



EVALUATION OF IN VITRO ANTIUROLITHIATIC ACTIVITY OF SYZYGIUM CUMINI

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ABSTRACT

The present study was undertaken to evaluate the in vitro antiurolithiatic activity of the medicinal plant *Syzygium cumini*. Butanolic extract showed its maximum efficiencies in the dissolution of calcium oxalate crystals. Our results have clearly indicated that the butanolic seed extract of *Syzygium cumini* was quite promising for further studies in this regard. In this study Neeri was used as standard drug.

KEYWORDS: In vitro antiurolithiatic activity, butanolic extract, urolithiasis, *Syzygium cumini*, Neeri.

1. INTRODUCTION

Plants provide food, raw materials for medicine and various other requirements for the very existence of life from the origin of human beings.^[1] Even the current conventional medicine is using a lot of plant derived chemicals as therapeutic agents. The overuse of synthetic drugs results in higher incidence of adverse drug reactions has motivated humans to return to nature for safe remedies. Herbs and herbal drugs have created interest among the people by its clinically proven effects.^[2] Therefore, there is a compelling need for detailed scientific validation of all traditional medicinal plant drugs to establish their efficacy and safety in light of modern science.

Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors.^[3] Urolithiasis is considered as the third most common affliction of the urinary tract. It refers to the solid non-metallic minerals in the urinary tract. It is a complex process that is a consequence of an imbalance between

promoters and inhibitors in the kidney. The formation of kidney stones involves several phytochemical events beginning with crystal nucleation, aggregation and end with retention within the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate stones representing up to 80% of the analyzed stones.^[4] Calcium containing stones may be in the form of pure calcium oxalate(50%) or calcium phosphate(5%) and a mixture of both(45%) followed by magnesium phosphate(15-20%), uric 5 acid(10%) and cystine(1%).^[5]

It is estimated that at least 10% of the population in the industrialized part of the world is afflicted by urinary tract diseases and among these kidney stones are common with an annual incidence of 0.5 -1.9%. About 12% of the population of India is expected to have urinary stones and out of that 50% of cases encounter loss of one or both 2 kidneys with or without renal damage up to some extent.^[2]

Stone disease is 2-3 times more common in males, than in females. It 5 has a reoccurrence rate of 70-81% in males and 47-60% in females.^[5] In spite of substantial progress in pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Kidney dialysis, endoscopic stone removal and extra corporeal shock wave lithotripsy are prohibitively costly and reoccurrence is quite common with these procedures.^[1]

Data from in vitro and in vivo clinical trials revealed that phytotherapeutic agents could be useful as alternative therapy in the management of urolithiasis. Medicinal plants and their products are more useful, because they promote the repair mechanism in natural way.^[1] Pharmacological and phytochemical prospecting of medicinal plants based on traditional knowledge can lead to the discovery of new drug and development of pharmacologically important products for human health care.^[6] Green medicines were safe and more dependable than the costly synthetic drugs, many of which have side effects.^[7]

The selected plant *Syzygium cumini* is native to the Indian Subcontinent, adjoining regions of Southeast Asia, China and Queensland.^[8] The vast number of literatures found in the database revealed that the extracts of different parts of jambolan showed significant pharmacological actions.^[9]

2. MATERIALS AND METHODS

Plant Material

The seeds of *Syzygium cumini* were collected in the month of August 2018 from Sangareddy village, Sangareddy dist. of Telangana, India. 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. 50g of powdered plant material was extracted with 500ml of butanol. The process of extraction was carried out up to 6 cycles, till the solvent in siphon tube of an extractor became colorless. The extract was filtered, 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 (KMnO₄), Sulphuric acid (H₂SO₄).

Investigation of In Vitro Antiurolithiatic Activity Test by Titrimetry

The experimental kidney stones of calcium oxalate (CaOx) were prepared in the laboratory by taking 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 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 0.1M 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 10mg standard drugs, i.e. Neeri. The 3rd group along with 1 mg of calcium oxalate contain butanolic extract. 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 1N sulphuric acid 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.^[10]

3. RESULTS AND DISCUSSION

Drug therapy has developed in response to population health care^[11] 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.^[12] 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.^[13] About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials.^[14] Plant based drug discovery programmes continue to provide an important source of new drug leads.^[15] 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.^[16]

This study evaluates the antiurolithiatic activity of butanolic extract of *Syzygium cumini*. The highest percentage i.e. 96% of calcium oxalate {CaOx} dissolution was observed. From this study, it was observed that butanolic extract of *Syzygium cumini* showed its highest dissolution of calcium oxalate. This study has given primary evidence for *Syzygium cumini* the plant which possess lithotriptic property. This in vitro study has given lead data and shown that butanolic extract was quite promising for further studies in this regard.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *Syzygium cumini* seeds extracts.

S.No	% of dissolution of calcium oxalate	
	GROUPS	<i>Syzygium cumini</i>
1.	Blank	0
2.	Positive Control	81
3.	butanolic extract	96



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



Figure 1(b): Decalcified Eggs.

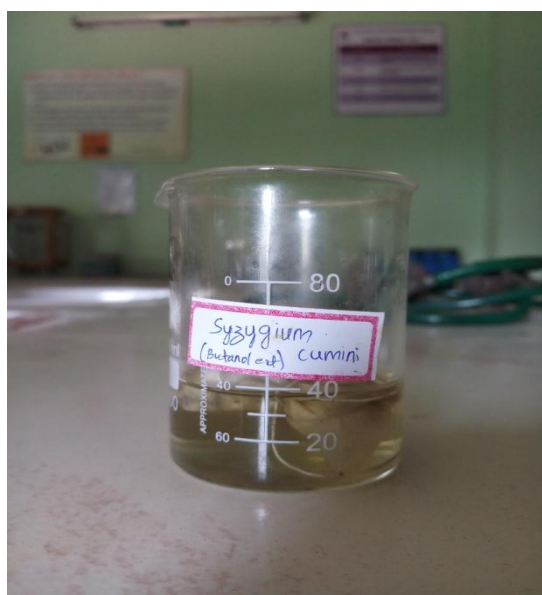


Figure 1(c): Egg membrane along with the contents suspended into the 0.1 M Tris buffer.

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

4. CONCLUSION

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

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