INTRODUCTION
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 urate stones. It is a worldwide problem, sparing no geographical, cultural and racial groups. It is a recurrent disease with a relapse rate of 50% [1]. The techniques for removal of calculi such as endoscopic stone removal lithotripsy and extracorporeal shock wave lithotripsy (ESWL), cause traumatic effect of shock waves, persistent residual stone fragments, acute renal disease, possibility of infection, which leads to decrease in renal function [2]. Therefore, anti-lithiatic drugs from natural sources have assumed greater importance as herbal alternatives which are cost effective with least side effects. The medicinal plants find application in pharmaceuticals, cosmetics, agriculture and food industry. The use of the medicinal herb for curing disease has been documented in history of all civilization. The plant medicine are in great demand in both developing as well as under developed countries for the health care because of their wide biological and medicinal activities, high safety margin and low cost [3, 4].

The market for Ayurvedic medicine is estimated to be expanding at the rate of 20% annually in India. The drugs of herbal origin have been used in traditional system of medicines such as Unani and Ayurveda since ancient time. The ayurvedic system of medicine uses about 700 species, Unani 700 species, Siddha 600 species, Amchi 600 species and modern medicine uses around 30 species.

Banana, an herbaceous flowering plants of the genus Musa. The generic name is derived from the Arabic word ‘mouz’. Musa species are grouped according to their ploidy level, that is, proportion of Musa acuminate (A) and Musa balbisiana (B) in their genome [5, 6]. In the present study, we selected a triploid cultivar Monthan (ABB), most familiar cooking type banana in Southern states of India [Fig. 1]. Ancient folk medicines used banana corm juice/ stem juice for all urinary tract problems including kidney stones. In order to ascertain the nature of bioactive components responsible for anti-lithiatic potential in the banana corm, they were extracted using solvents with different polarity. The study aims at testing the anti-lithiatic properties of banana corm extracts under in vitro conditions.

MATERIALS AND METHOD
Plant sample collection and preparation of extract
The corm of Monthan cultivar was collected from the Orchard of Tamilnadu Agriculture University, Coimbatore. The corms were cleaned and cut into thin slices and shade dried. The dried corms were powdered and passed through the coarse sieve (0.2mm). The powdered samples were used for extraction. An individual extraction of corm samples were carried out using solvents of increasing polarity such as petroleum ether, benzene, chloroform, ethyl acetate, ethanol, and water. The corm extracts were prepared by hot continuous extraction method using Soxhlet apparatus [7]. The extraction was repeated until the corm samples become colourless. The aqueous extract was prepared by the hot water percolation method [7] addition of 2g of the powdered sample in 100ml of distilled water and kept in a water bath at 60°C for 2 hours. Filtered and centrifuged twice and the supernatant was collected. All the extracts were evaporated in a water bath at 60°C. The residue was stored in an airtight container and refrigerated, which was utilized for the in vitro assays.

INHIBITION OF CALCIUM OXALATE CRYSTALLIZATION IN VITRO BY EXTRACT OF BANANA CULTIVAR MONTCHAN

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ABSTRACT
Objective: To study the effect of banana cultivar Monthan corm extract for its anti-lithiatic potential under in vitro condition.

Methods: Banana cultivar Monthan corm extracts of different solvents with varying polarity were tested for its anti-lithiatic potential. Kidney stone formation was studied under in vitro conditions using three critical assays such as crystal nucleation, aggregation and growth. The effect of extract on the formation and inhibition of crystals were observed spectrophotometrically and the results were presented in this paper.

Results: The results of the in vitro assays performed indicate that ethanol extract of Monthan readily prevented crystal nucleation, growth and aggregation.

Conclusion: Monthan corm juice is found to be an effective diuretic, and act as a promoter for inhibitors of crystallization.

Keywords: Lithiasis, Kidney stones, Musa, Banana, Monthan, Calcium oxalate, Nucleation, Crystal growth and aggregation.

EXPERIMENTAL WORK
In vitro assays
Formation of kidney stone involves three critical stages which include nucleation of calcium oxalate crystals, growth, and aggregation [8]. These three stages can be analyzed under in vitro conditions both in presence and absence of the corm extracts. In order to determine the maximum efficacy of the corm extracts, a varying concentration of all the extracts ranging from 50µg to 1600µg were utilized for these assays.

Nucleation assay
The stone formation begins with the occurrence of nuclei, therefore we chose the classical model for the study of oxalate crystallization
NEWLY FORMED CRYSTALS MAY COMBINE TO FORM A SMALL HARD MASS, CALLED CALKUS. THE PERCENTAGE INHIBITION OF CALCIUM OXALATE CRYSTAL GROWTH WAS EVALUATED IN THE PRESENCE OF ABACONIA CORM EXTRACTS BY THE PROCEDURE DESCRIPED BY CHAUDARY ET AL. [10], PAK ET AL. [11] AND FAROOQ ET AL. [12]. 4mM CALCIUM CHLORIDE AND 4mM SODIUM OXALATE WERE PREPARED SEPARATELY AT A FINAL CONCENTRATION OF 3mM/L AND 0.5mM/L RESPECTIVELY IN A BUFFER CONTAINING TRIS 0.5mM/L AND NaCl 0.15mM/L OF pH 6.5. BOTH THE SOLUTIONS WERE FILTERED }
Stone crystals bind to one another through a process known as aggregation or agglomeration promoted by strong chemicals and electrical forces. Adhered crystals were held in place and cannot be easily separated and this plays an important role in lithiasis. The inhibitory potential of the different solvents of increasing polarity as given in Figure 6 and 7 revealed that all the extracts exhibited inhibitory factor to a moderate level. Among them ethanol and methanol extract rendered good prevention against other extracts. The number and morphology of CaOx crystal when observed under light microscope (400X magnification) showed ethanol extract at its higher concentration (1600µg/ml) showed a greater potential towards crystal growth inhibition. Nirmaladevi et al [18] reported that crystal aggregation was inhibited by addition of aqueous extract of *Hibiscus rosa-sinensis*. 

**Fig. 3:** Effect of banana cultivar Monthan corm extracts on nucleation of CaOx. (Light microscopy, 400x)

**Fig. 4:** Effect of banana cultivar Monthan corm extracts on CaOx crystal growth.

**Fig. 5:** Effect of banana cultivar Monthan corm extracts on CaOx crystal growth. (Light microscopy, 400x)
Fig. 6: Effect of banana cultivar Monthan corm extracts on CaOx crystal aggregation.

Fig. 7: Effect of banana cultivar Monthan corm extracts on CaOx crystal aggregation. (Light microscopy, 400x)

It is also necessary to compare the effect of aqueous extract with that of the other extracts, since all the parts of banana plant being used in day to day life for various treatments and remedies. In this study, aqueous extract of corm juice was found dissolve crystal nucleation next to ethanol extract, whereas in crystal growth and aggregation it is third best when compared to ethanol and methanol extracts. Further studies need to be conducted in order to understand the regular intake of corm juice in diet may completely eliminate the urinary calculi formation.

CONCLUSION

The results clearly indicate that under in vitro conditions, the crystal nucleation, growth and aggregation, was found to express a concentration dependent inhibition. The results when compared among the other non-polar solvents, in all the assays, the ethanol corm extract of banana cultivar Monthan, inhibited crystal nucleation, growth and aggregation better. Critical condition of lithiasis can be treated using polar organic solvent such as ethanol which could render maximum protection thereby preventing injury/damage to kidney by the crystals.

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