



## ***In-vitro anti-lithiatic activity study of Tribulus terrestris fruits and Boerhaavia diffusa roots***

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### **Abstract**

*The different solvent extracts of Tribulus terrestris fruits and Boerhaavia diffusa roots were executed on generated calcium oxalate crystals by homogenous precipitation method for in-vitro anti-lithiatic activity. The alcoholic extracts of Tribulus terrestris fruits and Boerhaavia diffusa roots as shown significant activity on comparison to the synthetic drug Spironolactone, poly herbal formulation Cystone and to the other extracts of the drug, the furosemide was found to non active on formed crystals.*

**Key words:** *Tribulus terrestris* and *Boerhaavia diffusa*, Spironolactone, Cystone, antilithiatic.

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### **INTRODUCTION**

Urolithiasis otherwise urinary calculi a pathogenic continues to be more or less of an ambigue and the predicament found to an ancient and worldwide distributed. The different calculi are painful urinary disorders that start as salt/chemical crystals which precipitate out from urine. Under normal circumstances, the urine contains substances that prevent crystallization but for patients with this condition, these inhibitory substances are ineffective. Tiny crystals will pass out along with the urinary flow without causing problems. At least few of people will pass a kidney stone during their lifetime, producing some of the most severe pain possible, by increasing the stone concentration in the kidney. If the stone is large enough to block the tube (ureter) and stop the flow of urine from the kidney, it must be removed by surgery or other methods. It is also called Renal Calculus. Symptoms usually begin with intense waves of pain as a stone moves in the urinary tract. Typically, a person feels a sharp, cramping pain in the back and side in the area of the kidney or in the lower abdomen. Sometimes nausea and vomiting occur. Later, pain may spread to the groin. The pain may continue if the stone is too large to pass, blood may appear in

the urine and there may be the need to urinate more often or a burning sensation during urination. If fever and chills accompany any of these symptoms, which may lead to infections.

The plant drugs survey of *Tribulus terrestris* narrates that, the drug has been utilized for pre-clinical and clinical activities. *Tribulus terrestris* L. is a member of the family *Zygophyllaceae*. It is an annual herb about 30–70 cm high and has pinnate leaves (of unequal length), yellow flowers and characteristic stellate shaped carpel fruits. It is widely distributed in Africa, Western Asia, China, Japan, Korea, Europe, Kuwait and India. The extracts from this plant parts have been used traditionally in treating a variety of diseases including hypertension and coronary heart disease, ocular inflammation and infertility in both sex as an aphrodisiac and diuretics.[1] *Boerhaavia diffusa* (Nyctaginaceae family) is a herbaceous perennial plant, native of India and Brazil, where it was used for centuries as a medicinal plant by indigenous populations. The root of *B. diffusa* is used for the treatment of many diseases, such as liver disorders (jaundice, hepatitis, etc.), gastrointestinal disorders (as laxative), renal disorders (for calculations, cystitis and nephritis), and for the treatment of anaemia and of menstrual syndrome. The drug has recently been used as an adjuvant in an anticancer therapy.[2] Spironolactone is a synthetic 17-lactone drug which is a renal competitive aldosterone antagonist in a class of pharmaceuticals called potassium-sparing diuretics, used primarily to treat heart failure, ascites in patients with liver disease, low-renin hypertension, hypokalemia, and Conn's syndrome. On its own, spironolactone is only a weak diuretic, but it can be combined with other diuretics.[3] Furosemide,[4] a 'water pill,' is used to reduce the swelling and fluid retention caused by various medical problems, including heart or liver disease. It is also used to treat high blood pressure. It causes the kidneys to get rid of unneeded water and salt from the body into the urine. diuretic which is an anthranilic acid derivative. Chemically, it is 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid. The above synthetic drugs Spironolactone and Furosemide been used as a diuretic and most of the diuretic drugs have been used in urolithiasis treatment, so, it is been tried and compared with plant drug extract for the observation.

