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INVITRO ANTI-UROLITHIATIC POTENTIAL OF SAUROPUS ANDROGYNOUS AND POLYALTHIA LONGIFOLIA

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ABSTRACT

Despite advances in modern medicine, the urolithiasis continues to be a source of concern for mankind, as there is no effective treatment for kidney stones. In the present study we investigated antiurolithiatic activity of *Sauropus androgynous* and *Polyalthia longifolia* against calcium oxalate precipitation. Both the extracts are dissolved in water and the concentration of 100, 300, 500 and 700µg/ml are obtained. A mixture of 1 ml of artificial urine and 0.5 ml of extract solution is taken in the cell. A blank reading is taken and then 0.5 ml of 0.01 M sodium oxalate solution is added and immediately absorbance is measured for a period of the 10 minutes with 2 minutes interval at 620 nm. The results of ethanolic plant extracts of *Sauropus androgynous* exhibits dose and time-dependent % inhibition. The inhibition started by 100µg\ml with 15.77% and maximum inhibition 700 µg\ml was observed at 37.2%. The results of ethanolic plant extracts of *Polyalthia longifolia* exhibits dose and time-dependent % inhibition. The inhibition started by 100µg\ml with 22.14% and maximum inhibition 700 µg\ml was observed at 64.22% respectively. Both the extract shows significant dose and time-dependent % inhibition. This study emphasized the need to carry out in-depth pharmacological evaluations of this property in other dissimilar models and further isolation of chemical constituents responsible for antiurolithiatic activity.

KEYWORD: Urolithiasis, *Phyllanthaceae*, *Annonaceae*, Calcium oxalate, Cystone, Renal calculi.

INTRODUCTION

Urolithiasis is a condition arising from the formation of renal calculi when the urine is supersaturated with salt and minerals such as calcium oxalate, struvite (Ammonium magnesium phosphate), uric acid and cystine.[1] The worldwide incidence of urolithiasis is quite high and more than 80% of urinary calculi are calcium oxalate stone alone or calcium oxalate mixed with calcium phosphate. These urinary tract stones vary considerably in size from small 'gravel-like' stones to large staghorn calculi.[2]

The other factor that leads to urolithiasis/urinary tract stone production is the formation of Randall's plaques. Calcium oxalate precipitates form in the basement membrane of the thin loops of Henle; these eventually accumulate in the subepithelial space of the renal papillae, leading to a Randall's plaque and eventually a calculus.[3]

Urolithiasis is a condition in which kidney stones form from an excess of mineral deposits in the urinary tract. This disorder involves the crystallization of minerals in the kidneys, bladder, or ureters.[5] Urolithiasis arises when the equilibrium between kidney stone inhibitors (like magnesium) and promoters (such as uric acid) is disturbed in the kidneys.[6]

Kidney stones are known for being one of the most severe urologic ailments, affecting 5 to 15% of adults. Studies indicate that nephrolithiasis is more prevalent in men (12%) than in women (6%), with the highest incidence occurring between ages 20 and 40 for both genders. In developed countries, urinary stones impact 10-12% of the population. The frequency of these stones has been on the rise in recent years, with a trend toward younger ages of onset. With a prevalence exceeding 10% and a recurrence rate of about 50%, kidney stones pose a significant burden on healthcare systems.[4]

Sauropus androgynous

Sauropus androgynous popularly known as Katuk or Sweet leaf belongs to family *Phyllanthaceae*.[9] The leaves of this shrub have shown superior nutritive value compared to other leafy vegetables.[10] In India the leaves of this herb have been using in cooking as a vegetable because of its inexpensive source of dietary proteins.[11] The active compounds of *Sauropus androgynous* contains sauroposide which can also be detected i.e., alkaloids, flavonoids, carotenoids, ascorbic acid,

glycosides, tannins, saponins, terpenoids, phenols, steroids. It has high level of provitamin A, especially freshly picked leaves are rich in vit C and B, protein and minerals.[9] The decoction of its roots has also been using in nose ulcer and fever. The Leaves and roots of these shrub is also beneficial to cure anaemia, high blood pressure, eye troubles, to blood circulation and for regular bowel elimination.[12,16]

