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Evaluation of Diuretic and Anti-Urolithiatic Activities of Ethanolic Leaf Extract of *Sida Acuta*

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ABSTRACT

The present study was undertaken to investigate the Diuretic and antiurolithiatic activities of Ethanolic leaf extract of *Sida Acuta* in Albino rats. Ethanolic leaf extract was administered to experimental rats orally at doses of 200mg/kg and 400mg/kg (each p.o). Furosemide (5mg/kg) was used as a standard. The diuretic effect of the extract was evaluated by measuring the urine volume and determining sodium, potassium, chloride and bicarbonate contents. In *In vitro* antiurolithiatic activity Calcium oxalate crystallization was induced by the addition of 0.01M sodium oxalate solutions in synthetic urine. The effect of extract (100, 300 and 500µg/ml) was studied by time course measurement of absorbance. *In-vivo* Urolithiasis was induced in male rats by administering ethylene glycol (0.75%) in drinking water to groups II-V except normal control (Group I) for 28 days. Groups I, II and III served as normal control, positive control (hyperurolithiatic), and standard (cystone 750mg/kg), respectively, Groups IV and V served as curative regimen. Oxalate, calcium, phosphate were monitored in urine. Serum calcium, creatinine and uric acid were also recorded. The extract of *Sida acuta* was safe and exhibited no gross behavioral changes in the rats. A significant diuretic effect was observed from the experimental animals treated with extract of *Sida acuta* individually compared to the control. The results obtained suggest potential usefulness of extract of *Sida acuta* leaf as an antiurolithiatic agent.

Keywords: *Sida acuta*, Medicinal plants, Diuretic and Urolithiatic activity.

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INTRODUCTION

Diuretics are the agents which augment the renal excretion of sodium and either chloride or bicarbonate primarily and water excretion secondarily. The term saluretic is some time used to describe a drug that increases the renal excretion of sodium and chloride ions[1]. Diuretics play an important role in situations of hypercalciuria, edema, like acute and chronic renal failure, cirrhosis of liver, and acts as an antihypertensive agent. A number of diuretics like thiazides, furosemide, mannitol, and ethacrinic acid are used in practice[2]. Urolithiasis is the formation of stones in the urinary tract that prominently cause variable degree of pain, bleeding, and further may lead to secondary infection. It is one of the third most common afflictions found in humans[3]. *Sida acuta* (malvaceae), is an erect perennial shrub found throughout the hotter parts of Nepal and India. It is used for various medicinal purpose such as liver disorders, asthma, fever, headache (migrane), cough, cold, ulcer, anthelmintic, snake bite, urinary diseases, female disorders, antifertility agent and sedative, diuretic & abortifacient in ayurvedic preparations.[4,5]. Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system of medicine but they were not properly investigated[6]. As a step in this direction we focused our attention on search of herbal remedy and selected a medicinal plant named *Sida acuta* leaf for evaluating diuretic activity. The aim of the present work is to evaluate the Diuretic and Anti-Urolithiatic Activity of *Sida acuta* leaf extract.

MATERIALS AND METHOD

Collection of plant parts

The whole plant of *Sida acuta* was collected from the surroundings of Surampalem, East Godavari dist, Andhra pradesh. The plant was identified and authenticated by the botanist Mr. T. V. Raghavarao, Department of Botany SRVBSJB Maharanee College, Peddapuram, E.G.Dist. Andhra Pradesh.

Preparation of extract

The leaves of *Sida acuta* were collected in Surampalem, East Godavari district Andhra Pradesh India. The leaves were shade dried, pulverized and sieved through 40mesh. The powdered leaves were extracted with Ethanol in soxhlet apparatus. The extract obtained was evaporated under vacuum to remove the solvent completely. Then used for biological evaluation.

Diuretic activity

Adult albino rats of either sex were used for experiment. The animals were housed in standard metal cages provided with food and water *ad libitum*. The Institutional Animal Ethical Committee

approved the experimental protocol. The method described by Lipschtiz *et al*[7], and Kavimani *et al*[8], was employed for the evaluation of diuretic activity.

The rats were grouped into 4 groups 6 rats in each group. The groups are divided as follows.

