



EVALUATION OF ANTI-UROLITHIASIS ACTIVITY OF MELIA AZEDARACH.L LEAF EXTRACT USING IN-VITRO STRUVITE CRYSTAL GROWTH INHIBITION ASSAY

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ABSTRACT

Medicinal plants are widely used by the traditional medicinal practice to cure different diseases due to their worldwide availability and fewer side effects. *Melia azedarach.L* belongs to the family *Meliaceae* is a highly significant Medicinal plant found almost everywhere. The whole plant or its specific parts (Leaves, stem and roots) are known to have Medicinal properties and have a long history of use by indigenous and tribal people in India. Experimental and clinical studies prove that it has antioxidants, antimicrobial, anti-inflammatory, cardio protective, analgesics, anticancer, anti-ulcer, antipyretic and male contraceptive properties. Urolithiasis is one among the three prevalent disorders in the urinary system. The present study was aimed to grow struvite crystals in vitro using single diffusion gel growth technique and to understand the effects of *Melia azedarach.L* on its growth test drug was prepared at two different concentrations of 0.5 and 1% dispersed in 1.0 Magnesium acetate solutions were gently poured on the set gel in the test tube to enumerate the growth inhibition of struvite crystals. In the result of the study it was concluded that the test drug *Melia azedarach* has shown inhibitory activity on struvite crystals growth.

KEY WORDS: *Antiuro lithiatic, Melia azedarach, Siddha Medicine, Dieuritics*

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1. Introduction

A large number of people (upto 20% of the population worldwide) are suffering from urinary stones problem [1]. Urolithiasis (urinary calculi) is one among the three prevalent disorders in the urinary system. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate, followed by cystine, Struvite and Ammonium acid urate stones. It is a worldwide problem, sparing no geographical, cultural and racial groups [2].

Struvite (Magnesium Ammonium phosphate: $MgNH_4PO_4 \cdot 6H_2O$) is a complex mineral known to assume a number of natural morphological terms including coffin, wedge, short prismatic or short tubular form [3]. Struvite crystallization is related to the urinary tract infections by microorganism producing ureases [4]. They are mainly the microorganisms from species of proteus which are isolated in the cases of 70% of the so called infectious stones [5].

Traditionally, urolithiasis or kidney stones disease can be prevented or treated with herbs (herbal medicines or herbal extract) [6]. *Melia azedarach*. Linn (Meliaceae) properly known as Melia dubia cav, indian lilac and persian lilac is found in most of the tropical and Subtropical countries. [7] chemical constituents of the leaves include nimbinene, quercetin 3-o-b rutinoside, rutin, azaridine, meliacin which possibly contribute to its various uses in traditional medicine [8].

Melia azedarach is a rich source of Antibiotic, Diuretics, Anti-helminthic and Anti-urolithiatic. Each part of *Melia azedarach* has Medicinal properties [9]. But Anti-urolithiatic activity is rich in the leaves of *Melia azedarach* [10] however only a few studies have been conducted on the effects of herbs on the crystal growth of Struvite as one of the main components of renal calculi. Keeping in view of this such the present work of Anti-urolithiasis activity in *Melia azedarach*. L leaf extract using Struvite Crystal Inhibition Assay (in vitro) has been aimed.

TAXONOMY OF MELIA AZEDARACH

KINGDOM: Plantae

DIVISION : Magnoliophyta

CLASS : Magnoliopsida

ORDER : Sapindales

FAMILY : Meliaceae

GENUS : *Melia*

SPECIES : *Melia azedarach*

BOTANICAL NAME: *Melia azedarach*.L. [11,12]

2. Materials and Methods

2.1. Collection of Raw Drug

The leaves of *Melia azedarach* are collected from Tirunelveli District of TamilNadu during the month of April 2019 the plant was identified as *Melia azedarach* in Botany department of Maria Siddha Medical College and Hospital. The Voucher specimen of the plant was deposited as the college for further reference.