In Malaysia, traditional medicine uses this plant to soothe fevers, treat urinary troubles, and increase breast milk supply. It is known as the "multigreen" vegetable. due to its reputedly exceptional nutrition, vitamin content and traditional uses it is selected for antiurolithiatic activity.[7,8]

Polyalthia longifolia

Polyalthia longifolia cv. pendula (Annonaceae) is native to the drier regions of India and is locally known as "Ashoka". Ashoka (Latin name: Saraca asoka (Roxb) De Wilde) is also a Sanskrit name in Ayurveda of a drug used for the treatment of uterine disorders.[13] Literature report of few phytochemical screening tests on this plant shows the presence of saponins, carbohydrates, alkaloids, tannins, resins, steroids, glycosides and flavonoids as major phytochemical constituents.[14] *Polyalthia longifolia* is very versatile plant due to its chemical constituents which are responsible for its various pharmacological actions and medicinal properties like Anti-inflammatory activity, Anti-pyretic action, Anti-microbial activity, Anti-hepato-protective activity, Anti-oxidant activity.[15] *Polyalthia longifolia*, a key indigenous medicinal species in Indian herbal medicine and is widely employed in traditional remedies as a febrifuge and tonic. Nearly all parts of this plant are utilized in traditional medicine for treating various conditions, it is selected for anti-urolithiatic activity.

Antiurolithiatic activity

Experimental design

The effect of the extract on calcium oxalate crystallization is determined by the time course measurement of turbidity changes due the crystallization in artificial urine on the addition of 0.01M sodium oxalate solution. The precipitation of calcium oxalate at 37^0C and pH 6.8 has been studied by the measurement of turbidity at 620nm using UV/Visible spectrophotometer.[17]

Preparation of synthetic urine

For preparation of synthetic urine 3.8 gm of potassium chloride, 8.5 gm of sodium chloride, 24.5 gm of urea, 1.03 gm of citric acid, 0.34 gm of ascorbic acid, 1.18 gm of potassium phosphate, 1.4 gm of creatinine, 0.64 gm of sodium hydroxide, 0.5 gm of calcium chloride, 0.47 gm of sodium bicarbonate and 0.28 ml of sulphuric acid were added in 500 ml of de ionized water and stirred for 1 hr. The synthetic urine is stored until further use.[18]

Study without inhibitor

The volume of 1.0 ml of artificial urine is transferred into the cell and 0.5 ml of distilled water added to it and blank reading is taken. The 0.5 ml 0.01 M sodium oxalate is added to the previous volume and the measurement is determined immediately and recorded for 10 minutes.

Study with inhibitor

The extract is dissolved in water and the concentration of 100, 300, 500 and 700µg/ml are obtained. A mixture of 1 ml of artificial urine and 0.5 ml of extract solution is taken in the cell. A blank reading is taken and then 0.5 ml of 0.01 M sodium oxalate solution is added and immediately absorbance is measured for a period of the 10 minutes with 2 minutes interval at 620 nm.[17]

The % inhibition is calculated by using the following formula

% inhibition = absorbance of control – absorbance of test ×100/ absorbance of control

RESULT

Sauropus androgynous

Calcium oxalate crystallization inhibition by leaf extract of *sauropus androgynous*

The weight of *Sauropus androgynous* leaf powder = 75gmWeight of the extract obtained = 6.39 gm