Group	Treatment
Group-I (Control)	Treated with vehicle, acacia 0.5% orally
Group-II (Standard)	Treated with Furosemide 5mg/kg orally
Group-III (Test-1)	Treated with Ethanolic leaf extract *Sida cuta* 200mg/kg
Group-IV (Test-2)	Treated with Ethanolic leaf extract *Sida cuta* 400mg/kg

Immediately after administration the rats were placed in metabolic cages, one rat per cage. The metabolic cages were provided with a funnel and a beaker for urine collection and a mesh to separate the faeces from the urine. Before placing the bladder was emptied by pulling the base of tail of each rat[9]. The volume of urine collected was recorded after 5 hrs and urine was subjected to determine the sodium, potassium ions by flame photometry[10], chlorides and bicarbonates by titrimetric analysis[11], after 24 hrs the Saluretic, Natriuretic and diuretic indices were also calculated.

ANTI-UROLITHIATIC ACTIVITY

***In-vitro* Anti-Urolithiatic activity**

Experimental Protocol:

The effect of extract on Calcium oxalate crystallization was determined by the time course measurement of turbidity changes due to the crystallization in artificial urine on addition of 0.01M sodium oxalate solution. The Precipitation of calcium oxalate at 37°C and pH 6.8 has been studied by the measurement of turbidity at 620 nm using UV/Visible spectrophotometer[12].

Preparation of synthetic urine:

Synthetic urine was prepared by dissolving 3.8gm of potassium chloride, 8.5gm of sodium chloride, 24.5gmof urea 1.03gm of citric acid, 0.34gm of ascorbic acid, 1.18gm of potassium phosphate, 1.4gm of creatinine, 0.64gm of sodium hydroxide, 0.47gm of sodium bicarbonate and 0.28ml of sulfuric acid in 500ml of deionized water and stirred for 1hour and the synthetic urine was stored at -4^0c until further use[13].

Study without inhibitor:

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

Study with inhibitor:

The extract was dissolved in distilled water filtered through membrane filter and the concentration of 100, 300 and 500µg/ml was obtained. A mixture of 1ml of artificial urine and 0.5ml of extract solution was taken in the cell. A blank reading was taken and then 0.5ml of 0.01M sodium oxalate solution was added and immediately absorbance was measured for a period of the 10 minutes with 2 min interval at 620nm[14]. The % of inhibition was calculated using the following formula:

$$\% \text{ inhibition } = \frac{(\text{absorbance of control} - \text{absorbance of test})}{(\text{absorbance of control})} \times 100$$

***In-vivo* Anti-Urolithiatic Activity**

Ethylene glycol-induced hyperoxaluria method[15] was used to assess the antiurolithiatic activity in albino rats. Animals were divided into five groups containing six animals in each. Group I served as control and received regular rat food and drinking water ad libitum was fed to Groups II–V for induction of renal calculi for 28 days. Group III received standard antiurolithiatic drug, Cystone (750 mg/kg b.w.), from 15th day till 28th day[16]. Groups IV and V served as curative and received test drug 1 &2.

Group	Treatment for 1-14days	Treatment for 14-28days
Group-I (Control)	Received regular rat food and drinking water ad libitum	received regular rat food and drinking water ad libitum
Group-II (calculi induced)	Ethylene glycol (0.75%) in drinking water	Ethylene glycol (0.75%) in drinking water
Group-III (Standard)	Ethylene glycol (0.75%) in drinking water	Ethylene glycol (0.75%) in drinking water + Cystone (750 mg/kg b.w.)
Group-IV (Test-1)	Ethylene glycol (0.75%) in drinking water	Ethylene glycol (0.75%) in drinking water + Ethanolic extract (200 mg/kg b.w.)
Group-V (Test-2)	Ethylene glycol (0.75%) in drinking water	Ethylene glycol(0.75%) in drinking water + Ethanolic extract (400 mg/kg b.w.)

RESULTS AND DISCUSSION

Diuretic activity

The results obtained in diuretic activity were shown in Table 1 & 2. Table 1 and figure 1 shows urine volume (mL/5hrs) and excretion of electrolytes sodium, potassium, chlorides and bicarbonate ions in urine.

