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Evaluation of *In Vitro* Antiurolithiatic Activity of *Laurus nobilis* Leaves

*Chiluveri Sanjuna
Mittapalli Anjali
Mukhera Prasad
Narayanolla Sandhya
Joolakanti Himabindhu
Ramanjaneyulu K

Department of Pharmacognosy, Vishnu Institute of Pharmaceutical Education
and Research, Narsapur, Medak, Telangana, India

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ABSTRACT

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

KEYWORDS: *In vitro* antiurolithiatic activity, Water extract, Urolithiasis, *L. nobilis*, Neeri

INTRODUCTION

Kidney stones are hard, solid particles that form in the urinary tract. In many cases, the stones are very small and can pass out of the body without any problems. However, if a stone (Even a small one) blocks the flow of urine, excruciating pain may result, and prompt medical treatment may be needed. Recurrent stone formation is a common part of the medical care of patients with stone disease. Calcium- containing stones, especially calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate are the most commonly occurring ones to an extent of 75-90% followed by magnesium ammonium phosphate (Struvite) to an extent of 10- 15%, uric acid 3-10% and cystine 0.5-1% [1].

In most of the cases the commonly occurring stones are calcium oxalate or magnesium ammonium phosphate type. Helps in spontaneous passage of calculi by increasing urine volume, pH and anti-calcifying activity. Balance the Inhibitor and promoter of the crystallization in urine and affects the crystal nucleation, aggregation and growth (Crystallization inhibition activity). Relieves the binding mucin of calculi (Lithotriptic activity) Improved renal function [2].

Laurus nobilis (family Laureaceae), commonly known as 'tej patta' is widely distributed in tropical and subtropical regions. *L. nobilis* has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties, which is widely used in folk medicine for the treatment of various diseases [3]. The ash of the leaves is used for strengthening the teeth and gums. Vinegar prepared from the juice of the ripe fruit is an agreeable stomachic and carminative and used as diuretic and it is also useful in spleen enlargement and an efficient astringent in chronic diarrhea. The leaves are rich in acylated flavonol glycosides, quercetin, myricetin, myricitrin, myricetin 3-O-4-acetyl-L-rhamnopyranoside, triterpenoids, esterase, galloyl carboxylase and tannin [4].

MATERIALS AND METHODS

Plant Material

The leaves of *L. nobilis* collected in the month of January 2019 from Venkatapur, Medak dist. of Telangana, India. The plant was authenticated by M. Malla Reddy [M.sc, M. Phil in botany] retired lecturer in botany, Vikarabad, Telangana. The leaves were washed with tap water and dried under shade.

Preparation of Plant Extract

The leaves 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 water. 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 extract was 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 H₂SO₄ 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 10 mg standard drugs, i.e. Neeri. The 3rd, 4th groups along with 1 mg of calcium oxalate contain methanol 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 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 un-dissolved 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 of water extract of *L. nobilis*. The highest percentage i.e. 98% of CaOx dissolution was observed in water extract. Water extract of *L. nobilis* was found to be more effective in dissolution of calcium oxalate than standard drug Neeri. From this study, it was observed water extract of *L. nobilis*

showed dissolution of calcium oxalate. This study has given primary evidence for *L. nobilis* as the plant which possess lithotriptic property. This *in vitro* study has given lead data and shown that water extract are quite promising for further studies in this regard.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *Laurus nobilis* leaves extracts

S. No	% of dissolution of calcium oxalate	
	GROUPS	<i>L. nobilis</i>
1.	Blank	0
2.	Positive Control	81
3.	Water extract	98



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

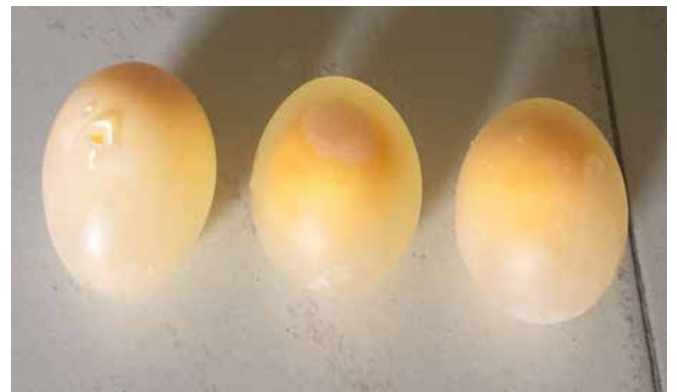


Figure 1(a): Decalcification of egg shell in 10% Acetic acid overnight



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 *L. nobilis* by using the standard drug, Neeri. The work was performed by using *in vitro* antiurolithiatic model for calculating percentage dissolution of kidney stone. Water leaf extract of *L. nobilis* shows highest dissolution than standard drug Neeri. This study has given primary evidence for *L. nobilis* as the plant which possess antiurolithiatic property.

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