Cystone [5] has been reported to be useful in urolithiasis, as it corrects the crystalloid and colloid balance and also acts by disintegrating calculi and crystals. The action of disintegration is due to its action on mucin, which binds particles together in a calculus. In addition, Cystone helps by flushing the urinary passage by virtue of its diuretic action. It also relaxes smooth musculature of the urinary tract, thereby relieving spasms. Thus the patient is relieved of the agony of colics and pain. Cystone works in cases of urolithiasis due to its comprehensive activity that leads to downward passage of calculi.

The formulation of Cystone (The Himalaya Drug Co.) contains extracts of:

Didymocarpus pedicellata 65 mg  
Saxifraga ligulata 49 mg  
Rubia cordifolia 16 mg  
Cyperus scariosus 16 mg  
Achyranthes aspera 16 mg  
Onosma bracteatum 16 mg  
Vernonia cinerea 16 mg  
Shilajeet (purified) 13 mg  
Hajrul yahood bhasma 16 mg

Hajrul yahood bhasma is prepared with *Ocimum basilicum*, *Tribulus terrestris*, *Mimosa pudica*, *Dolichos biflorus*, *Pavonia odorata*, *Equisetum arvense*, *Tectona grandis* seed. The tablet formulation which as been used to compare the effect against drugs and extracts used.

## MATERIALS AND METHODS

### Plant materials

The drug *Tribulus terrestris* fruits and *Boerhaavia diffusa* roots collected from Natural Remedies Pvt Ltd Bangalore India and identified and authenticated by NISCOM, New Delhi. The collected were coarse powdered using the pulverizer, this coarse powder was stored in air tight container preceding to extraction. The Spironolactone and Furosemide drugs with required standards of quality were procured from Aventis Pharma Ltd., Cystone, a polyherbal formulation was secured from Pharmaceutical supermarket market.

**Preparation of extract** Subjected for sequential extraction process using soxhlet apparatus with different solvent system like, pet ether, chloroform, ethanol, and water, and there residual extracts were collected.

### Experimental Work

The experiment consisted of the following test tubes of 10 ml capacity and marked the tubes as control and tests into 12 groups, each group has 6 test tubes, in each tube 1ml of calcium chloride anhydrous and 1ml sodium oxalate were added to the tubes and 2 ml of tris buffer (disodium hydrogen phosphate and potassium dihydrogen phosphate) adjusted at 7.4 pH which to the kidney pH and incubated at 36.7<sup>0</sup>C over night.[6,7] The next day the test tubes were centrifuged for 10min to decant to remove top liquid layer. The calcium oxalate crystal formed in the test tube were checked using the compound microscope under 45x magnification, the crystal formed were resembling the prisms shape, to this 5ml (5mg/ml) equivalent to 25mg to each test tube of the different sequential extracts of plant *Tribulus terrestris* and *Boerhaavia diffusa* were induced to the tubes and at the same quantity the synthetic drugs Spironolactone, Furosemide and the Poly herbal formulation Cystone were administered to the test tube, all the above treating agents was administered as aqueous suspension using tween 60 as suspending agent and again it was incubated 36.7<sup>0</sup>C for 3 days on the fourth day all the test tubes were taken and checked for dissolution of the crystals under the microscope at the same superimposition, to this test a drop of con Hcl were added to separate the oxalate ion [8] calcium [9] and both the ions were spectroscopically analyzed.

**Groupings:**Group- I ⇒ generated calcium oxalate crystals and referred as control, Group- II ⇒ generated calcium oxalate crystals + 5ml **Furosemide**, Group- III ⇒ generated calcium oxalate crystals + 5ml **Spironolactone**, Group-IV ⇒ generated calcium oxalate crystals + 5ml **Cystone**, Group-V ⇒ generated calcium oxalate crystals + 5ml **Pet Ether Extract of *Tribulus terrestris***, Group-VI ⇒ generated crystals + 5ml **Chloroform Extract of *Tribulus terrestris***, Group-VII ⇒ generated crystals + 5ml **Alcohol Extract of *Tribulus terrestris***, Group- VIII ⇒ generated crystals + 5ml **Water Extract of *Tribulus terrestris***, Group- IX ⇒ generated calcium oxalate crystals + 5ml **Pet Ether Extract of *Boerhaavia diffusa***, Group- X ⇒ generated crystals + 5ml **Chloroform Extract of *Boerhaavia diffusa***, Group-XI ⇒ generated crystals + 5ml

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**Alcohol Extract** of *Boerhaavia diffusa*, Group-XII  $\Rightarrow$  generated crystals + 5ml **Water Extract** of *Boerhaavia diffusa*.

