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Review Article

Assessment of Potential Antiurolithiatic Activity of Some Selected Medicinal Plants by *In vitro* Techniques

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Abstract

Now a day's 80% of global population depend on medicinal plants. Apart from the general medicines, ability to utilize the medicinal properties from the nature by extracting their active constituents is found to be more beneficial with less or no side effects. Preva-lence of renal calculi is most common in population these days. Different causes of calculi are hereditary factors, metabolic disorders, infections and urinary obstructions. This may lead to major complications like uremia, pyelonephritis, kidney failure etc. Based on the literature survey, three medicinal plants Semecarpus anacardium, Terminalia chebula and Tinsopora cordifolia were selected, which were claimed in traditional medicine to dissolve renal stones. Antiurolithiatic activity of chloroform, methanol and aqueous extracts was estimated by Kramer and Tisdall method. Semi-permeable membranes obtained by treating farm eggs were used in the study. The amount of calcium oxalate dissolved in Tris buffer was selected as the criterion to assess the anti-urolithiatic activity. The chloroform extract of all the three plants showed potential anti-rolithiatic activity when compared with standard drug.

Keywords: Antiurolithiatic activity; Semecarpus anacardium; Traditional medicine; Renal calculi; Chloroform extract; Semi-permeable membranes

Introduction

Nephrolithiasis or presence of renal stones is an important health problem in the adult population. The incidence of urolithiasis is quite high worldwide. More than 80% of urinary calculi encountered are because of calcium oxalate stones alone or calcium oxalate mixed with calcium phosphate. Different types of calculi include renal calculi, gall bladder calculi, pancreatic calculi, liver calculi etc. The medi-cal aid available at present is either costly or suffers from serious side-effects. In contrast, traditional medicines are less costly and have offered a substitute for nephrolithiasis [1].

Semecarpus anacardium has hypocholesterolemic activity, antispermatogenic effect, anti-atherogenic effect, hypoglycemic, fungi static activity [2], myocardial protective activity [3], used in arthritis [4], antimicrobial activity [5], serum cholinesterase inhibitory activity [6]. Shows antibacterial, astringent, purgative, laxative, stomachic activity, it is used in treatment of asthma, piles, cough, healing of wounds, scalds, α-amylase activity [7], chronic fever, hepatitis, anemia, oozing skin lesions, obesity, improves memory, used in dysuria and urinary stones, significant renoprotective [8], ulcerogenic activity [9], aging principles [10], anticonvulsant activity [11]. *Tinospora cardifolia* is effective in leprosy, ulcers, heals the herpes lesions, indigestion, irritable bowel syndrome, good antispasmodic and cardiotonic, aphrodisiac, strengthens the urinary system and increases the resistance of inner layers of bladder and urethra to fight repeated urinary tract infections, used in HIV infection [12]. Immunostimulatory properties [13,14], anti-hepatotoxic, antistress and antioxidant properties.

A thorough study of literature revealed that *Semecarpus anacardium*, *Terminalia chebula* and *Tinospora cordifoliaare* used in traditional medicine to dissolve mineral calculi. The present work was taken up to give a scientific base for the traditional uses claimed.

Materials and Methods

The seeds of *Semicarpus anacardium*, fruits of *Terminalia chebula*, and the stems of *Tinospora cordifolia* were all collected from the local market near Kaleshwara Rao market, Vijayawada, Krishna district. They were authenticated in the botany department of Siddhartha MahilaKalasala, Vijayawada, and a voucher specimen was preserved in Kommareddy VenkataSadasiva Rao Siddhartha College of Pharmaceutical Sciences for future reference.

Chloroform, methanol, Dragendroff's reagent, Mayer's reagent, Wagner's reagent, tannic acid solution, α-naphthol, conc.sulphuric acid solution, copper sulphate, sodium hydroxide, Fehling's solution A & B, potassium hydroxide solution, picric acid, conc. hydrochloric acid, Calcium chloride dihydrate, sodium oxalate, potassium permanganate, oxalic acid, Trispowder were procured from Qualigen Fine Chemicals.

Extraction

Shade dried plant material was pulverized and about 100 gm of the powder was extracted successively with chloroform, ethanol and finally with water using Soxhlet extractor. The crude extract was dried below a temperature of 60°C and stored in refrigerator until use. The % yield was calculated.

Phytochemical screening of the extracts

The plant extracts obtained were dissolved in sufficient quantity of a mixture of DMSO and Tween80 solution. Phytochemical analysis was carried out following standard procedures [15].

The results of these tests were given in the Table 2.

