



Research Journal of Pharmaceutical, Biological and Chemical Sciences

Urinary Calculi Dissolving Activity of Indian Herbs- An In Vitro Study: A Step towards Rationalization.

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ABSTRACT

"The garden is the poor man's apothecary" is an old and famous proverb. India, right from beginning has been known for the use of natural flora for the medicinal purpose. But, the systematic study with reference to modern techniques, standardization and calculation of dose has been always a problem with this natural treasure. "The revival interest in herbal medicine is a worldwide phenomenon". Plants of Indian origin namely *Saxifragae granulata*, *Tribulus terrestris*, *Solanum xanthocarpum*, *Rhamnus prushinae* (Refer figures and illustrations 1-4) were studied with respect to their in vitro urinary calculi dissolving activity. The aqueous and alcoholic extracts of roots and aerial parts of the plants were used in the studies. Reduction in weights of urinary stones, percentage weight loss, stone dissolution rate and amount of calcium and phosphate released were used as parameters in this study. The experiment has given significant results statistically and graphically by employing the Paired T test.

Keywords: *S. granulata*, *T. terrestris*, mean dissolution rates, statistical analysis.

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INTRODUCTION

The most important phosphate containing calculi involved in urinary stone disease are apatite, brushite, and struvite. Overall phosphate stones accounts for about 20 % of all urinary stones and rank first among the list of the recurrent calculi. The most widely used therapy involving any type of phosphate stone is calculus removal. However, they are associated with injury to the urinary tissue. Stone dissolution through herbal extracts may be tried as an option even in the advent of more sophisticated modalities in the treatment of the urolithiasis. [1-2]

The four plants, namely *Saxifragae granulata*, *Tribulus terrestris*, *Solanum xanthocarpum* and *Rhamnus prushinae* are found mainly in tropical Asia and Africa. *T. terrestris* mainly contains flavanoid glycosides, saponin glycosides and diosgenin while, *S. granulata* mainly shows the presence of tannic acid, Gallic acid and albumin. The plants are known for their diuretic and cardio tonic activity. Currently these plants are used in the herbal commercial preparations for kidney stones treatment and hence the study aims at standardizing and customizing the activity of extracts of these plants towards calculi dissolving using an in vitro method. [1-4]

MATERIALS AND METHODS

Collection of materials

Branded Herbal powders and herbal formulations were purchased from local medical shop.

Methodology of extraction

Alcoholic and aqueous extracts of different concentrations were obtained by the procedure given by J.B. Harbones [5] (Photochemical method: A guide to Modern Techniques of Plant Analysis, 2nd edition, Champan & Hall Ltd. London,1984,11,12)

The urinary calculi of an average weight (0.5 – 0.7gm) were subjected to the extracts of different concentrations to determine different parameters (in triplicates). These parameters were compared to the locally marketed formulation. [6]

Methods used for estimation of calcium and phosphate:

The surgically removed urinary calculi were subjected to different extracts obtained from plants at different conc. (30-70 mg/ml for aq. and 5-50 mg/ml for alc. extracts) and were analyzed for their reduction in weight, percentage wt. reduction, dissolution rates, release of calcium and phosphate after every 24 hrs.

Calcium estimation was done by Calcium estimation method of Clark & Collip [7] whereas phosphate estimation was done by using spectrophotometric method (Murphree2002) [8].

Stone analysis:

Surgically removed stones were obtained from the patients and were analyzed on the basis of the color and appearance of the stone. **(Refer table1)** [9].The calcium estimation was done by using Clark and Collip method⁷ while phosphate estimation was done using the spectrophotometric method [8]. The stones were found to have the calcium and phosphate and uric acid was found to be absent or was present in negligible amount **(Practical Biochemistry, Calculi Analysis)**.

Statistical analysis using paired T test:

PAIRED T TEST:

The data so obtained was compared with the local marketed preparation by applying the paired t test (Microsoft excel 2007).

The T value found for the alc. & aq. extracts of *S. granulata* and *T. terrestris* respectively is greater than that 100 compared to that of the commercial T value (6.965) implying that they are significant. While extracts of *R.prushinae* and *S. xanthocarpum* was found to be equiefficacious to the commercial preparation with T value less than that of 6.695. [10]

RESULT & DISCUSSION

Mean dissolution rates of urinary calculi with all 4 plants' extracts (alcoholic. and aqueous) were found as per the table (Refer table 1) which implies that commercial preparation and the *S. xanthocarpum*, *R. prushinae* extracts are comparable.