2.2. Methodology

An aqueous solution of 0.5M Ammonium dihydrogen phosphate was admixed with the sodium metasilicate solution of specific gravity 1.05 in 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% conc of test drug 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 serves 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 growth of crystal were carried out for five consecutive days. [13,14]

3. Results and Discussion

For the treatment of urolithiasis, there exists a large number of plants products as a prophylactic or curative agent in ethnomedicine but there were very few plants which have been studied extensively. The main drawback in the development of standard drug may be attributed to multi factorial nature of kidney stone and different chemicals composition of renal stones. In vitro crystals system are widely used to study processes of crystal nucleation growth and aggregation [15]

Struvite crystals with different morphologies like dendritic type, prismatic type, rectangular platelet

type, needle type was grown in the gel [16]. The single diffusion gel growth technique was adopted to evaluate anti-urolithiasis potential of study drug MN2. Test drug was prepared at two different concentrations of 0.5 and 1 % dispersed in 1.0 M Magnesium acetate solutions.

Table 1: Average Length of the Crystal in different medium

S.No	Medium	Average Length of the Crystals in cm
1	Control Gel medium	2.02 ± 0.2387
2	Gel medium + 0.5 % MAZ	1.66 ± 0.3647
3	Gel medium +1% MAZ	0.92 ± 0.164

Values represent mean ± SEM of triplicates

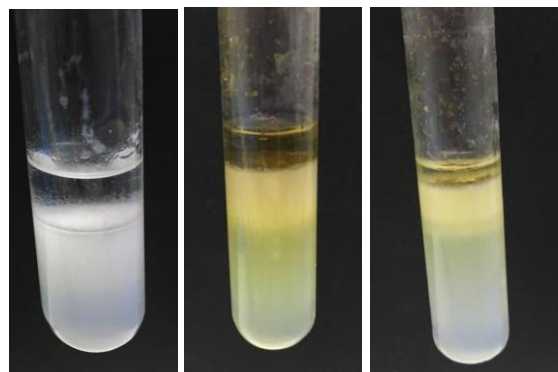


Fig. 1

Fig. 2

Fig. 3

Figure 1 shows growth of Struvite crystals in control gel medium. It was prepared about 5ml of 1.0M Magnesium acetate without test drug were added as supernatant to control tube which serves as crystal controls group. Figure 2 and 3 shows growth of struvite crystals in gel medium with 0.5 of 1% of MN2 which is prepared by 5ml of supernatant solutions of 0.5 and 1 % concentration of test drug in 1.0M Magnesium acetate were gently poured on the set gels in test tubes to enumerate the growth inhibition of Struvite crystals. Then the growth of Crystal was carried out for five consecutive days. After 5 days to observe the size variation of Struvite crystals. It was shown on the figure 4. ‘A’ indicates size variations of Struvite crystals in control gel medium, ‘B’ indicates size variations of Struvite crystals in gel medium with 0.5 % of MN2 and ‘c’ indicates size variations of Struvite crystals in gel medium with 1% of MA2.



Fig. 4 Size variation of Struvite crystals

A - Size variation of Struvite crystals in Control Gel medium , B- Size variation of Struvite crystals in Gel medium with 0.5 % of MAZ, C- Size variation of Struvite crystals in Gel medium with 1 % of MAZ

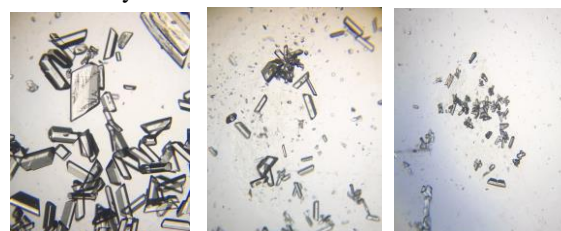


Fig. 5

Fig. 6

Fig. 7

After fragmented Struvite crystals size were observed using under microscope. Figure 5 shown control gel medium Figure 6 and 7 shown gel medium with 0.5 and 1% MA2. Table 1 reported on average length of the crystal in different medium. From the study to observe the average size of the crystal was higher in control medium (2.02cm).The average size of the crystal was significantly decreased in 0.5 % of test drug(1.66cm).The average size of the crystal was much reduced in 1%of test drug (0.92cm).From the results of the study it was concluded that the test drug MA 2 has promising anti-urolithiasis property in the tested medium.

4.Conclusion

Natural component might prove to be potentially beneficial but comparatively less toxic than present day drugs. Melia azedarach is easily available and low cost Medicinal plant. It has rich in Antiurolithiatic activity. More research work can be done on humans so that a drug with multifarious effects will be available in the future market.

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5. References

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