### Elemental Ions Analysis

#### Test for oxalate

The determination of oxalic acid in 0.5ml of generated crystals samples, the co-precipitated oxalic acid with calcium sulphate, which is reduced to glycollic acid by boiling with dilute sulphuric acid and a zinc pellet and estimated colorimetrically with chromotropic acid at 570 nm. [8]

#### Test for Calcium

In acid medium, calcium binds with O-Cresolphthalein Complexone (O-CPC) to produce a purple colour, which absorbs at 570 nm is proportional to the concentration of calcium. [9]

#### Microscopical studies

On revise, before adding the dilute Hcl, the different extracts and the other agents treated on the regenerated calcium oxalate crystals in test tubes were observed under the 10X magnification using the photographic microscope and pictures were taken and pragmatic effects of the agents were studied. the crystals formed is rated on assumption as per to the scores ranges from 0-4 marks which on comparison to the control group-1 with the others treated groups and the crystals formation are denoted by the arrow marks(Fig: 1-12).

#### Data analysis

The data of urinary and renal parameters were expressed as mean  $\pm$  SEM. The results were analyzed statistically using one way ANOVA. The minimum level of significance was fixed at  $P < 0.05$ . Comprasion were made between the group I with the groups II to XII.

## RESULTS AND DISCUSSION

The *in-vitro* lithiatic activity which has been carried, where the calcium oxalate crystals was generated by the sodium oxalate and calcium chloride on incubation at 36.7<sup>0</sup>C with tris phosphate buffer at 7.4 pH. The generated crystals were treated with the different agents mentioned above in experimental part into twelve groups and group-I kept as control and others groups as treated, the estimation of calcium and oxalate were carried out, on comparison, the group-I with other groups found that the alcoholic extract of both plants *Boerhaavia diffusa*, *Tribulus terrestris* has shown significant and better action in dissolving the crystals, the cystone and spironolactone which has shown restrained activity, where as other extract and furosemide were much equivalent to the control and found non significant on measuring the ion content.(Table1).

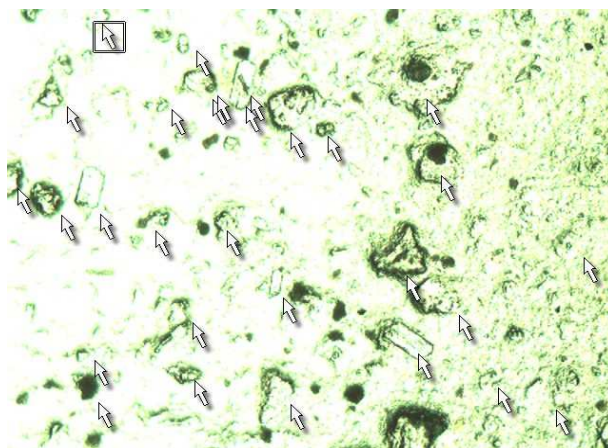
**Table 1: Effect of the drugs and extracts treated on generated calcium oxalate analysis**