TABLE 1: Chemical composition present in the renal stones

Stones name	Stones color	Chemical composition present in the renal stones
A	Brownish blue	Calcium oxalate di-hydrate ,calcium phosphate
B	Light yellow	Calcium oxalate mono hydrate ,calcium oxalate di-hydrate
C	Yellowish brown	Calcium oxalate monohydrate with phosphates
D	White yellow	Calcium oxalate monohydrate, Phosphates, Calcium oxalate di-hydrate
E	Brownish white	Calcium oxalate monohydrate

Statistical analysis of the present study was done by paired T test. The statistical evaluation of paired T test was carried out at 2 degree of freedom and 1 % level of significance. This analysis implies that alcoholic and aqueous extracts of *S. granulata* & *T. terrestris* are found

to be significant in percent rate dissolving activity of urinary calculi where as all the others are found to be equiefficacious with that of the commercially available urolytic herbal preparation.

Table 2: Mean dissolution rates for the alc. and aq. extracts of the plants

Name of plant	Mean dissolution rates in mg/day	
	Alcoholic	Aqueous
S. granulate	20	3.75
T. terrestris	1.5	1
S. xanthocarpum	0.175	0.225
R. prushinae	0.3	0.175
Commercial preparation (aq. Suspension)	0.2	

Table 3: Weight reduced in mg. for the alc. and aq. Extracts

Name of plant	Wt. reduced in mg.	
	Alcoholic	Aqueous
S. granulata	78.5	14.89
T. terrestris	58.32	38.11
S. xanthocarpum	7	
R. prushinae	12	7
Commercial (aq. Suspension)	8	

Urological calcium phosphate calculi were poorly dissolved by S. xanthocarpum & R. prushinae and the had low dissolution rate .The most effective extracts were found to be of S. granulata and T terrestris which showed 0.80-1.23% stone weight reduction over 72 hours exposure.

The study has shown that S. granulata & T, terrestris can dissolve calcium phosphate stones effectively in vitro at precise dilution of their chemical components. Based on these findings it is suggested that the extracts of these plants may become useful complements to the modern techniques of the stone fragmentation.

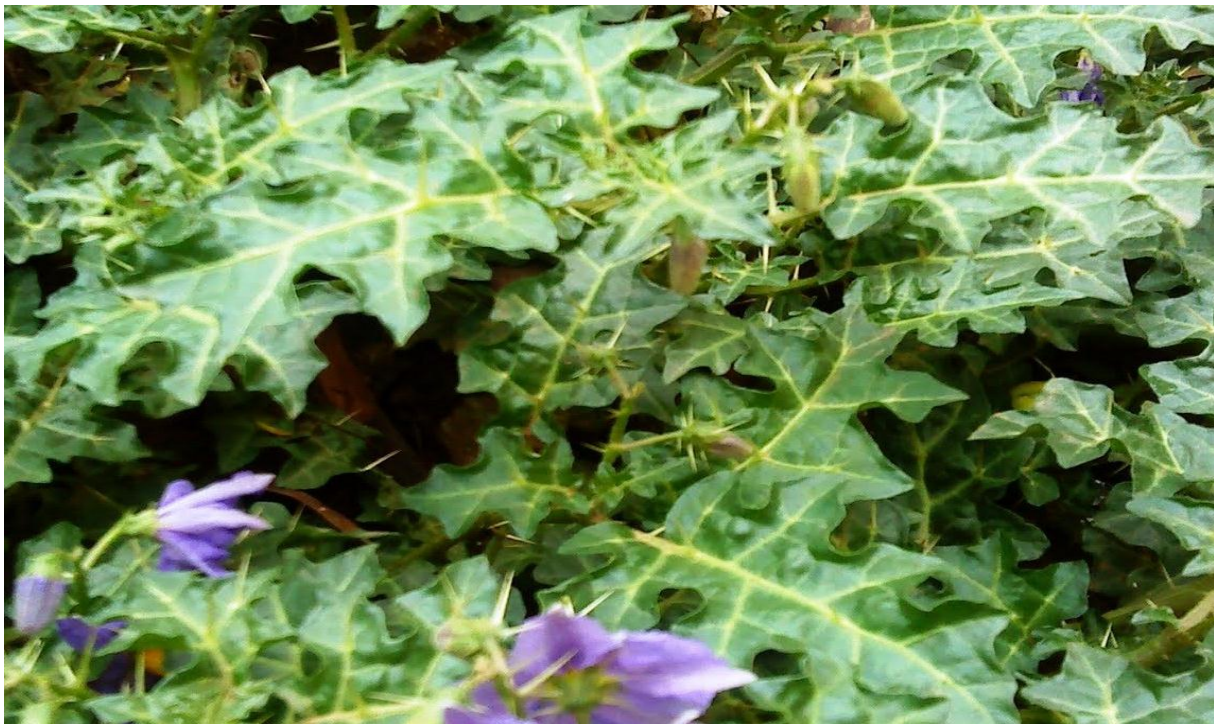
Graphical results:

Graphical results (Refer graph 5 in figures and illustrations and Table 2) imply that among the extracts S. granulate and T. terrestris have a good urine calculi dissolving activity compared to that of R. prushinae, S. xanthocarpum & the commercial preparation. The activity was studied for 5 days. The S. granulata & T terrestris extract was found to be about 10-5 times significance over the commercial and other extracts graphically.