Table 1: Comparison of diuretic effect of *Sida acuta* to that of Control

Group	Volume of urine (mL) after 5hrs	Na^+ µmoles/Kg	K^+ µmoles/Kg	Cl^- µmoles/Kg	HCO_3^- µmoles/Kg
Group I (Control)	0.15±0.04	183.33±0.35	121.48±0.48	98.66±0.59	9.97±0.17
Group II (Standard)	0.74±0.01**	232.14±0.65*	144.34±0.20*	152.52±0.39**	25.36±0.33**
Group III (*Sida acuta*- 200mg/kg)	0.31±0.03	180.23±0.03	124.93±0.45	140.98±0.33*	24.24±0.53*
Group IV *(Sida acuta*- 400mg/kg)	0.52±0.06*	196.21±0.52	128.76±0.53	158.92±0.39***	27.10±0.39***

Values are expressed as Mean ± SEM; n=6 (number of animals in each group); $p<0.001$.

All comparisons are made with that of control.

Table 2 and figure 2 shows the saluretic, natriuretic and diuretic indices of ethanol extract of leaf *Sida acuta*. From the results it can be observed that ethanolic extract of leaf *Sida acuta* shown significant diuretic effect by increasing the urine output and increased excretion of electrolytes sodium, potassium, chlorides and bicarbonate ions in urine when compared to that of control.

Table 2: Comparison of Saluretic, Natriuretic & Diuretic indices of *Sida acuta* to that of control.

Sl.no.	Group	Saluretic Index [Na^+ +Cl^-]	Natriuretic Index[Na^+/K^+]	Diuretic Index
1	Group I (Control)	272.02	1.42	-
2	Group II (Standard)	384.66	1.61	4.9
3	Group III *(* SA -200mg/kg)	321.21	1.45	2.0
4	Group IV *(* SA -400mg/kg)	355.13	1.53	3.4

Diuretic Index = {volume of urine in test group/volume of urine in control group}

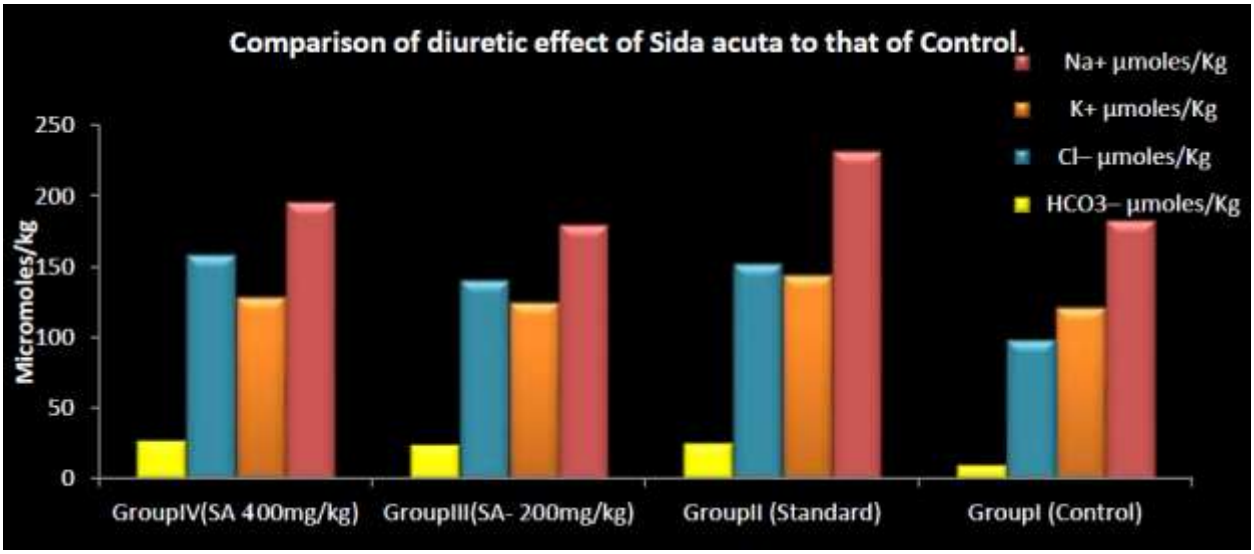

Figure 1: Comparison of diuretic effect of Ethanolic leaf extract of *Sida acuta* to that of Control.

