive - Streptococcus aureus
(b) Gram-negative - E.coli
(c) Fungus – Candida albicans

4.7 ***Antiuriolitiatic Activity:***

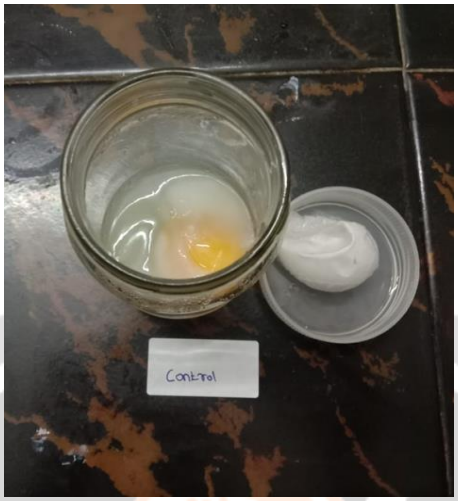
Investigation of in vitro antiurolithiatic activity test by titrimetry The experimental kidney stones of calcium oxalate (CaOx) were prepared in the laboratory by taking an equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2N H2SO4. Both were allowed to react in sufficient quantity of distilled water in a beaker, the resulting precipitate was calcium oxalate. The precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water and dried at 60 °C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed it together in the semi-permeable membrane of the egg as shown in the model designed given below. This was allowed to suspend in a conical flask containing 100 ml of 0.1M Tris buffer. The first group served as blank containing only1 mg of calcium oxalate. The second group served as a positive control containing 1 mg of calcium oxalate and along with the 10 mg standard drugs, The 3rd, 4th groups along with 1 mg of calcium oxalate contain methanolic and aqueous, extracts. The conical flasks of all groups were kept in an incubator preheated to37 °C for 2 h. Remove the contents of semi-permeable membranes from each group into separate test tubes, add 2 ml of 1Nsulphuricacid to each test tube and titrated with 0.9494 N KMnO4 till a light pink colour end point obtained. The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment, in the beginning, to know the total quantity of dissolved calcium oxalate by various solvent extracts.

5. RESULT AND DISCUSSION

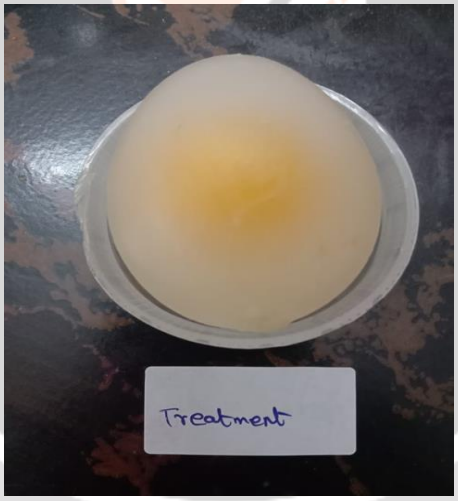
These are among the promising areas of research and development of medicines from the vast highly potential plant resources. Plants are also attractive sources for the development of novel and very effective and safe therapeutic agents against kidney procumbens. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. Unlike allopathic medicines which target is only one aspect of urolithiatic pathophysiology, most of the plant-based therapy has been shown to be effective at different stages of stone pathophysiology.

About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials. Plant-based drug discovery programmes continue to provide an important source of new drug leads. Lithiasis (stone formation) is an important cause for acute and chronic renal failure, includes both nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or both). Among the various kinds of stones identified, calcium stones occur mainly in Men, while phosphate stones formation is more in women. This study evaluates the antiurolithiatic activity of Ethanolic and Aqueous extract of (Aegle marmelos). The highest percentage i.e. 88% of calcium oxalate {CaOx} dissolution was observed in Ethanolic extract followed by an Aqueous extract which had a percentage dissolution of calcium oxalate was 70%. Both Ethanolic extracts of Aegle marmelos were found to be more effective in the dissolution of calcium oxalate than standard drug Neeri.

From this study, it was observed that Aqueous and Ethanolic extracts of Aegle marmelos showed their highest dissolution of calcium oxalate. Ethanolic extract was found to be even more effective than aqueous extract in the dissolution of calcium oxalate. This study has given primary evidence for Aegle marmelos as the plant which possess lithotriptic property. This in vitro study has given lead data and shown that Aqueous and Ethanolic extracts are quite promising for further studies in this regard.



(a)



(b)

Figure 6:
(a) Control - 88%
(b) Treatment - 70%

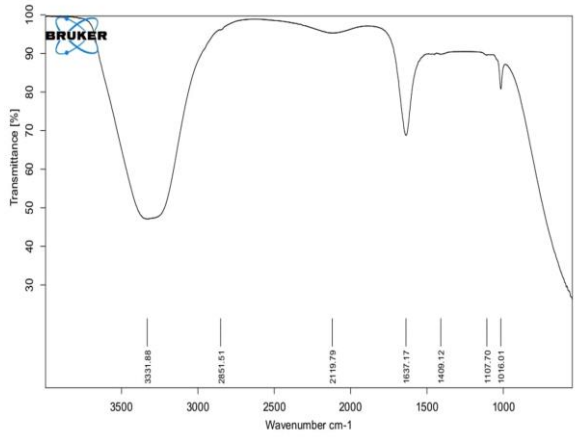


Figure 7: Graph

6. CONCLUSION

The Antiurolithic activity work was performed by using in vitro antiurolithiatic model for calculating percentage dissolution of kidney stone in the study. The study of Antiurolithic activity was carried out for synthesized Zinc nanoparticle used in the Aegle marmelos. This Zno were screened with 150 µg/ml showed the 70% curable.

Therefore, this medicinal plant has the property of curing the stone formation and urinary track infection at higher concentration of the samples.

When its compared with the control the result revealed that *Aegle marmelos* extract as the good yield for anti-urolithic activity.

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