% yield = Weight of the extract ÷ Weight of powder×100

= 6.39/75 × 100

= 8.52% w/w

Absorbance value of *sauropus androgynous*

Determination of plant extract

Sl.No	% Inhibitionat time inmins	Without inhibition	With inhibition 100	% Inhibition 100%	With inhibition 300	% Inhibition 300%	With inhibition 500	% Inhibition 500%	With inhibition 700	% Inhibition 700%
1	0	0.357	0.285	20.16%	0.253	29.13%	0.228	36.13%	0.209	41.45%
2	2	0.357	0.296	17.08%	0.253	29.13%	0.230	35.57%	0.216	39.49%
3	4	0.357	0.297	16.08%	0.268	24.92%	0.235	34.17%	0.229	35.85%
4	6	0.357	0.304	14.84%	0.279	21.84%	0.235	34.17%	0.229	35.85%
5	8	0.357	0.304	14.84%	0.279	21.84%	0.247	30.81%	0.231	13.%
6	10	0.357	0.318	10.92%	0.282	21%	0.247	30.81%	0.231	13.16%

Influence of extract of *Sauropus androgynous* on Calcium oxalate precipitation

Concentration of extract (µgm/ml)	% Inhibition
100	15.77%
300	24.64%
500	33.61%
700	37.2%

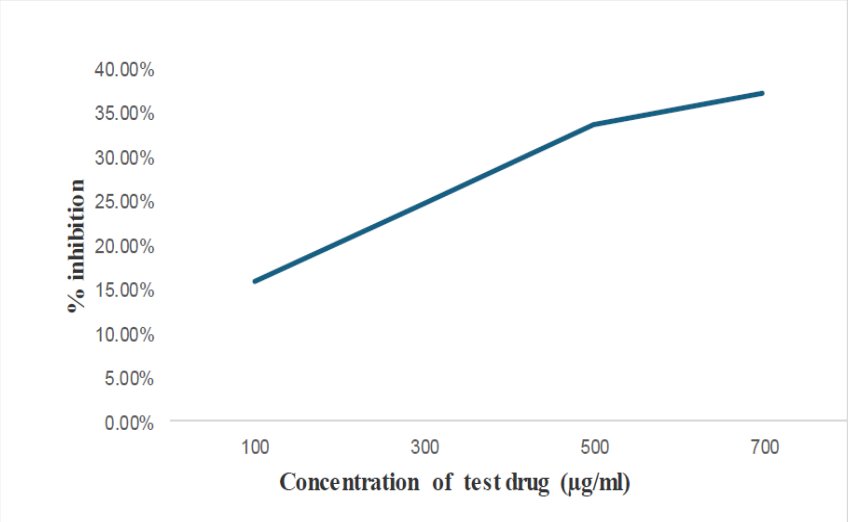

Figure: Effect of extract on calcium oxalate precipitation.

Polyalthia longifolia

Calcium oxalate crystallization inhibition by seed extract of *polyalthia longifloia*

The weight of *Polyalthia longifolia* seeds powder = 150gm

Weight of the extract obtained = 4.65 gm

% yield = Weight of the extract ÷ Weight of powder×100

= 4.65/150 × 100

= 3.1% w/w

Absorbance value of *polyalthia longifolia*

Determination of plant extract

Sl.no	% Inhibitionat time inmins	Without inhibition	With inhibition	% Inhibition	With inhibition	% Inhibition	With inhibition	% Inhibition	With inhibition	% Inhibition
			100	100%	300	300%	500	500%	700	700%
1	0	0.11	0.07	31.8%	0.06	44.5%	0.03	65.4%	0.023	79.0%
2	2	0.11	0.08	24.3%	0.06	38.6%	0.04	59.4%	0.025	77.4%
3	4	0.11	0.08	22.3%	0.07	35.3%	0.04	56.2%	0.033	70.5%
4	6	0.11	0.09	19.4%	0.07	34.5%	0.05	49.5%	0.046	59.2%
5	8	0.11	0.09	18.4%	0.07	32.4%	0.06	44.7%	0.054	52.6%
6	10	0.11	0.09	16.5%	0.08	30.4%	0.068	40.86%	0.069	41.1%

Influence of extract of *Polyalthia longifolia* on Calcium oxalate precipitation

Concentration of extract (µgm/ml)	% Inhibition
100	22.14%
300	35.99%
500	50.22%
700	64.22%

