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IN VITRO EVALUATION OF ANTI-UROLITHIC ACTIVITY OF *Tectona Grandis* L.F BARK EXTRACTS

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Abstract

Urolithiasis is derived from the Greek words ouron (urine) and lithos (stone). It is a condition of formation of calculus in the urinary system, i.e. in the kidney, ureter, and urinary bladder or in the urethra. It is estimated that approximately 2% of the population experiences renal stones disease at some time in their life with male-female ratio of 2:1, because of enhancing capacity of testosterone and inhibiting capacity of oestrogen in stone formation. Modern synthetic medicines are very effective in curing a number of diseases but also cause serious side effects. Crude drugs are less efficient with respect to cure of disease but are relatively free from side effects. A number of medicinal plants are evaluated mainly against calcium phosphate & calcium oxalate type kidney stones, employing various experimental models of Urolithiasis.

Keywords: Urolithiasis, Anti- urolithic, antilithogenic.

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INTRODUCTION

Kidney stone is one of the most important problems in different countries over the world. They are affected by different factors like nutrition, age, drug history and other environmental and family factors. Its recurrent renal disease affect 4-8% in UK, 15% in US, 20% in industrialized population are suffering with the problem of urinary stone formation [12]. Generally there are five different types of stones of which calcium oxalate is the most common (80%), struvite stone (10%), uric acid stone (9%) and (1%) is due to Cystine and ammonium ureter [11]**.** Calcium stone are usually dark brown in colour and small (less than a centimeter), ovoid, hard, with granular rough surface. About 15% of urinary calculi are made up of magnesium-ammonium calcium phosphate often called struvite stones, mixed stones or triple phosphate stones. Struvite stones are yellow- white or gray colour and they tends to be soft and friable and irregular in shape. Approximately 6% of urinary calculi are made up of uric acid. Uric acid calculi are radiolucent unlike radio- opaque stones and are smooth, yellowish brown, hard and often multiple on cut section, they showed laminated structure. Cystine stones are very rare; they are caused by cystinuria, a genetic kidney disease and they comprise less than 2% of urinary calculi. Cystine stones are yellowish and waxy, small, rounded, smooth and often multiple [11].

PLANT PROFILE [4, 9, 10]

Biological source: Tectona grandis L.f.

Family **:** Verbenaceae

Chemical constituents

It contains Tectoquinone, lapachol, deoxylapachol and its isomertectoleafoquinone. Steroidal compounds as Betulinic acid, tectoinols A&B, saqualene, polyisoprene-a-tolylmethylether, tectogandone, monoterpene. Anthraquinone glycosides and Rutin ,quercitin,Tannic acid, gallic acid, ferulic acid ellagic acid caffeic acid [7,8].

Fig. 1: Leaves of Tectona grandis L.f.

Fig. 2: Flower of Tectona grandis L.f.

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MATERIALS AND METHOD

Collection of Plant Material

The bark of Tectona grandis L.f. was collected from the medicinal garden of Dev Bhoomi Institute of Pharmacy and Research, Dehradun (Uttarakhand). The plant specimen was identified and authenticated by taxonomist Dr. S. K. Srivastava, Botanical Survey of India (BSI), Dehradun (Uttarakhand), with the Acc. No. 115374.

Preparation of Extract [5, 6]

For alcoholic and aqueous extract the powdered material 10gm & 100gm respectively mixed with absolute alcohol 100ml & 1000ml distilled water with occasional shaking and left for 7 days. After 7 days it was filtered through double layered muslin cloth. The filtrate collected was concentrated by evaporation and it was further evaporated in a china dish at room temperature. It was then stored in glass bottles and labeled for further use. For hydro alcoholic extract the powdered material (10g) was mixed with 100 ml distilled water & ethanol (1:1). Stirred thoroughly and left for 7 days in an air tight glass container. After 7 days it was filtered through double layered muslin cloth. The filtrate collected was then concentrated by evaporation and it was further evaporated in a china dish at room temperature. It was then stored in glass bottles and labeled for further use.