S.NO	GROUP NO	TREATMENT DETAILS	
1	GROUP 1	TCC	*Terminaliachebula* chloroform extract
2	GROUP 2	TCM	*Terminaliachebula* methanol extract
3	GROUP 3	TCA	*Terminaliachebula* aqueous extract
4	GROUP 4	SAC	*Semecarpusanacardium* chloroform extract
5	GROUP 5	SAM	*Semecarpusanacardium* methanol extract
6	GROUP 6	SAA	*Semecarpusanacardium* aqueous extract
7	GROUP 7	TiCC	*Tinosporacordifolia* chloroform extract
8	GROUP 8	TiCM	*Tinosporacordifolia* methanol extract
9	GROUP 9	TiCA	*Tinosporacordifolia* aqueous extract
10	GROUP 10	STD	Tamsulosin Hydrochloride
11	GROUP 11	CONTROL	----

S.No	Extract	% Yield
1	TCC	0.35
2	TCM	10.97
3	TCA	7.01
4	SAC	26.6
5	SAM	4.58
6	SAA	4.37
7	TiCC	0.69
8	TiCM	1.58
9	TiCA	19.42

Table 1: % Yield of various extracts.

Experimental protocol

Preparation of Calcium Oxalate by Homogenous Precipitation

Equimolar solutions of calcium chloride dihydrate (A.R) dissolved in distilled water and sodium oxalate (A.R) dissolved in 10 ml of 2N H_2SO_4 and distilled water were prepared. Sufficient quantity was allowed to react in a beaker with slight aid of heat until more precipitation occurs. The resulting precipitate was calcium oxalate which was freed from traces of sulphuric acid by treating with ammonia solution. Finally it was washed with distill water and dried at temperature 60°C for 4 hrs.

Preparation of the semi permeable membrane from farm eggs

The semi permeable membrane of eggs is separated following procedure available in literature [16]. This membrane lies in between the outer calcified shell and the inner contents like albumin and yolk. Shell was removed chemically by placing the eggs in 2M HCl for 24 hrs, which caused complete decalcification. Further, washed with distill water and carefully with a sharp pointer, a hole is made on the top and the contents squeezed out completely from the decalcified egg. It was washed thoroughly with distilled water, and placed in ammonia solution, in the moistened condition for a while and then rinsed with distill water. Finally it is stored in refrigerator at a pH of 7-7.4.

Method

In-vitro antiurolithiatic activity can be studied by inducing calcium oxalate stone formation and studying the effect of plant extracts on their ability to decrease the amount of calcium oxalate [17,18,19 and 20]. In the present study Kramer and Tisdall method was used to evaluate In-vitro antiurolithiatic activity. The experimental protocol is depicted in figure 1.

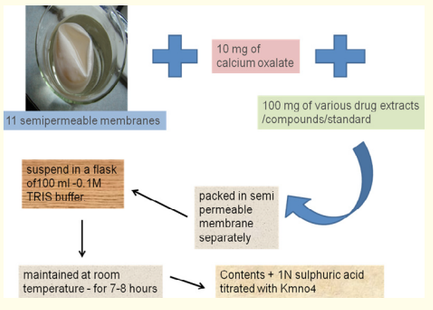

Figure 1: Experimental protocol.

A total of 11 semi permeable membranes were prepared. Exactly 10 mg of calcium oxalate and 100 mg of various drug extracts / compounds/standard were weighed and packed in semi permeable membrane separately and carefully sutured. This was allowed to suspend in a conical flask containing 100 ml of 0.1M TRIS buffer. All the conical flasks were maintained at room temperature undisturbed for 7-8 hours. The contents remaining in the semi permeable membrane were transferred into a test tube. 2 ml of 1N sulphuric acid was added and titrated with KMnO4 till a light pink color was obtained. The results are displayed in figure 2.

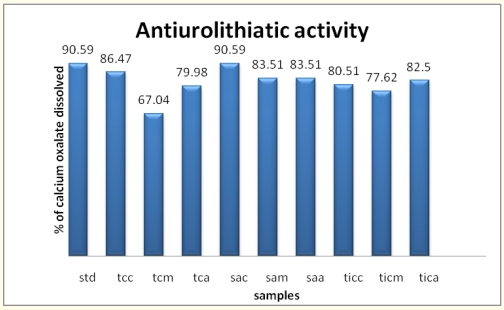

Figure 2: Antiurolithiatic activity of different samples.