Saxifraga granulata(4" *6")



Tribulus terrestris(4" *6")

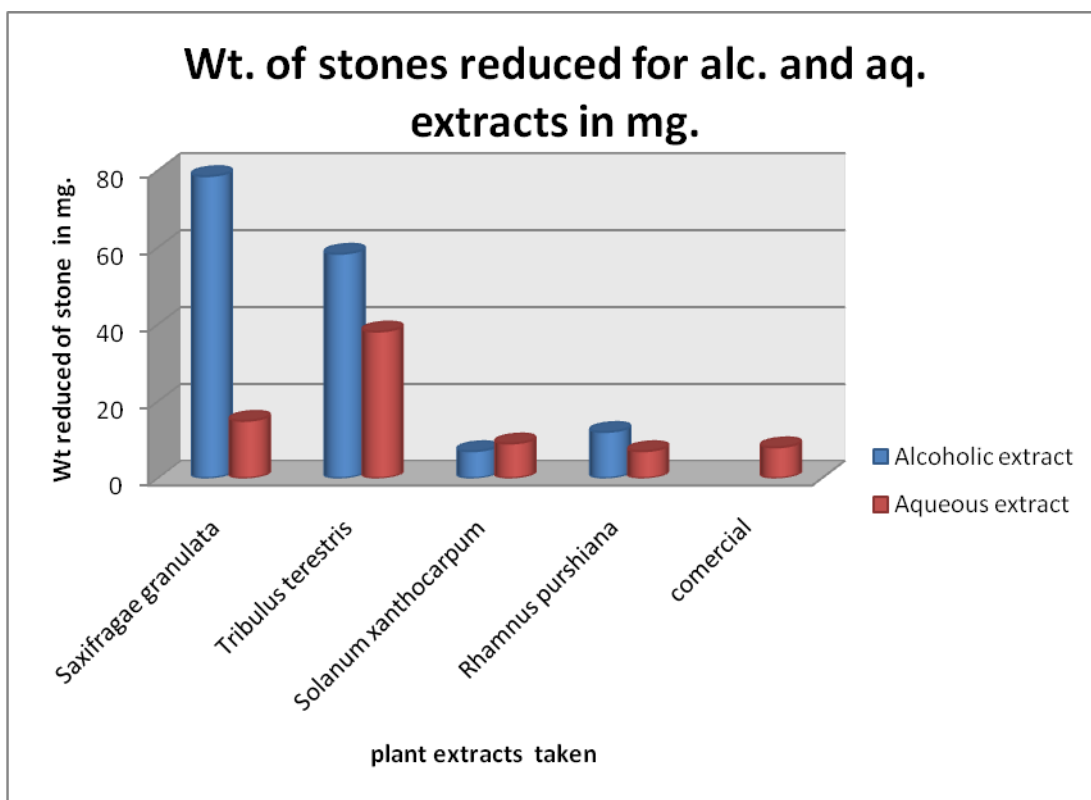


Solanum xanthocarpum(4" *6")



Rhamnus prushinae

Graphical representation for the reduction in weight of urinary stones for alcoholic and aqueous extract:\





CONCLUSION

Surgical removal of the urinary stones has always been painful and frightful for the patients. The above results can be of importance in using these drugs for getting rid of these problems. Nature today is a need of an hour as allopath and its side effects can be overcome by using these drugs.

The results are to be further assessed for the other plant extracts for estimating the further urinary calculi dissolving activity.

ACKNOWLEDGEMENTS

Dr. S.B. Bhise (Principal, Sinhagad college of Pharmacy, Lonawala, Pune), Dr. C.R. Patil (R.C. Patel college of pharmacy, Dhule), Dr. Dadasaheb Khokare (University Department of Pharmaceutical Sciences, Nagpur), Dr. Suhas Kulkarni (Karad) for their support and help, Dr. Pardeshi (M.D. Gynecologist, Jijai hospital, Aurangabad) and Dr. Kulkarni (M.D. Urologist, Hegdewar hospital, Aurangabad) for providing us surgically removed urinary stones. Also thankful to Adwait Deshpande and Jadhav Sheetal, for their cooperation during the studies.

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