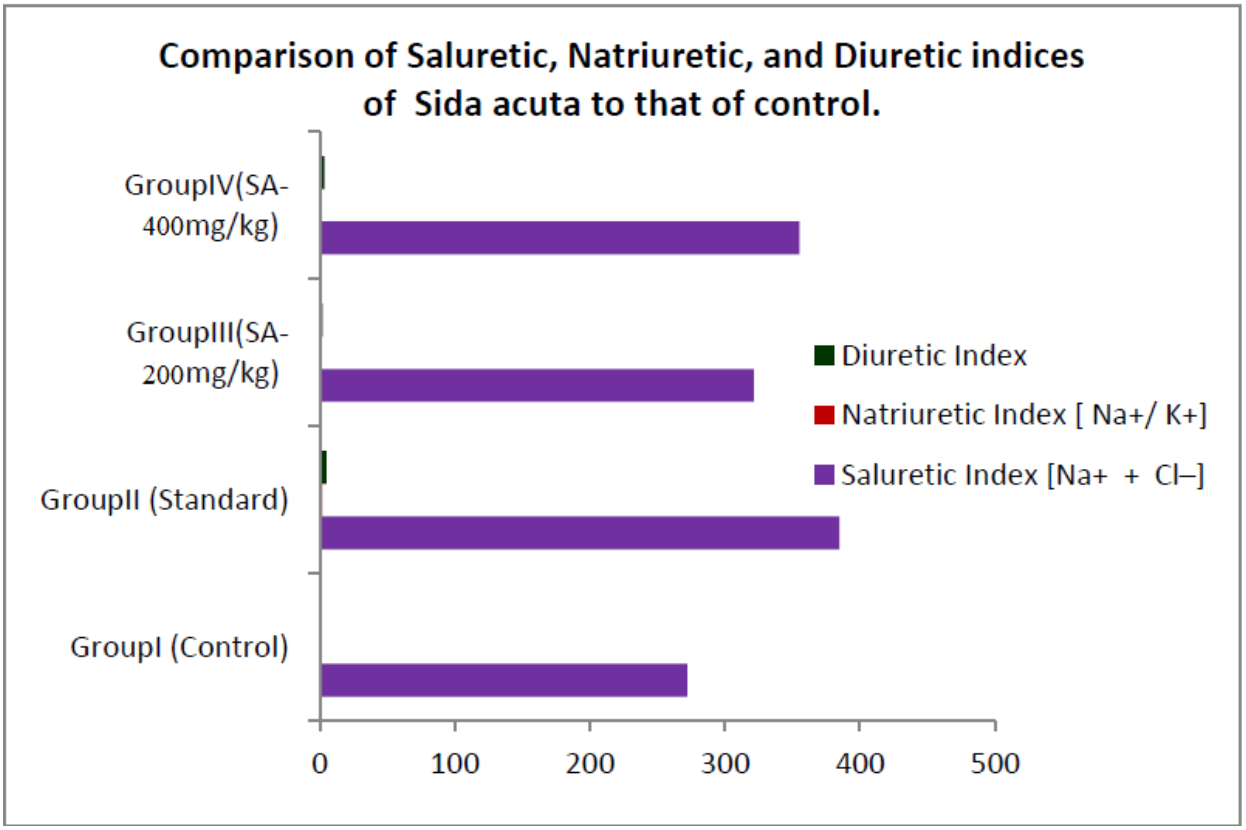

Figure 2: Comparison of Saluretic, Natriuretic, and Diuretic indices of Sida acuta to that of control.