Equivalent factor:

1 ml of 0.9494 N KMnO4 equivalent to 0.1898 mg of Calcium.
Percent of mineral phase dissolved in the presence of test sample was calculated as follows: % Dissolved = [(C-T)/C]*100
Where C = precipitate of calcium oxalate remained in control
T = precipitate of calcium oxalate remained when test solution is used.

be, *Semecarpus anacardium*-26.6%, *Terminalia chebula*-0.35%, *Tinospora cordifolia*-0.69%. The % yield with methanol was found to be, *Semecarpusana cardium*-4.58%, *Terminalia chebula*-10.97%, *Tinospora cordifolia*-1.58%. The % yield with distilled water was found to be, *Semecarpusana cardium*-4.37%, *Terminalia chebula*-7.01%, *Tinospora cordifolia*-19.42%. Among the three samples *Semecarpusana cardium* was found to yield the highest amount of chloroform extract (26.6%), *Terminalia chebula* yielded highest amount of methanol extract (10.97%) and *Tinospora cordifoliagave* the highest amount of aqueous extract (19.42%).

The results of phytochemical screening are shown in Table 2. The plant extracts showed the presence of alkaloids, carbohydrates, fats and fixed oils, glycosides like anthraquinone glycosides, cardiac glycosides, saponin glycosides, tannins, steroids and terpenoids.

The amount of calcium oxalate dissolved was selected as the criterion to evaluate antiurolithiatic activity. The results are shown in figure 2. The amount of calcium oxalate dissolved with

Test name	Semicarpusanacardium			Teminaliachebula			Tinosporacordifolia		
	SAC	SAM	SAA	TCC	TCM	TCA	TICC	TICM	TICA
Dragendroff's test	+	-	+	-	+	-	+	+	-
Mayers test	+	-	-	+	+	-	+	+	+
Wagner's test	+	+	+	+	+	+	+	-	-
Hager's test	-	-	-	+	+	-	+	-	-
Tannic acid test	-	-	-	+	+	-	+	-	-
Molish test	-	+	+	+	+	+	+	+	+
Fats and fixed oil	+	+	+	+	+	+	+	+	+
Anthraquinone glycosides	+	+	+	-	+	+	+	+	+
Cardiac glycosides	+	+	+	-	+	-	+	+	+
Saponin glycosides	-	+	+	-	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	-
Steroids and terpenoids	+	+	+	+	+	+	+	+	+

+ indicates presence
- indicates absence

Table 2: Preliminary phytochemical screening

Results and discussion

The results of extraction and yield of various extracts is depicted in Table 1. The plant material was extracted with chloroform, methanol and water. The % yields of chloroform extracts were found to

standard drug was 90.59%. Among the tested extracts of *Terminalia chebula*, % calcium oxalate dissolved with chloroform extract was 86.469%, methanol extract 67.035%, and aqueous extract 79.98%. The ability to dissolve calcium stones by extracts of *Semecarpusana cardium* was as follows: with chloroform extract

90.59%, methanol extract 83.51%, and aqueous extract 83.51%. Among the extracts of *Tinospora cordifolia*, % calcium oxalate dissolved with chloroform extract was 83.51%, methanol extract 77.62%, and aqueous extract 83.51%. The chloroform and aqueous extracts (100 mg) were found to possess significant antiurolithiatic activity when compared to the standard.

The chloroform extract of the *Semecarpus anacardium* was found to have significant antiurolithiatic activity when compared to other extracts. The activity was comparable with the standard drug Tamsulosin hydrochloride -0.2 mg. The increasing order of antiurolithiatic activity with various extracts tested was STD & SAC > TCC > SAM & SAA > TiCA >TiCC > TCA > TiCM >TCM.

Among the different extracts tested, chloroform extracts of all the three plants were found to exhibit potential antiurolithiatic activity. Medicinal plants with proven antiurolithiatic activity were found to contain alkaloids, tannins, steroid, and terpenoids as chief principles [21,22 and 23]. The antiurolithiatic activity reported for the chloroform extracts of *Semecarpusana cardium*, *Terminalia chebula* and *Tinospora cordifolia* may be because of the non-polar active principles residing in these plants. This *in vitro* study has given a very valuable data and showed that these extracts possess quite promising potential antiurolithiatic activity.

Conclusions

The incidence of renal stones is increasing alarmingly in developing countries. There are several herbal remedies for the treatment of urolithiasis. Based on the data available in the literature *Semecarpusana cardium*, *Terminalia chebul*a and *Tinsopora cordifolia* were selected. These herbs are used in ethnomedical practice in the treatment of urinary problems and renal stones. The study has given a basic scientific evidence for the traditional uses of these plants in dissolving renal stones. Further, in vivo studies are required to strengthen the work and prove their therapeutic usefulness.

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Conflicts of interest

None.

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