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Evaluation of *In Vitro* Antiurolithiatic Activity of *Manilkara zapota* Seeds

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ABSTRACT

The present study was undertaken to evaluate the *in vitro* antiurolithiatic activity of the medicinal plant seeds of *Manilkara zapota.* Methanolic extracts showed their maximum efficiencies in the dissolution of calcium oxalate crystals. Our results have clearly indicated that the methanolic extracts of *M. zapota* of seeds were quite promising for further studies in this regard. In this study Neeri was used as standard drug.

KEYWORDS: *In vitro* antiurolithiatic activity, Methanolic extract, Urolithiasis, *Manilkara zapota,* Neeri

INTRODUCTION

Urolithiasis is commonly referred as stone formation in any part of the urinary tract such as kidneys, ureters, urinary bladder and urethra. It is one of oldest, most frequent and highly recurrent disease and was initially found in the tombs of Egyptian mummies dating back to 4000 BC. Epidemiological studies revealed that urolithiasis is more common in men than in women and is more prevalent between the ages of 20 to 40 in both sexes. Calcium containing uroliths are known as brushite, whewellite, weddellite, whitlockite and carbonate apatite. Struvite and newberyite are magnesium containing whereas ammonium acid urate, mono sodium urate monohydrate, uric acid anhydrous, uric acid mono and dehydrate are commonly existing urate stones. Medicinal plants are considered as a rich source of therapeutic agents due to the belief and observations regarding their traditional use for the prevention of various ailments. Various research findings and data from different part of the globe are contributing and helping the scientific community in evaluating and establishing the pharmacological activities of these plants [1].

Urinary stone formation affects 10-12% of the population in industrialised countries. From epidemiological data, Calcium Oxalate (CaOx) is the most common component of the calculi. The formation of such concretions involves several physicochemical events, e.g. nucleation, growth and aggregation, but the mechanism(s) of these processes remain incompletely understood. It is widely known that a patient who has one kidney stone is more likely to develop another. Therefore, the prevention of recurrence (Often up to 60%) is crucial. Unfortunately, despite considerable progress in medical therapy, there is no satisfactory drug to treat kidney stones. Many patients still undergo surgery to remove the stones; thus in Morocco, as in many countries, most patients (≈70%) use medicinal plants as an alternative therapy for many diseases, including lithiasis [2].

Nowadays stone formation is the oldest and serious painful urologic disease with significant prevalence in the population due to change in lifestyle and dietary factors. Stone formation or lithiasis is characterized by calculi formation. It has two main types such as nephrolithiasis and Urolithiasis. Calculi formation in urinary bladder, ureter or any part of urinary tract rather than kidney is known as Urolithiasis while nephrolithiasis is characterized calculi formation in kidney. Generally, calcification for the formation of bone and teeth takesplace in controlled biological situations. Uncontrolled pathological crystallization occurs when solvent becomes supersaturated formation of precipitates in the body called as kidney stones [3].

There are many theories that explain the pathogenesis of stone formation, for example, the super saturation theory and the inhibitors theory. Super saturation occur when there is an abundance of solute in a solution. Although, Urolithiasis is a multifactorial dis-ease, nutrition,

especially fluid intake, with several underlying disorders of metabolism: that is why diet is an important treatment, especially in the prevention of recurrences. Epidemiological studies reveal that about 80% of all kidney stones are composed of calcium salts (75% calcium oxalate), while about 5% are pure uric acid. Kidney stones have previously been linked to higher rates of high blood pressure, obesity, diabetes and other heart disease risk factors. Researchers have speculated that the Dietary Approach is to Hypertension (DASH) style diet can, also prevent kidney stones. The main components of the DASH diet includes fruit, vegetables, nutsandle gumes, low fat dairy, whole grains, and lower intake of salt, sweetened drinks, red meat and processed meat. The DASH style diet may reduce stone risk by increasing urinary citrate and volume. The small associations between higher DASH score and lower relative super saturation of calcium oxalate and uric acid suggesting that unidentified stone inhibitors in dairy products and/ or plants.

Sapotaceae is a family of some 35-75 ill-defined genera and about 800 species, most are tropical trees. *Manilkara zapota* (L.) Van Royen (Synonyms: *Manilkara zapotilla, Manilkara achras, Mimusopus manilkara, Achras zapota,* and *Achras sapota*), growing abroad, was subjected to chemical study resulted in isolation of flavonoids, tannins (Mainly from unripe fruits), triterpenes, and saponins (Mainly from the seeds). Also, the plant was reported to exhibit antioxidant, antimicrobial, and analgesic activities [4].

MATERIALS AND METHODS

Plant Material

The seeds of *Manilkara zapota* was collected in the month of march 2019 from Venkatapur village, Medak dist. of Telangana, India. The plant was authenticated by M. Mallareddy [M.sc, M.phil in botany] retired lecturer in botany. The seeds were washed with tap water and dried under shade.

Preparation of Plant Extract

The seeds were shade dried and powdered. The crude plant extract was prepared by Soxhlet extraction method. 50 g of powdered plant material was extracted with 500 ml of ethanol. The process of extraction was carried out up to 6 cycles, till the solvent in siphon tube of an extractor became colorless. The two extracts were filtered separately, and evaporated to dryness using rotary evaporator. Further the dried extracts were maintained in a refrigerator at 4°C for further antiurolithiatic activity.

