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Evaluation of In Vitro Antiurolithiatic Activity of *Adhatoda vasica* (Vasaka)

			
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ABSTRACT

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



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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 characterized clinically by colicky pain (renal colic) as manifest by hematuria. Major risk factors responsible for the nephrolithiasis are inadequate urinary drainage, microbial infections, the 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 dysfunction¹.

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 kidneys².

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 phosphate³.

The size and nature of crystals govern 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. The 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 common⁴. Various factors such as age, sex, industrialization, socioeconomic status, diet and environment, influences urolithiasis in terms of both site, and the physicochemical composition of the calculi⁵.

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 medicine. Even today, plants provide a cheap source of drugs for the majority of the 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 Studies⁶⁻⁷.

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 acid(10%) and cystine(1%)⁸.

Adhatoda vasica Nees (family Acanthaceae), commonly known as Vasaka or Arusha. The Vasaka plant perennial, evergreen and highly branched with an unpleasant smell and bitter taste, the plant lives for multiple seasons and retains its leaves throughout the year. It is a shrub 1.0 m to 2.5 m in height, with opposite ascending branches. The drug contains stem, leaf, flower, fruit, and seeds^{9,10}.

Adhatoda vasica is the source of vitamin C and has medicinal uses, mainly antispasmodic, fever reducer, anti-inflammatory, anti-bleeding, bronchodilator, antidiabetic, disinfectant, anti-jaundice, oxytocic, and expectorant¹¹. Most of these attributes fall mainly into respiratory therapy category for cold, asthma, bronchitis and tuberculosis. Antioxidant activity of plants might be due to their phenolic compounds¹².

2. MATERIALS AND METHODS:

PLANT MATERIAL

The leaves of *Adhatoda vasica* 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 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. 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 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 the equimolar solution of calcium chloride to 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 the semi-permeable membrane of the 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. The first group served as blank containing only 1 mg of calcium oxalate. The second group served as a 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 methanolic 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 color 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 extracts¹³.

3. RESULTS AND DISCUSSION:

Drug therapy has developed in response to population health care¹⁴ needs. There are many crucial areas in medicine such as liver diseases, arthritis, old age related problems, certain viral infections and cancer where 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 effects¹⁵. Unlike allopathic medicines which target is only one aspect of urolithiatic pathophysiology, most therapy has been shown to be effective at different stages of stone pathophysiology¹⁶. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials¹⁷. Plant-based drug discovery programmes continue to provide an important source of new drug leads¹⁸. 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 women¹⁹.

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

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *Adhatoda vasica* leaves extracts.

% of dissolution of calcium oxalate		
S. No.	GROUPS	<i>Adhatoda vasica</i>
1.	Blank	0
2.	Positive Control	81
3.	Methanolic extract	98.8

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



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



Figure 1(b): Decalcified Eggs

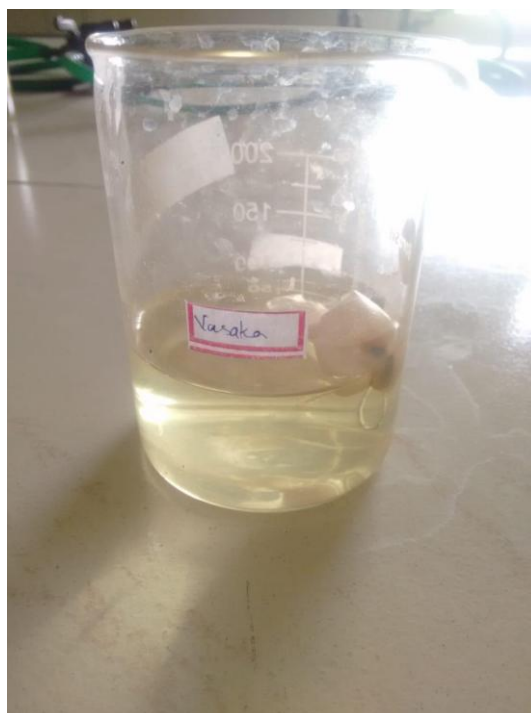


Figure 1(c): Egg membrane along with the contents suspended into the 0.1 M Tris buffer.

4. CONCLUSION:

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

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