Parameters mg/generated crystals in test tube	Group- I Control Generated Calcium oxalate	Group- II 5mg/ml Furosemide	Group- III 5mg/ml Spiranolactone	Group- IV 5mg/ml Cystone
Oxalate	<b>15.02 ±0.45</b>	<b>14.78<sup>d</sup> ±0.66</b>	<b>10.53<sup>c</sup> ±0.66</b>	<b>6.77<sup>b</sup> ±0.50</b>
Calcium	<b>6.20 ±0.29</b>	<b>5.24<sup>d</sup> ±0.20</b>	<b>3.01<sup>c</sup> ±0.25</b>	<b>2.66<sup>b</sup> ±0.21</b>
Parameters mg/generated crystals in test tube	Group- V 5mg/ml pet ether extract of Tt	Group- VI 5mg/ml chloroform Extract of Tt	Group- VII 5mg/ml alcohol extract of Tt	Group- VIII 5mg/ml water extract of Tt
Oxalate	<b>15.44<sup>d</sup> ±0.44</b>	<b>14.87<sup>d</sup> ±0.79</b>	<b>3.97<sup>a</sup> ±0.32</b>	<b>6.66<sup>b</sup> ±0.57</b>
Calcium	<b>5.84<sup>d</sup> ±0.31</b>	<b>6.15<sup>d</sup> ±0.10</b>	<b>2.30<sup>a</sup> ±0.19</b>	<b>2.48<sup>b</sup> ±0.16</b>
Parameters mg/generated crystals in test tube	Group- IX 5mg/ml pet ether extract of Bd	Group- X 5mg/ml chloroform extract of Bd	Group- XI 5mg/ml alcohol extract of Bd	Group- XII 5mg/ml water extract of Bd
Oxalate	<b>16.29<sup>d</sup> ±0.30</b>	<b>15.19<sup>d</sup> ±0.53</b>	<b>4.92<sup>a</sup> ±0.33</b>	<b>8.57<sup>b</sup> ±0.35</b>
Calcium	<b>6.03<sup>d</sup> ±0.07</b>	<b>5.35<sup>d</sup> ±0.12</b>	<b>2.43<sup>a</sup> ±0.16</b>	<b>2.36<sup>b</sup> ±0.21</b>

Comparison were made between the group I with the groups II to XII; Statistical significance: a = <sup>ns</sup> P > 0.05, b = \*P < 0.05, c = \*\*P < 0.01, d = \*\*\*P < 0.001; Bd => *Boerhaavia diffusa*; Tt => *Tribulus terrestris*

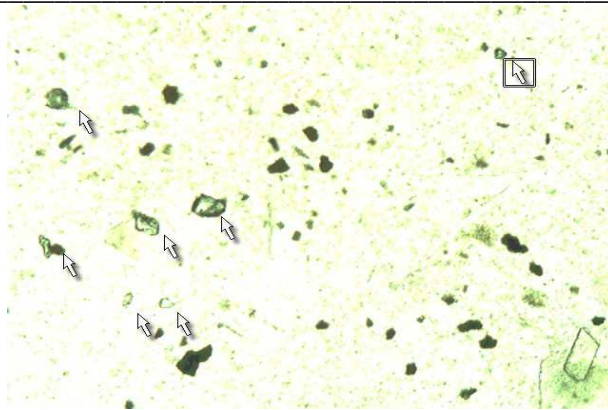
## CONCLUSION

Both the ions which was analyzed under visible spectroscopy. on detail in-vitro study, it was found that the alcoholic extracts of the both plants *Tribulus Terrestris* and *Boerhaavia diffusa* has shown significant anti-lithiatic activity in dissolution of regenerated calcium oxalate crystals.

