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Evaluation of *in vitro* anti urolithiatic activity of *Sesbania aculeata*

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Abstract

Aim: The Aim of present study was evaluation of *In vitro* anti urolithiatic activity of *Sesbania aculeata*.

Method: In the present study, Titrimetry method was used to assess the anti urolithiatic activity of Ethanolic extract of *Sesbania aculeata*. In this study Neeri was used as standard drug.

Result & Discussion: It was observed that the calcium oxalate crystals dissolution was observed in the ethanolic extract of *Sesbania aculeata*. It was found that ethanolic extract of *Sesbania aculeata* has more efficient to dissolve calcium oxalate.

Conclusion: It can be concluded that the ethanolic leaf extract of *Sesbania aculeata* has shown significant anti urolithiatic activity when compared to Neeri.

Keywords: Anti urolithiatic activity, ethanolic extract, *Sesbania aculeata*, calcium oxalate, Neeri

Introduction

Urolithiasis refers to the solid nonmetallic minerals in the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate. The formation of these stones involves several physicochemical events, beginning with crystal nucleation, aggregation, and ending with retention within the urinary tract^[1].

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 calculi) 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^[2] and environmental factors like hot and dry climatic conditions^[3]

Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factor^[4] Urinary calculi are the third prevalent disorder in the urinary system. It is calculated that nearly 10% of the population of the industrialized world is affected by urinary tract stone disease. Kidney stones account for 0.5 to 1.9 % of clinical cases in industrialized countries^[5].

Urine analysis 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 stone is one of the major types which occupy about 75% of the total population^[6].

Sesbania aculeata is a species of flowering plants in the pea family, Fabaceae. Fabaceae or Leguminosae is an important and third largest family of flowering plants, which is commonly known as the legume family, pea family, bean family or pulse family. The name 'Fabaceae' comes from the defunct genus Fabaceae, Fifty species of *Sesbania* have been described in tropical and subtropical regions of the world *Sesbania aculeata*, is the species most commonly found in India. Locally, it is known by the name Dhaincha, Danchi and Dunchi. It is an erect, low annual sub shrub and reaches up to height of one to two meters. It has fibrous, pithy stems with long leaves. The leaves are pinnate, 1.2–2.5 cm long, 0.3 cm wide and are glabrous. It bears purple-spotted yellow flowers from September to November in Indian climatic conditions It produces pods which contain light brown beans^[7].

Oven-dry fiber of *Sesbania aculeata* is reported to contain 0.71% ash, 0.94% fats and waxes, 2.3% nitrogenous matter, 9.76% pentosan, 16.3% lignin and 85.2% holocellulose

(63.6% alpha cellulose) [8]. seeds of the genus *Sesbania aculeata* are reported to contain trypsin inhibitor and chymotrypsin inhibitors. The leaves of *Sesbania aculeata* yields good concentration of (+) - pinitol which is an anti-diabetic agent. [9] It also has several medicinal uses and used in treatment of various eye, skin and inflammations [10]

Sesbania aculeata is used for soil erosion control, hedges, intercropping "mother plants," nitrogen fixation, and windbreaks, for fodder and fuel wood. It is used for manufacturing of paper, particle boards, pipes, ropes and as sizing and thickening agent. Lately, the *Sesbania aculeata* plant is used as biomass and supplies 128 kW of electricity at 240 V. in Bihar [11].

Materials and methods

Plant materials

The leaves of *Sesbania aculeata* were collected from marvelly (Vil), vatpally (Mdl), sangareddy (Dist) of Telangana in the month of August 2017. 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 of plant were dried under shade and crushed in pulveriser and powdered. These powdered plant material was extracted with ethanol in a soxhlet apparatus for 72 hours. After complete the extraction, the extracts were cooled at room temperature and filtered and evaporated to dryness using rotary evaporator.

Chemicals used: Neeri, Sodium oxalate, Tris buffer, Calcium chloride, Potassium permanganate (KMnO₄), Sulphuric acid (H₂SO₄).

Investigation of *in vitro* anti urolithiatic 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 groups along with 1 mg of calcium oxalate contain aqueous, extract. The conical flasks of all groups were kept in an incubator pre heated to 37°C for 2 hrs. 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 [12].



Fig 1(a): Decalcification of egg shell in 10%



Fig 1(b): Decalcified Eggs Acetic acid overnight.



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

Fig 1: *In vitro* experimental model setup to evaluate anti urolithiatic activity

Results and discussion

In the present study, Titrimetry method was used to assess the anti urolithiatic activity of ethanolic extract of *Sesbania aculeata* the dissolution percentage, i.e. 56.9% of calcium oxalate (CaOx) dissolution was observed in ethanolic extract. From this study, it was observed that ethanolic extract of *Sesbania aculeata* leaves showed anti urolithiatic activity. This study has given primary evidence for *Sesbania aculeata* the plant which possesses lithotriptic property. This *In vitro* study has given lead data and shown that ethanolic extract of *Sesbania aculeata* is quite promising for further studies in this regard.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *In vitro* anti urolithiatic activity of Ipomoea aquatic leaves extracts % of dissolution of calcium oxalate

S. No	Groups	<i>Sesbania aculeata</i>
1.	Blank	0
2.	Positive control	81
3.	Ethanol extract	56.9

Conclusion

In vitro anti urolithiatic model was performed for calculating percentage dissolution of kidney stone. The dissolution of calcium oxalate crystals by ethanolic extract of *Sesbania aculeata* was studied by using the standard drug, Neeri. It can be concluded that the ethanolic leaf extract of *Sesbania aculeata* has shown significant anti urolithiatic activity when compared to Neeri.

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