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ANTI-UROLITHIATIC ACTIVITY OF *Tribulus terrestris* LEAVES

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Abstract

A kidney stone, also known as a renal calculus is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. Urolithiasis is a complex process that occurs from series of several physicochemical event including super-saturation, nucleation, growth, aggregation and retention within the kidneys. Data from in-vitro, in- vivo and clinical trials reveal that phytotherapeutic agents could be useful as either alternative or an adjunct therapy in the management of Urolithiasis. Medicinal plants / natural products are more useful for body because they promote the repair mechanism in natural way. Various plant species of *Tribulus terrestris*, have been reported to posses antiurolithiatic property**.** In this study ethenol extracts of *Tribulus terrestris Leaves.* Linn and standard for dissolving kidney stones- calcium oxalate by an in-vitro model. To check their potential to dissolve experimentally prepared kidney stones- calcium oxalate by an in-vitro model for *Tribulus terrestris* and cystone as a standard compound collected from market**.** Ethenoic fraction was more effective in dissolving calcium oxalate (48.5±0.022%). Reference standard-formulation Cystone was found to be more effective (53.5±0.02 %).

Keywords: *Tribulus terrestris* Leaves, Polyherbal Formulation, Kidney Stones, Urolithiatic, Calcium Oxalate.

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INTRODUCTION

Kidney acts as a filter for blood, removing waste products from the body and helping to regulate the levels of chemicals which are important for body functions. The urine drains from the kidney into the bladder through a narrow tube called the ureter. When the bladder fills and there is an urge to urinate, the bladder empties through the urethra, a much wider tube than the ureter. In some people, the urine chemicals crystallize and form the beginning, or a nidus of a kidney stone. These stones are very tiny when they form, smaller than a grain of sand but gradually they can grow to a quarter inch or larger. 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]. Herbs and herbal drugs have efficient pharmacological action and potent effects on body. Also, the overuse of synthetic drugs, which results in higher incidence of adverse drug reactions, has motivated humans to return to nature for safe remedies.Tribulus terrestris belongs to the family Zygphyllaceae is a valuable herb known for its application in the folk medicine in various parts of the world. This plant is extremely rich in substances having potential biological significance, including: saponins, flavonoids, alkaloids, and other nutrients. The fruit and root of Tribulus terrestris (Caltrop fruit) contains pharmacologically important metabolites such as phytosteroids, flavonoids, alkaloids and glycosides . Preparations based on the saponin fraction of T. terrestris are used for treatment of infertility and libido disorders in men and women, as well as for treatment of cardiac diseases. Food supplements containing T.terrestris extracts are marketed in USA and Europe with claim of a general stimulating action. The other reported pharmacogolical activities include antioxidant, antihypertensive, antimolluscicidal, aphrodisiac,

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antidiabetic, anthelmintic, anticancer, antifungal, CNS stimulant activity The study have been undertaken to evaluate Tribulus terrestris .Linn leaves extracts and cystone as a standard for their possible potential to dissolve experimental kidney stone using a modified in vitro model to isolate the chemical constituent responsible for the activity [3-10].

MATERIAL & METHOD

Collection of Plant Material

Tribulus terrestris leaf were collected from botinical garden in MAM College Of Pharmacy, Kesanapalli, Narsaraopet, Guntur (Dt), Andhra Pradesh, India and used for this study

Extraction of plant material

Collected leafs were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were shade dried then made into fine powder, this powdered samples (100g/100ml) in ethenol, and distilled water for overnight at room temperature., soxhlet apparatus are used for this extraction. The extract from these solvents are soaked and evaporated under pressure. The leaf extracts were concentrated at 50°C and the residue obtained was stored at 4°C.

Extraction and Isolation

The leaves of about 60 gms of powder was extracted with ethenol and aqueous in soxhlet. All extracts were concentrated on a water bath and residue was dried in a desiccator All the prepared extracts were subjected to qualitative chemical tests to detect the presence of different classes of phyto constituents.

PHYTOCHEMICAL ANALYSIS OF THE EXTRACT

Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods. In brief, the phytochemicals such as tannins, alkaloids, saponins, flavonoids, terpenoids, and phenols/polyphenols were qualitatively determined as following:

Test for alkaloids (mayer's test)

2.0ml of extract was measured in a test tube to which picric acid solution was added. The formation of orange coloration indicated the presence of alkaloids

Test for cardiac glycosides (keller-killani test)

5ml of plant extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. A brown ring of the interface indicates a deoxysugar characteristic of

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cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer which shows the presence of Cardiac glycosides.

Test for tannins

The substance (extracts) mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of Tannins.

Test for saponins

Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H2SO4. Formation of yellow color observed in each extract indicated the presence of flavonoids.

Test for Steroids

One gram of the test substance (plant extracts) was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated sulphuric acid was added along the sides of the test tube. Appearance of green colour indicates the presence of Steroids.

Test for terpenoids (salkowski test)

5ml of each plant part extract was mixed in 2 ml of chloroform, and concentrated H2SO4 (3ml) was carefully added to form a layer. Formation of reddish brown coloration at the interface shows the positive results for presence of terpenoids.

Test for Reducing Sugars

One gram of the aqueous extract was weighed and placed into a test tube. This was diluted using 10 ml of deionised distilled water. This was followed by the addition of Fehling's solution. The mixture warmed to 40ºC in water bath. Development of brick-red precipitate at the bottom of the test tube was indicative of the presence of a reducing sugar. Same procedure was repeated using dimethyl sulphoroxide (DMSO) as the diluent for the ethanolic extract.

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Test for Resins

Two grams of the ethanolic extract was dissolved in 10ml of acetic anhydride. A drop of concentrated sulphuric acid was added. Appearance of purple colour, which rapidly changed to violet, was indicative of the presence of resins. Same procedure was repeated using the aqueous extract of the plant material.

