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Evaluation of *In Vitro* Anti Urolithiatic Activity of *Lagenaria siceraria*

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ABSTRACT

The present study was explores that evaluation of *in vitro* antiurolithiatic activity of *Lagenaria siceraria* seeds. It was observed that the highest calcium oxalate crystals dissolution was observed in the methanolic extract of *L. siceraria.* It was found that methanolic extract of *L. siceraria* seeds has more efficient to dissolve calciumoxalate. In this study Neeri was used as standard drug.

KEYWORDS: Antiurolithiatic activity, *Lagenaria siceraria*, Calcium oxalate

INTRODUCTION

Urolithiasis defined as the urinary stone originating anywhere in the urinary tract. Medicinal plants are established as renewable sources with antiurolithiatic effects. There are many marketed formulations which are having antiurolithiatic activity, some of them are Cystone, Calcuri and Chandraprabha bati. These formulation have been widely used clinically to dissolve urinary calculi in the kidney and urinary bladder. Apart from these, there are series of other traditional plants available and have been scientifically assessed for their antiurolithiatic activity [1]. Kidney stone is one of the most prevalent diseases worldwide and calcium oxalate has been shown to be the main component of the majority of stones formed in the urinary system of the patients. Traditional knowledge on use of medicinal plants for curing chronic diseases is proving its worth to modern society too [2].

Kidney stone formation or urolithiasis is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation, and retention within the kidneys. Epidemiological data have shown that calcium oxalate is the predominant mineral in a majority of kidney stones. Among the treatments used are Extracorporeal Shock Wave Lithotripsy (ESWL) and drug treatment. Even improved and besides the high cost that imposes, compelling data now suggest that exposure to shock waves in therapeutic doses may cause acute renal injury, decrease in renal function and an increase in stone recurrence. In addition, persistent residual stone fragments and the possibility of infection after ESWL represent a serious problem in the treatment of stones [3]. Urolithiasis is the process of stone formation in the kidney, bladder, and/or urethra [4]. Urolithiasis is one of the important constraints in livestock as well as human health globally since last decades. With its multi-factorial etiology, high rate of reoccurrence and treatment failure, urinary stone diseases provides a medico-vet challenge [5]. Oxalic acid is biosynthesized from ascorbic acid, glycolate and glyoxylate in the metabolism of higher plants. A significant loss of minerals is more prevalent in the body when it is consumed in large content of oxalate rich foods. When calcium ions present in the body bind with free oxalic acid/oxalate it precipitate as insoluble calcium oxalate crystals and may lead to hypocalcaemia and urolithiasis. Generally kidney stones are comprised of high concentration of calcium oxalate with subsequent minute amount of calcium carbonate, calcium phosphate [6].

Renal calculi formation is one of the most common urological disorders. Urinary stone disease is a common disease, which affects 10-12% of the population in industrialized countries. In males, the highest prevalence of the disease occurs between the age of 20 and 40 years, while in females, the highest incidence of the disease occurs later [7].

In spite of substantial progress in the pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Endoscopic stone removal and extracorporeal shock wave lithotripsy are prohibitively costly and recurrence is quite common with

these procedures. 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 [8].

Plants provide food, raw materials for medicine and various other requirements for the very existence of life from the origin of human beings [9]. The majority of the global population utilizes medicinal plants for their health care. 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 [10].

The bottle gourd (*Lagenaria siceraria*) is popularly known as lauki, ghia or dudhi in India. Its consumption is advocated by traditional healers for controlling diabetes mellitus, hypertension, liver diseases, weight loss and other associate [11].

L. siceraria (Molina) Standl. is a vegetable food also used as a traditional medicine. It is reported to have immunomodulatory, hepatoprotective, cardioprotective, antioxidant, anti-stress and adaptogenic, antihyperlipidemic, analgesic, and anti-inflammatory properties. A novel protein, Lagenin (20 kDa), isolated from seeds is reported to have antitumor, antiviral, antiproliferative, and anti-HIV activities. The consumption of bottle gourd can be considered to improve human health [12]. It is used as vermifuge purgative diuretic and it is also recommended for increasing lactation for lactating women. Fruits are also used in treatment of cancer, pain, ulcer, fever, pectoral cough, asthma and other bronchial disorders [13].