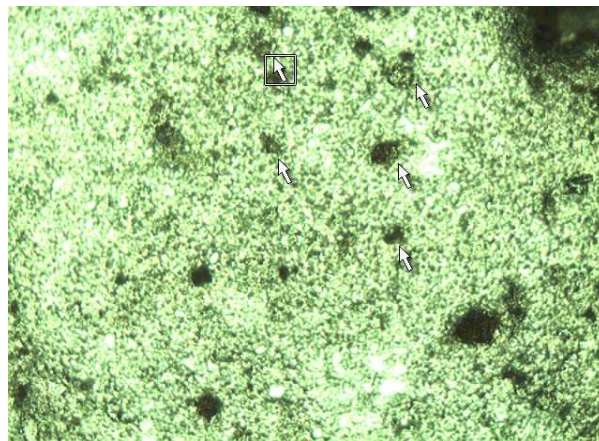


**Fig: 1 CONTROL Group-I: The huge number of calcium oxalate crystals creation were seen and scored 4**

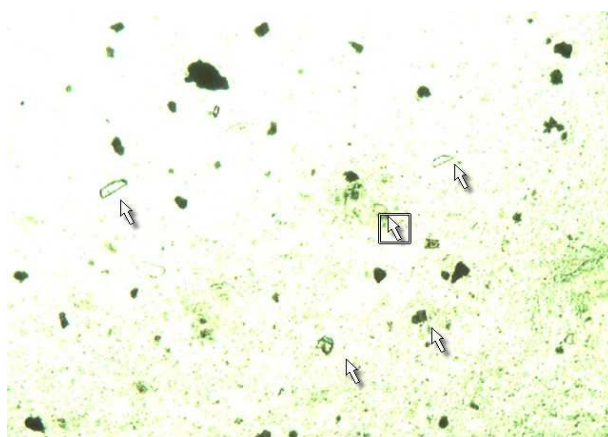




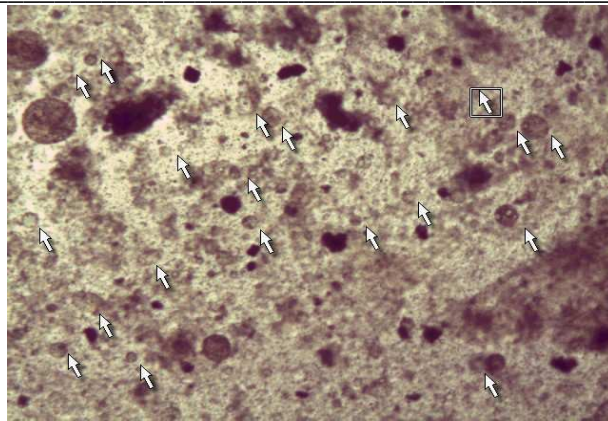
**Fig: 2 FUROSEMIDE Group II:** The huge number of calcium oxalate crystals creation were not dissolved by the drug and scored 4



**Fig: 3 SIRANOLACTONE Group III:** The number of calcium oxalate crystals dissolutions was less by the drug and scored as 2



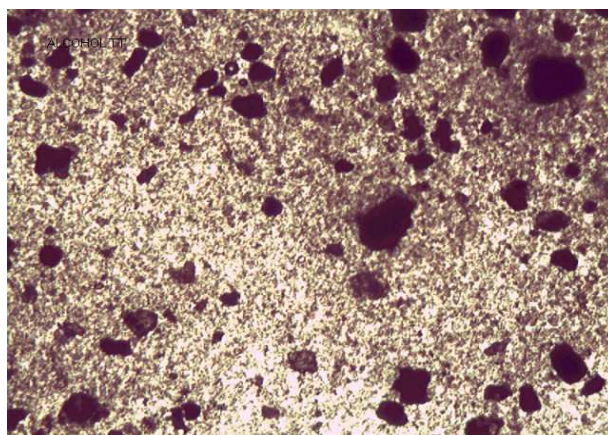
**Fig: 4 CYSTONE Group -IV** The number of calcium oxalate crystals dissolution more the polyherbal formulation and scored as 1



**Fig: 5 PET ETHER EXTRACT of *Tribulus terrestris* Group V: The huge number of calcium oxalate crystals creation found undissolved by the extract and scored 4**



**Fig: 6 CHLOROFORM EXTRACT of *Tribulus terrestris* Group VI: The number of calcium oxalate crystals creation found undissolved by the extract and scored 4**

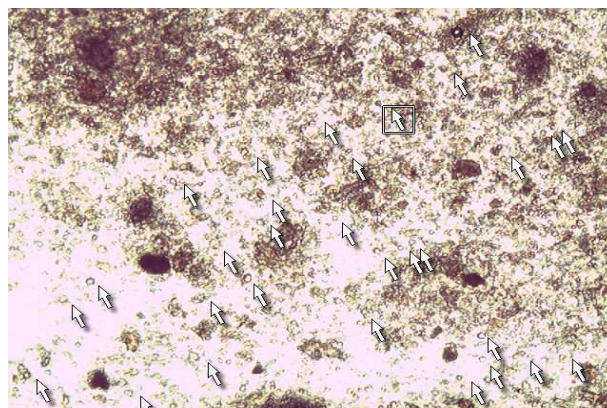


**Fig: 7 ALCOHOL EXTRACT of *Tribulus terrestris* Group VII: The number of calcium oxalate crystals was dissolved maximum by the extract and scored 0**