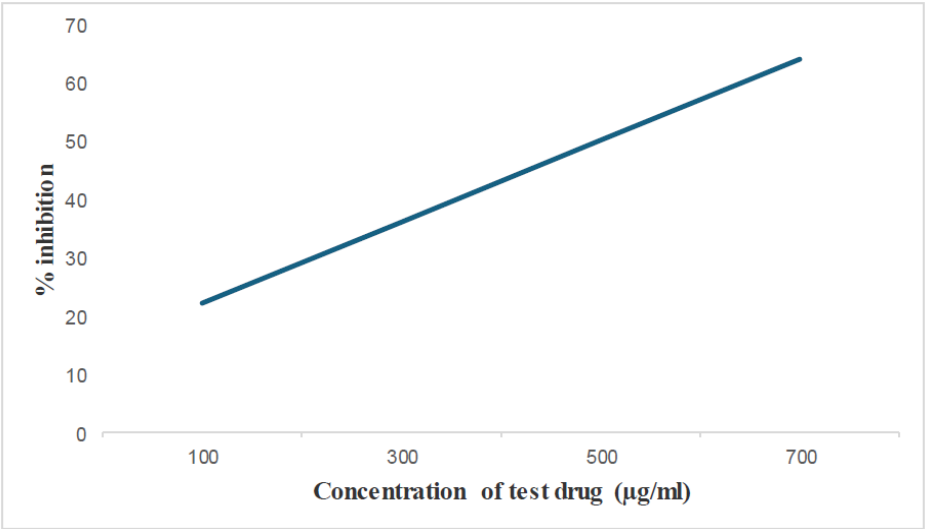

Figure: Effct of extract on calcium oxalate precipitation.

Determination of standard drug

Sl.No	% Inhibition at time in mins	Without inhibition	With inhibition 100	% Inhibition 100%	With inhibition 300	% Inhibition 300%	With inhibition 500	% Inhibition 500%	With inhibition 700	% Inhibition 700%
1	0	0.295	0.173	41.35%	0.130	55.93%	0.101	65.76%	0.052	82.37%
2	2	0.299	0.189	36.78%	0.146	51.17%	0.09	69.89%	0.065	78.26%
3	4	0.305	0.218	28.52%	0.163	46.55%	0.112	63.27%	0.071	76.72%
4	6	0.342	0.256	25.14%	0.171	43.93%	0.14	59.06%	0.089	73.97%
5	8	0.380	0.287	24.47%	0.227	40.26%	0.167	56.05%	0.11	71.05%
6	10	0.397	0.307	22.67%	0.252	36.52%	0.20	49.62%	0.124	68.76%

Influence of Standard drug on Calicum oxalate precipitation

Concentration of extract (µg/ml)	% Inhibition
100	29.82%
300	45.72%
500	60.60%
700	75.18%

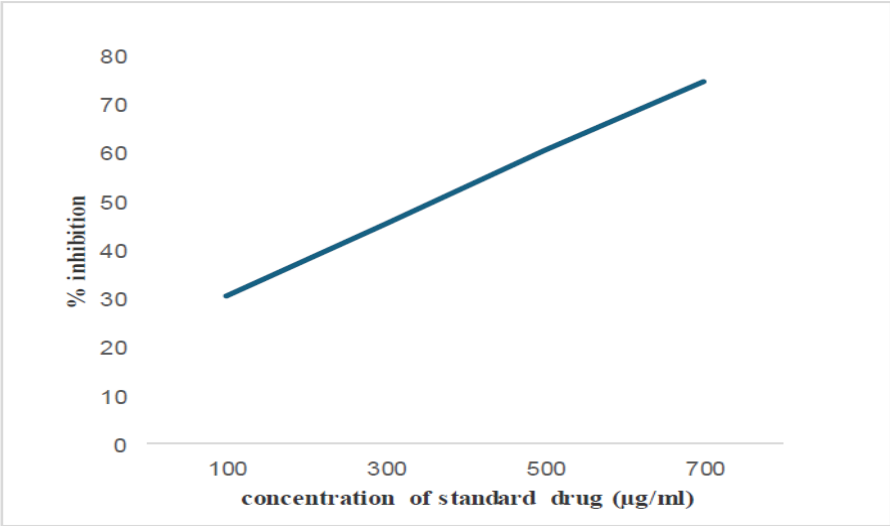

Figure: Effect standard drug on calcium oxalate precipitation.