MATERIALS AND METHODS

Plant Material

The seeds were collected from market, Narsapur (Mondel), Medak (Dist.) of Telangana in the month of February 2019. The plant was authenticated by M. Malla reddy (M.SC, M.phil in botany) retired lecturer in botany, vikarabad, telangana. The seeds were washed with tap water and dried under shade.

Preparation of Plant Extract

The seeds of plant were dried under shade and crushed in pulveriser and powdered. These powdered plant material was extracted with methanol in a soxhlet apparatus for 72 hours. After complete the extraction, the extracts were cooled at room temperature, filtered and evaporated to dryness using rotary evaporator.

Chemicals Used

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

Investigation of *In Vitro* Antiurolithiatic Activity 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°C. The dissolution percentage of calcium oxalate wasevaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed it together in semipermeable 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 group along with 1 mg of calcium oxalate contains methanolic extracts. The conical flasks of all groups were kept in an incubator preheated to 37°C for 2 h. Remove the contents of semipermeable membranes from each group into separate test tubes, add 2 ml of 1 N sulphuricacid 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 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 [14].

RESULTS AND DISCUSSION

In the present study, Titrimetric method was used to assess the antiurolithiatic activity of methanolic extract of *L. siceraria* seeds. The dissolution percentage, i.e. 99% of CaOx dissolution was observed in methanolic extract. From this study, it was observed that methanolic extract *L. siceraria* seeds showed antiurolithiatic activity. This study has given primary evidence for the plant which possess lithotriptic property. This *in vitro* study has given lead data and shown that methanolic extract of seeds of *L. siceraria* is quite promising for further studies in this regard.

Table 1: Shows % dissolution of Calcium Oxalate (CaOx) by *in vitro* antiurolithiatic activity of *Lagenaria siceraria* seeds extracts

	% of dissolution of calcium oxalate	
S. No	**GROUPS**	*Lagenaria siceraria*
1.	Blank	0
2.	Positive control	51
3.	methanol extract	99

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

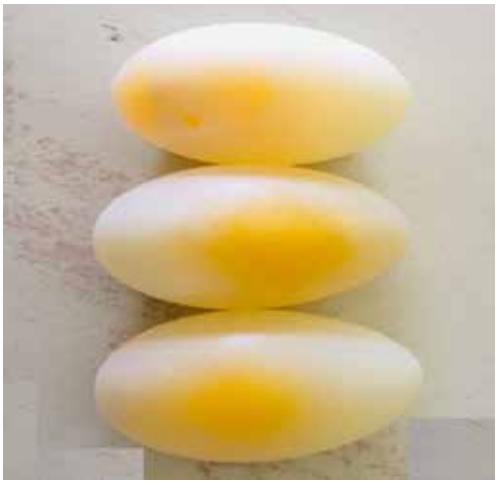

Figure 1(a): Decalcification of egg shell in 10% Acetic acid overnight

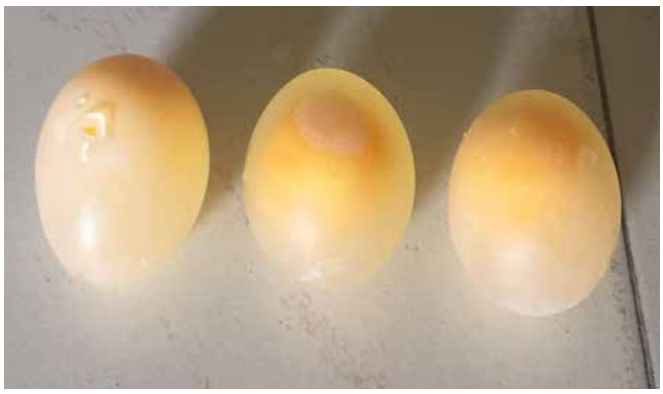

Figure 1(b): Decalcified Eggs

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

CONCLUSION

In the present work, the dissolution of calcium oxalate crystals by methanolic extract of *L siceraria* was studied by using the standard drug, neeri. The work was performed by using *in vitro* antiurolithiatic model for calculating percentage dissolution of kidney stone and methonolic extraxt of *L siceraria* seeds had more activity than stadard drug. This study has given primary evidence for *L siceraria* as the plant which possess antiurolithiatic property.

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