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Research Article

EVALUATION OF *IN VITRO* ANTI UROLITHIATIC ACTIVITY OF *IPOMEA AQUATICA*

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ABSTRACT

Aim: The Aim of present study was evaluation of *in vitro* anti urolithiatic activity of *Ipomoea aquatica*. Method: In the present study, Titrymetry method was used to assess the anti urolithiatic activity of Ethanolic extract of *Ipomoea aquatica*. 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 *Ipomoea aquatica*. It was found that ethanolic extract of *Ipomoea aquatica* has more efficient to dissolve calcium oxalate. Conclusion: It can be concluded that the ethanolic leaf extract of *Ipomoea aquatica* has shown significant anti urolithiatic activity when compared to Neeri.

KEYWORDS: antiurolithiatic activity, ethanolic extract, *Ipomoea aquatica,* calcium oxalate, Neeri.

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, 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]and environmental factors like hot and dry climatic conditions[2].

Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factor[3] Urinary calculi are the third prevalent disorder in the urinary system. It is calculated that nearly10% 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[4].

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[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].

Ipomoea aquatica is a semiaquatic, tropical plant grown as a vegetable for its tender shoots and leaves. It is found throughout the tropical and subtropical regions of the world, although it is not known where it originated. This plant is known in English as water spinach, river spinach, water morning glory, water convolvulus, or by the more ambiguous names Chinese spinach, Chinese Watercress, Chinese convolvulus, swamp cabbage or kangkong in Southeast Asia[7].

Ipomoea aquatica grows in water or on moist soil. Its stems are 2–3 metres (7–10 ft) or longer, rooting at the nodes, and they are hollow and can float. The leaves vary from typically sagittate (arrow head-shaped) to lanceolate, 5–15 cm (2–6 in) long and 2–8 cm (0.8–3 in) broad. The flowers are trumpet-shaped, 3–5 cm (1–2 in) in diameter, and usually white in colour with a mauve centre. Propagation is either by planting cuttings of the stem shoots that will root along nodes or planting the seeds from flowers that produce seed pods[8,9].

Water spinach, Ipomoea aquatic Forssk. (Convolvulaceae), is an aquatic or semi-aquatic Edible herb[10]. I.aquatica is used traditionally against various disorders like diabetes, liver malfunction, constipation and in the treatment of arsenic and heavy metal poisoning[10,11] .Literature reviews revealed the occurrence of significant amounts of phenolic compounds, flavonoids, saponins, β-carotene and ascorbic acid in I.aquatica[10].Genus Ipomoea (Convolvulaceae) are used in traditional system of medicine all over the world and species Ipomoea aquatic Forsk widely used as ailment in the treatment of liverdiseases[12] and constipation[13] *Ipomoea aquatica* contains several phytoconstituents such as vitamins, including A, B, C, E, and "U" (S-methyl methionine) and is used to treat gastric and intestinal Disorders[14].

MATERIALS AND METHODS

PLANT MATERIALS

The leaves of *Ipomoea aquatica* 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 ($KMnO_4$), 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°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 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 0.1M 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 10mgstandard drugs, i.e. Neeri. The 3rd, 4th groups along with 1 mg of calcium oxalate contain methanolic and aqueous, extracts. The conical flasks of all groups were kept in an incubator preheated to37oC for 2 h. Remove the contents of semipermeable membranes from each group into separate test tubes, add 2 ml of 1Nsulphuricacid to each test tube and titrated with 0.9494 N KMnO4 till a light pink color 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[15].

RESULTS AND DISCUSSION

In the present study, Titrymetry method was used to assess the anti urolithiatic activity of ehanolic extract of *Ipomoea aquatica*. The dissolution percentage, i.e. 64% of calcium oxalate (CaOx) dissolution was observed in ethanolic extract. From this study, it was observed that ethanolic extract of *Ipomoea aquatica* leaves showed anti urolithiatic activity. This study has given primary evidence for Ipomoea aquatic the plant which possesses lithotriptic property. This in vitro study has given lead data and shown that ethanolic extract of Ipomoea aquatic 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 aquatica leaves extracts

	% of dissolution of calcium oxalate	
Sl.No.	**Groups**	***Ipomoea aquatica***
1.	Blank	0
2.	Positive control	81
3.	Ethanol extract	64



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 anti urolithiatic activity

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 *Ipomoea aquatica* was studied by using the standard drug, Neeri.

It can be concluded that the ethanolic leaf extract of *Ipomoea aquatica* has shown significant anti urolithiatic activity when compared to Neeri.

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