Evaluation for Anti-urolithiatic Activity

Step-1: Preparation of experimental kidney stones (Calcium oxalate stones) by homogenous precipitation:

Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Sodium oxalate (AR) in 10ml of 2N H2SO4 were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10ml of (2N H2SO4), was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium phosphate. Both precipipitates freed from traces of sulphuric acid by Ammonia solution. Washed with distilled water and dried at 60 0C for 4 hours.

Step -2: Preparation of semi-permeable membrane from farm eggs:

The semi - permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. Then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Step-3: Estimation of Calcium oxalate

Weighed exactly 1mg of the calcium oxalate and 10mg of the extract/compound/standard and packed it together in semi evaluation Permeable membrane by suturing as shown in Model design Figure. This was allowed to suspend in a conical flask containing 100ml 0.1 M TRIS buffer. One group served as negative control (contained only 1mg of calcium oxalate). Placed the conical flask of all groups in an incubator, preheated to 37 0C for 2 hours, for about 7-8 hours. Removed the contents of semi-permeable membrane from each group into a test tube. Added 2 ml of 1 N sulphuric acid and titrated with 0.9494 N KMnO4 till a light pink colour end point obtained.1ml of 0.9494 N KMnO4 equivalents to 0.1898mg of 4 Calcium. The amount of undissolved calcium

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oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substance(s) could dissolve.

RESULT

Qualitative chemical tests indicated the presence of phenolic compounds, flavnoids, steroids and Saponin in extracts of *Tribulus terrestris* Linn. On basis of this fraction we performed in vitro Anti-Urolithiatic Activity by comparing different extracts of *Tribulus terrestris* with standard. % Dissolution of Calcium oxalate table is given below

Table 1: % Dissolution of Calcium oxalate

S.No.	GROUP	% Dissolution of Calcium oxalate
1	Blank	0
2	Ethanol extract	49± 0.024
3	Aqueous extract	58.5±0.022
4	Standard(cystone)	73.5±0.038

Table 2: Phytochemical Screening

Phytochemicals	Ethanol extract	Aqueous extract
Alkaloids	+	+
Cardiac Glycosides	+	+
Saponins	+	+
Tannins	+	+
Flavnoids	+	+
Steroids	+	+
Terpenoids	-	-
Reducing Sugars	-	-
Resins	-	-

(+) = Positive (-) = Negative

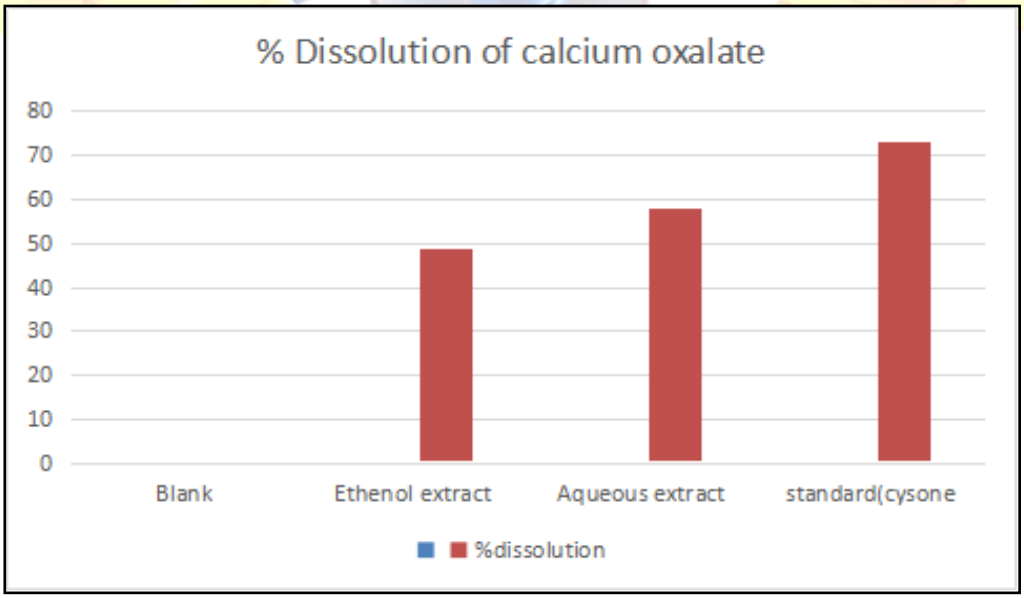

Fig.1: Dissolution of Calcium Oxalate

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These graphical represent shows % Dissolution of calcium oxalate by in vitro Anti-Urolithiatic Activity of extracted fraction of *Tribulus terrestris*. L drug. An aqueous extract at 10mg concentration produced higher dissolution of calcium oxalate as compare to other fraction. Standard shows higher dissolution as compare to others.

DISCUSSION

This study evaluate that antiurolithiatic activity of different extracts of *Tribulus terrestris* Linn leaves and isolated phenolic compound, steroidal compound. The study of the urinary chemistry with respect to the stone-forming minerals will provide a good indication of the risk of stone formation. From the study results it is observed that aqueous fraction show highest dissolution of calcium oxalate in comparison to other fractions. This study has given primary evidence for *Tribulus terrestris* L. as plant which possess lithotriptic property. This in vitro study has given lead data, and shown that phenolics and steroids form aquous fraction is quite promising for further work in this regard.

CONCLUSION

Urolithiasis has been performed for *Tribulus terrestris* Linn and marketed polyherbal formulation Cystone. And perform work by using In Vitro antiurolithiatic Model for calculating % dissolution of kidney stone. And aquous extract is giving higher % dissolution of calcium oxalate crystal.

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