***In-vitro* antilithogenic activity**

Method [1-3]

Test tubes of 10 ml capacity were used and marked the tubes as control and test. 5 groups (each group of 6 tubes), in each tubes 1ml of calcium chloride (Merck specialties Pvt. Ltd., Mumbai) anhydrous and 1ml sodium oxalate (CDH, New Delhi) were added along with 2 ml of Tris buffer (disodium hydrogen phosphate and potassium dihydrogen phosphate) and adjusted at 7.4 pH which to the kidney pH and incubated at 36.7^0C over night. The next day the test tubes were centrifuged for 10min., decanted to remove top liquid layer. The calcium oxalate crystals formed in the test tube were checked using the compound microscope under 45X magnification. The crystal formed resembled prisms shape. 5ml (5mg/ml) (equivalent to 25mg) of extracts of plant Tectona grandis L.f. were introduced to the tubes and the same amount of Poly herbal formulation Cystone was added to the test tube. All the above treating agents were administered as aqueous suspension using tween-80 as suspending agent and incubated at 36.7^0C for 3 days. On the fourth day all the test tubes were taken and checked for dissolution of the crystals under the microscope

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at the same magnification, to this test a drop of con. HCl was added to separate the oxalate ion, calcium and both the ions were spectroscopically analyzed. Crystal dissolution was observed under 45X microscope and prism shape calcium oxalate crystals were sized / measured by eye piece and stage micrometer. Mean size of more than 50 crystals were observed.

Elemental Calcium Analysis

Calcium, in an alkaline medium, reacts with o-cresolphthalein to form an intense chromophore which absorbs light at 575 nm (570-580 nm). Magnesium and iron are excluded by complexing with 8-hydroxyquinoline.The U.V. spectrophotometer was set with the parameters given along with the kit. The working, standard and test solutions were Prepared as per the protocol, Incubated for 5 min at room temp. Mixed and read at 575 nm.

Calculation

$$\frac{\text{mg}}{\text{dl}} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard} \frac{\text{Mg}}{\text{dl}}$$

RESULTS

The effect of various extract (5mg/ml-5ml) / Cystone (5mg/ml-5ml) on calcium oxalate crystal size dissolution generation was determined by microscopy and chemical analysis. The prism shape calcium oxalate crystals were sized/ majored by eye piece of stage micrometer. Mean size of more than 50 crystals were observed as described in following tables.

Table 1: Generated calcium oxalate crystals size in control group

Sl. No.	1	2	3	4	5	6	7	Total
1	20	11	14	19	15	18	10	107
2	10	11	21	16	30	21	17	126
3	12	20	9	10	8	15	35	109
4	14	15	25	29	5	11	20	114
5	13	17	13	15	14	18	26	116
6	24	22	8	10	12	30	22	128
7	7	30	20	28	10	9	17 & 14	135

Mean – 835/50 = 16.72µm

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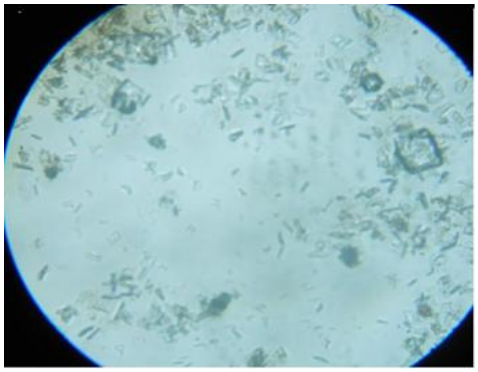

Fig.3: Observed calcium oxalate crystal in control untreated group under 45Xmagnification

Table 2: Calcium oxalate crystal size in standard control (Cystone) group.

Sl.No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
1	7	10	8	13	3	2	6	49
2	6	14	16	4	10	5	8	63
3	12	11	12	4	8	20	8	75
4	20	9	25	7	5	6	2	74
5	14	15	3	9	4	6	3	54
6	21	8	4	8	8	9	7	65
7	10	13	6	5	6	4	6 & 6	56

Mean –436/50 = 8.72µm

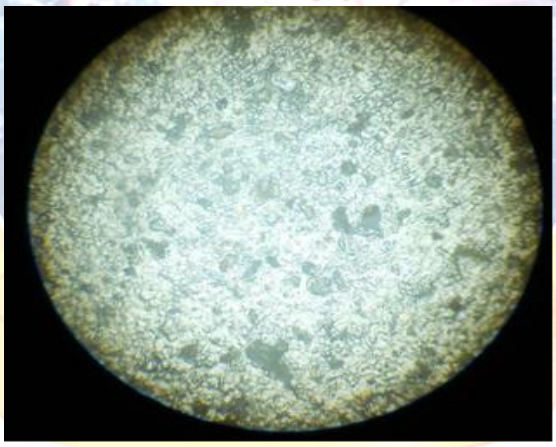

Fig.4: Observed calcium oxalate crystal in Cystone treated group under 45Xmagnification

