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EVALUATION OF IN VITRO ANTIUROLITHIATIC ACTIVITY OF *LINUM USITATISSIMUM*

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ABSTRACT

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

KEYWORDS: In vitro antiurolithiatic activity, Ethanolic extract, urolithiasis, *Linum usitatissimum*, Neeri.

1. INTRODUCTION

Urolithiasis is the third most common disorder of the urinary tract, is defined as the formation of sediment in the urinary tract consisting of one or more of the poorly soluble crystalloids of urine. It is a worldwide problem particularly common in parts of United States, South Africa, India and South East Asia. Approximately 2% of the world population experiences renal stone disease with a male-female ratio of 2:1 and the peak incidence is observed in 2nd to 3rd decade of life. Renal calculi are characterised clinically by colicky pain (renal colic) as manifest by hematuria. Major risk factors responsible for the nephrolithiasis are inadequate urinary drainage, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities i.e.; deficiency of Vitamin-A, excess of vitamin D, metabolic diseases like hyperparathyroidism, cystinuria, gout, intestinal dysfunction.[1]

Urolithiasis refers to the solid nonmetallic minerals in the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate. Urolithiasis is a complex

process that is a consequence of an imbalance between promoters and inhibitors in the kidneys.[2]

Nephrolithiasis or renal stone disease remains a significant health problem in the adult population, with serious medical consequences, throughout a patient's lifetime. The worldwide incidence of urolithiasis is quite high, and more than 80% of urinary calculi are calcium oxalate stones alone or calcium oxalate mixed with calcium phosphate.[3]

The size and nature of crystals governs overall clinical manifestations of this complaint whereas urinary chemistry is one of the important factors in determining the type of crystals formed and the nature of macromolecules included on the surface of the crystals. Calcium oxalate stones make up the majority as they account for 70-80% of all kidney stones. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are highly costly and with these procedures recurrence is quite common.[4] Various factors such as age, sex, industrialization, socioeconomic status, diet and environment, influences urolithiasis in terms of both site, and the physico-chemical composition of the calculi.[5]

Thus a drug for the prevention of this disease or its recurrence would be of great interest. Medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheap source of drugs for majority of world's population. Several pharmacological investigations on the medicinal plants used in traditional antiurolithic therapy have revealed their therapeutic potential in the in vitro models ~ 19 ~ Journal of Medicinal Plants Studies.[6-7]

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%).[8]

Flax (*Linum usitatissimum*), also known as **common flax** or **linseed**, is a member of the genus *Linum* in the family Linaceae. It is a food and fiber crop cultivated in cooler regions of the world. The textiles made from flax are known in the Western countries as linen, and traditionally used for bed sheets, underclothes, and table linen. The oil is known as linseed oil. In addition to referring to the plant itself, the word "flax" may refer to the unspun fibers of the flax plant. The plant species is known only as a cultivated plant.[9]

Flax is grown for its seeds, which can be ground into a meal or turned into linseed oil, a product used as a nutritional supplement and as an ingredient in many wood finishing products. Flax is also grown as an ornamental plant in gardens. Moreover, flax fibers are used to make linen. The Latin name of the species, *usitatissimum*, means "most useful".[10]

Flax fibers taken from the stem of the plant are two to three times as strong as cotton fibers. Additionally, flax fibers are naturally smooth and straight. Europe and North America both depended on flax for plant-based cloth until the 19th century, when cotton overtook flax as the most common plant for making rag based paper. Flax is grown on the Canadian prairies for linseed oil, which is used as a drying oil in paints and varnishes and in products such as linoleum and printing links.

Linseed meal, the byproduct of producing linseed oil from flax seeds, is used to feed livestock. It is a protein-rich feed for ruminants, rabbits, and fish.[11]

Flaxseeds produce a vegetable oil known as flaxseed oil or linseed oil, which is one of the oldest commercial oils. It is an edible oil obtained by expeller pressing and sometimes followed by sovent extraction. Solvent-processed flaxseed oil has been used for many centuries as a drying oil in painting and varnishing.[12]

2. MATERIALS AND METHODS

Plant Material

The seeds of *Linum usitatissimum* was collected in the month of march 2018 from Narsapur village, Medak dist. of Telangana, India. The plant was authenticated by D.Venkateshwara Rao, Deputy Director, Telangana. Forest Academy, Dullapally, Hyderabad, Rangareddy District. 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 ethanol and water individually. 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 4ºC for further antiurolithiatic activity.