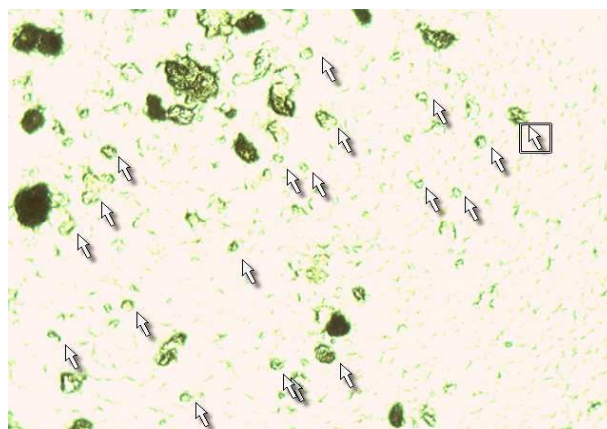




**Fig: 8 WATER EXTRACT of *Tribulus terrestris* Group VIII: The number of calcium oxalate crystals was dissolved less by the extract and scored 2**

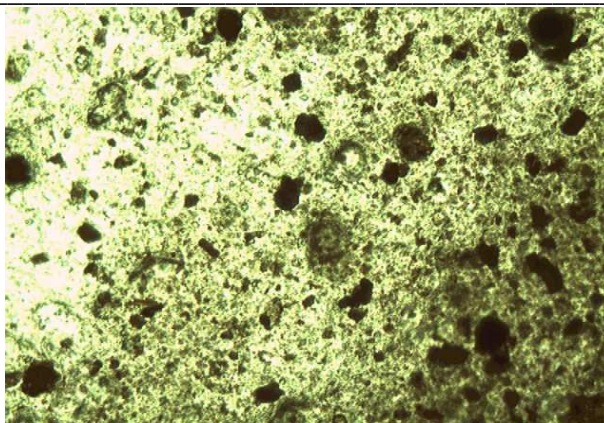


**Fig: 9 PET ETHER EXTRACT of *Boerhaavia diffusa* Group IX: The huge number of calcium oxalate crystals formation was not dissolved by the extract and scored 4**

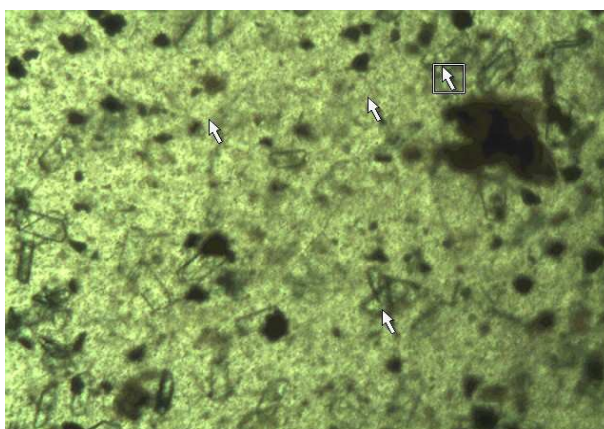


**Fig: 10 CHLOROFORM EXTRACT of *Boerhaavia diffusa* Group X: The number of calcium oxalate crystals formation was not dissolved by the extract and scored as 4**





**Fig:11 ALCOHOL EXTRACT of *Boerhaavia diffusa* Group XI: The number of calcium oxalate crystals was dissolved maximum by the extract and scored as 0**



**Fig:12 WATER EXTRACT of *Boerhaavia diffusa* Group XII: The number of calcium oxalate crystals formation dissolved were less by the extract and scored 2**

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