Table 3: Calcium oxalate crystal size in aqueous extract treated group

Sl. No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
1	16	13	5	19	8	5	14	80
2	4	16	14	16	6	10	4	70
3	8	4	6	11	4	8	13	54
4	16	7	14	8	15	4	12	76
5	5	18	4	14	3	16	6	66
6	4	4	17	10	4	5	18	62
7	17	15	8	6	3	5	8 & 5	67

Mean – 475/50 = 9.50µm

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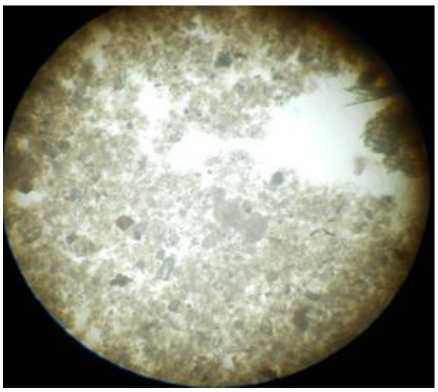

Fig.5: Observed calcium oxalate crystal in aqueous extract treated group under45X magnification

Table 4: Calcium oxalate crystal size in hydro alcoholic extract treated group

Sl. No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
1	4	8	16	10	7	9	4	58
2	14	9	14	8	14	8	18	85
3	5	16	15	16	7	13	8	80
4	6	6	4	7	6	11	6	46
5	7	16	24	14	5	8	15	89
6	17	13	8	5	15	7	5	70
7	4	4	5	16	5	6	14 & 6	60

Mean –488/50 = 9.76µm

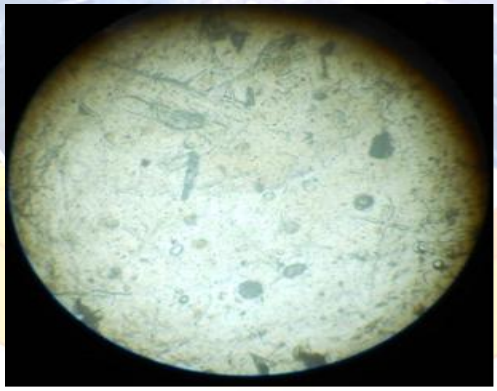

Fig. 6: Observed calcium oxalate crystal in hydroalcohalic extract treated groupunder 45X magnification.

Table 5: Calcium oxalate crystal size in alcoholic extract treated group

Sl. No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
1	5	13	16	15	15	14	3	81
2	14	12	18	16	14	14	12	100
3	13	20	4	8	3	5	14	67
4	4	11	15	4	14	16	6	70
5	14	10	13	16	12	7	15	87
6	3	7	9	4	13	8	4	50
7	12	18	14	16	13	19	13 & 11	116

Mean –571/50 = 11.42µm

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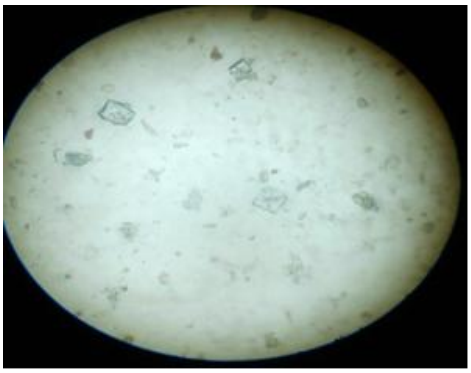

Fig.7: Observed calcium oxalate crystal in alcoholic extract treated group under45X magnification

Table 6: Effect of Tectona grandis L.f. bark extracts on generated calciumoxalate mean crystals size

Sl. No.	Group		Mean crystal size µm ± SEM
1	Group	1 Normal control	16.72±1.009
2	Group 2	Standard Cystone	8.72±1.900
3	Group 3	Aqueous extract	9.50±1.373
4	Group 4	Hydroalcohalic extract	9.76±1.217
5	Group 5	Alcoholic extract	11.42±1.142