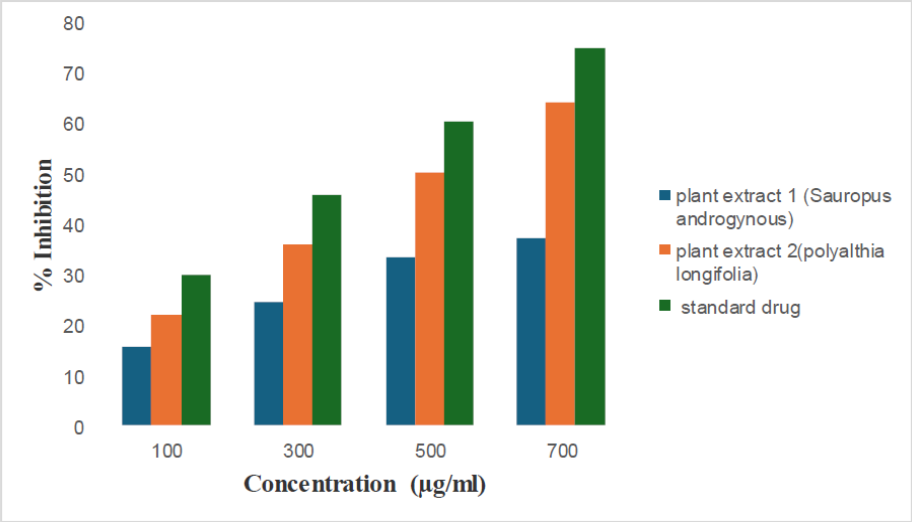

Figure: Comparison of standard drug and plant extracts.

DISCUSSION

The results of *In-vitro* Anti-Urolithiasis activity of ethanolic plant extracts of *Sauropus androgynous* exhibits dose and time-dependent % inhibition. The inhibition started by 100µg\ml with 15.77% and maximum inhibition 700 µg\ml was observed at 37.2%.

The results of *In-vitro* Anti-Urolithiasis activity of ethanolic plant extracts of *Polyalthia longifolia* exhibits dose and time-dependent % inhibition. The inhibition started by 100µg\ml with 22.14% and maximum inhibition 700 µg\ml was observed at 64.22%.

The results of *In-vitro* Anti-Urolithiasis activity of standard drug (Cystone) exhibits dose and time-dependent % inhibition. The inhibition started by 100µg\ml with 30.43 % and maximum inhibition 700 µg\ml was observed at 74.89%.

Both the extract shows significant dose and time-dependent % inhibition. when extracts compared with standard drug, the ethanolic extract of *Polyalthia longifolia* exhibits maximum % of inhibition and significantly reduces the calcium oxalate precipitation. both the drugs reduce precipitation and shows the anti-urolithiatic property. further studies on isolation and screening of this drugs are required.

CONCLUSION

Based on results, it has concluded that the ethanolic extract of *Sauropus androgynous* and *Polyalthia longifolia* of doses 100, 300,500 and 700 µg/ml has possessed Antiurolithiatic property. The ethanolic extract of *Polyalthia longifolia* with same dose shown significant percentage of inhibition than the ethanolic extract of *Sauropus androgynous.*

This study emphasized the need to carry out in-depth pharmacological evaluations of this property in other dissimilar models and further isolation of chemical constituents responsible for antiurolithiatic activity.

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