Chemicals Used

Neeri, Sodium oxalate, Tris buffer, calcium chloride, Potassium permanganate ($KMnO_4$), Sulphuric acid (H_2SO_4).

Investigation of *In Vitro* Antiurolithiatic Activity Test by Titrimetry

The experimental kidney stones of CaOx were prepared in the laboratory by taking equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2 N H_2SO_4. 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 semi permeable membrane of egg as shown in the model designed given below. This was allowed to suspend in a conical flask containing 100 ml of 0.1 M Tris buffer. First group served as blank containing only 1 mg of calcium oxalate. The second group served as positive control containing 1 mg of calcium oxalate and along with the 10 mg standard drugs, i.e. Neeri. The 3rd, 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 to 37°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 $KMnO_4$ 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].

RESULTS AND DISCUSSION

Drug therapy has developed in response to population health care [6] needs. There are many crucial areas in medicine such as liver diseases, arthritis, old age related problems, certain viral infections and cancer where the conventional medicine is devoid of satisfactory treatment. 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 [7]. Unlike allopathic medicines which target is only one aspect of urolithiatic pathophysiology, most of plant based therapy have been shown to be effective at different stages of stone pathophysiology [8]. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials [9]. Plant based drug discovery programmes continue to provide an important source of new drug leads [10]. 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 [11].

This study evaluates the antiurolithiatic activity methanolic extract of seeds of *M. zapota*. The percentage i.e. 99% of CaOx dissolution was observed in methanolic extraxct. Of seds of *M. zapota* Methanolic extract of *M. zapota* was found to be more effective in dissolution of calcium oxalate than standard drug Neeri. From this study, it was observed that methanolic extracts of showed their highest dissolution of calcium oxalate. Methanolic extract was found to be effective in dissolution of calcium oxalate. This study has given primary evidence for *M. zapota* 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.

Table 1: Shows % dissolution of Calcium Oxalate (CaOx) by *Manilkara zapota* leaves extracts

	% of dissolution of calcium oxalate	
S. No	**GROUPS**	*Manilkara zapota*
1.	Blank	0
2.	Positive Control	81
3.	Methanolic extract	99

Figure 1: *In vitro* experimental model setup to evaluate antiurolithiatic activity

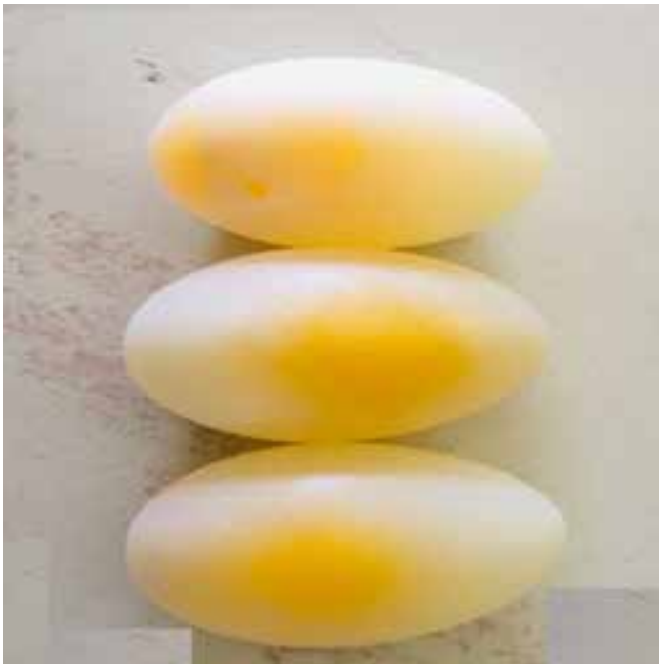

Figure 1(a): Decalcification of egg shell in 10% Acetic acid overnight

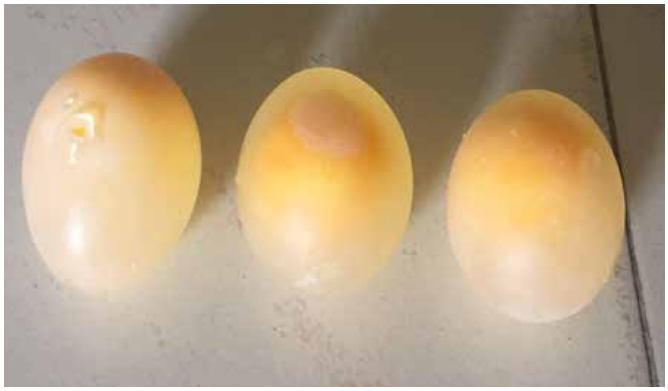

Figure 1(b): Decalcified Eggs

Figure 1(c): Egg membrane along with the contents suspended into the 0.1 M Tris buffer

CONCLUSION

In vitro urolithiasis has been performed on the selected plant *M. zapota* by using the standard drug, Neeri. The work was performed by using in vitro antiurolithiatic model for calculating percentage dissolution of kidney stone. Methanolic seed extracts of *M. zapota* shows highest dissolution than standard drug Neeri. This study has given primary evidence for *M. zapota* as the plant which possess antiurolithiatic property.

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