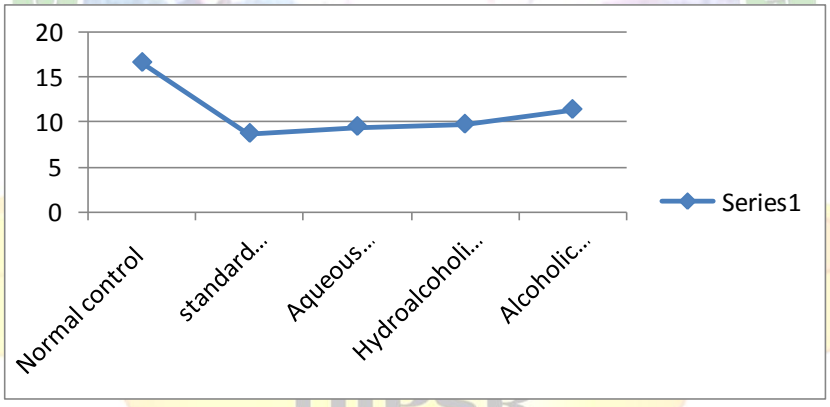

Fig.8: Effect of Tectona grandis L.f. bark extracts on generated calcium oxalatemean crystals size

Table 7: Effect of Tectona grandis L.f. bark extracts treatment on free calcium concentration

S. No.	Group	Calcium (mg/dl)
1	Normal control	8.71
2	Standard control (Cystone)	31.030
3	Test -1 (Aqueous extract)	27.412
4	Test -2 (Hydro-alcoholic extract)	25.125
5	Test -3 (Alcoholic extract)	19.824

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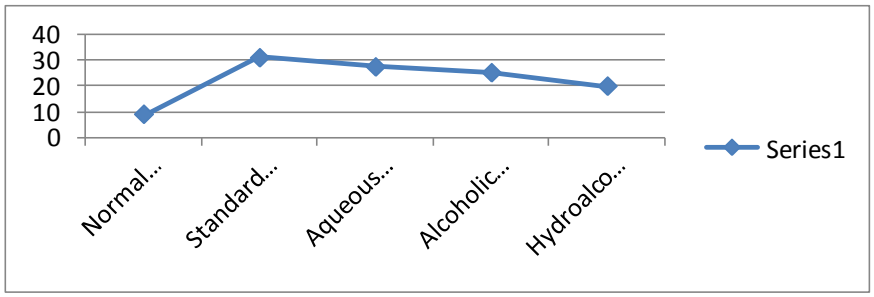

Fig. 9: Effect of Tectona grandis L.f. bark extracts treatment on free calcium concentration.

Method 2

In this method microscopically we observed that due to influence of drug treatment on both the reactants. It was not proper calcium oxalate crystal growth compared to normal reaction setup. Thus the formed crystals size was very minute measuring less than 5 µm at 45X magnification and the number of crystal size was very less. That is this drug may help in prophylactic regimen of urolithiasis.

DISCUSSION

The aqueous and hydroalcohalic extracts of Tectona grandis L.f. bark inhibited the precipitation of calcium and oxalate. The results of our study clearly showed the utility of Tectona grandis L.f. bark for the treatment of renal calculi. In microscopical examination the reduction of crystal size in aqueous extract was more significant than both hydroalcohalic and alcoholic extracts compared to normal untreated crystals. All three test extracts decreased the crystal size with the potency order of aqueous, hydroalcohalic and alcoholic extract respectively with comparable results of standard Cystone drug treatment. Since our test drug extract showed activity less than standard but the results of aqueous and hydroalcohalic extract were very near and significant to the standard drug. Hydroalcohalic extract also shown mild activity than compared to standard. Simultaneously elemental calcium ion analysis also supporting the result outcomes in the same way as discussed above.

CONCLUSION

In the present study the plant Tectona grandis L.f. was selected for its considerable flavonoid content and an attempt was made to evaluate anticalcinogenic potential. Different extracts of Tectona grandis L.f. showed anticalcinogenic action in growth of calcium oxalate crystals. Aqueous extract was more potent than alcoholic extract and hydro-alcoholic extract at tested crystal dissolution and quantitative elemental ion analysis in vitro study.Although, to establish Tectona grandis L.f. as an anticalcinogenic drug studies with other methods of evaluation of

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urolithiatic action needs to be undertaken, but still the Tectona grandis L.f. is a potential lead in search of active compounds for treatment of urolithiasis.

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