Chemicals Used

Neeri, Sodium oxalate, Tris buffer, calcium chloride, Potassium permanganate (KMnO4), Sulphuric acid (H_2SO_4).

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 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 60^0C. 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 only1 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 ethanolic extract. The conical flasks of all groups were kept in an incubator preheated to37^oC for 2 h. Remove the contents of semi permeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuricacid 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 substracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved calcium oxalate by various solvent extracts.[13]

3. RESULTS AND DISCUSSION

Drug therapy has developed in response to population health care[14] 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.[15] 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.[16] About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials.[17] Plant based drug discovery programmes continue to provide an important source of new drug leads.[18] 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.[19]

This study evaluates the antiurolithiatic activity of Ethanolic seeds extract of *Linum usitatissimum*. The highest percentage i.e. 98.8% of calcium oxalate {CaOx} dissolution was observed in Ethanolic extract. Ethanolic extract of *Linum usitatissimum* was found to be more effective in dissolution of calcium oxalate than standard drug Neeri. From this study, it was observed that Ethanolic seeds extract of *Linum usitatissimum* showed their highest dissolution of calcium oxalate. This study has given primary evidence for *Linum usitatissimum* as the plant which possess lithotriptic property. This in vitro study has given lead data and shown that Ethanolic seeds extract of *Linum usitatissimum* was quite promising for further studies in this regard.

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

	% of dissolution of calcium oxalate	
S.No	**GROUPS**	***Linum usitatissimum***
1.	Blank	0
2.	Positive Control	81
3.	Ethanolic extract	99.6

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

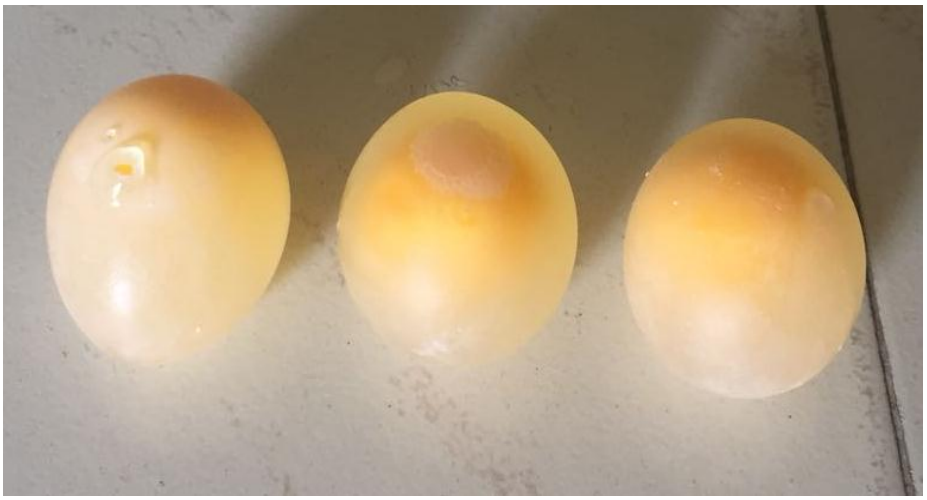

Figure 1(b): Decalcified Eggs.

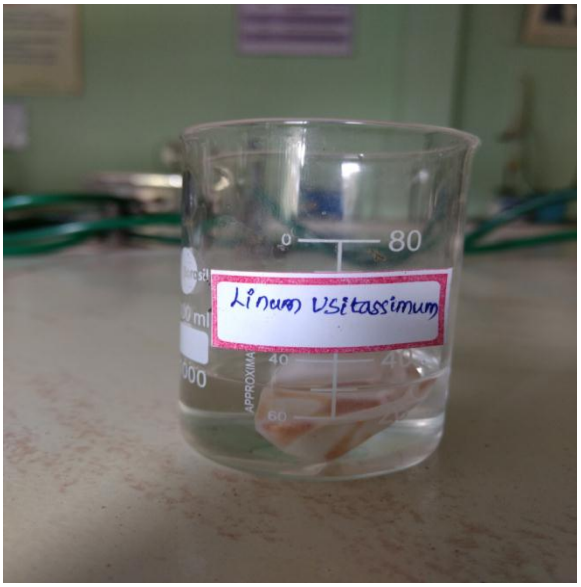

Figure 1(c): Egg membrane along with the contents suspended into the 0.1 M Tris buffer.

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

4. CONCLUSION

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

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