Anti-Urolithiatic Activity

***In-vitro* method**

In *in-vitro* antiurolithiatic activity, Calcium oxalate crystallization was induced by the addition of 0.01M sodium oxalate solutions in synthetic urine. The effect of extract (100, 300 and 500µg/ml) was studied by time course measurement of absorbance at 620 nm for ten minutes by means of a spectrophotometer. Ethanolic extract showed inhibition at 0 min 22.56, 36.25 and 42.68%), and maximum inhibition of the crystallization of calcium oxalate at 10 min (46.25, 56.35 and 70.92%). The results of in *In-vitro* Antiurolithiatic activity of *Sida acuta* leaf extract are given in Table 3 and Figure 3

Table 3 *In-vitro* Antiurolithiatic Activity of Ethanolic Leaf Extract of *Sida acuta*

	100 µg\ml	**300 µg\ml**	**500 µg\ml**
0 min	22.56	36.25	42.68
2 min	27.64	35.25	50.62
4 min	37.14	45.14	60.25
6 min	37.56	48.23	63.25
8 min	43.12	50.23	67.50
10 min	46.25	56.35	70.92

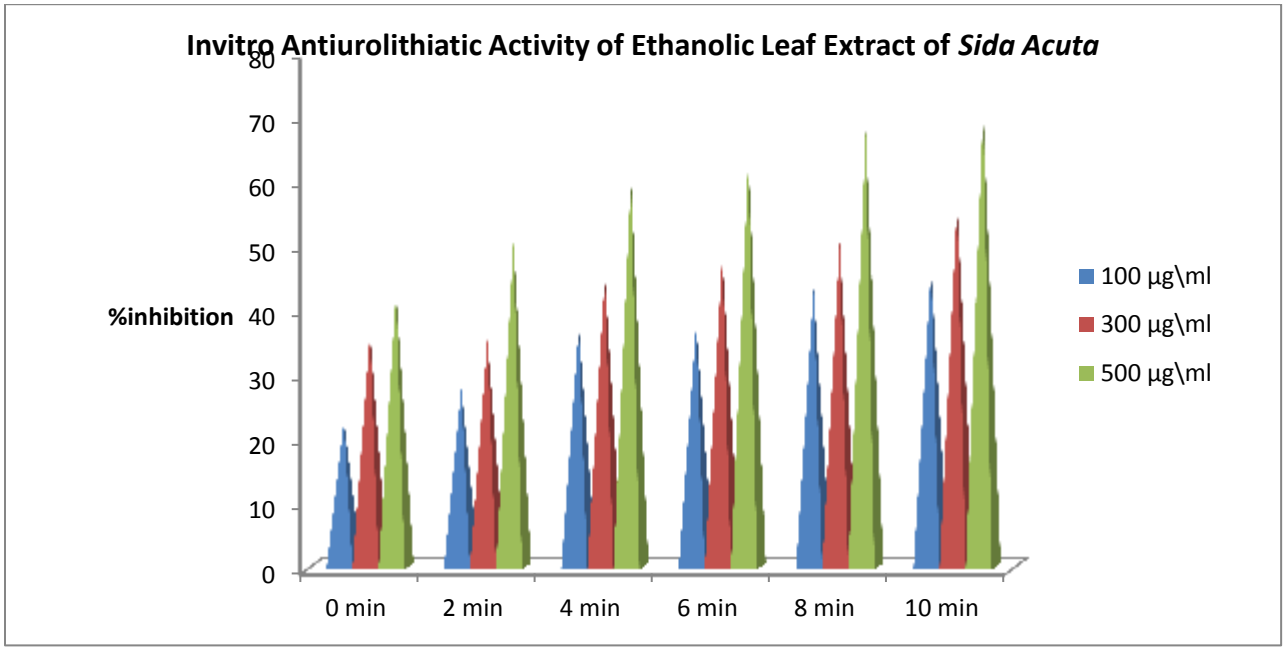

Figure 3: *In-vitro* Antiurolithiatic Activity of Ethanolic Leaf Extract of *Sida acuta*

***In-vivo* method**

In *in-vivo* Anti-Urolithiatic activity of *Sida acuta* leaf extracts shows dose dependent decrease in the levels of calcium, phosphate and oxalate in urine and calcium, creatinine and uric acid in serum were given in the below table No.4 and Fig 4.

Table No.4: *In-vivo* Anti-Urolithiatic activity of *Sida acuta* leaf extracts

S. No.	Group	Urine			Serum		
		Calcium	Phosphate	Oxalate	Calcium	Creatinine	Uric acid
1	Group I (Control)	3.5±1.15	18±2.08	4.2±1.04	8±1.52	0.6±0.14	3.2±1.04
2	Group II (calculi Induced)	15±0.14	39±0.14	7.6±2.08	16±2.08	38±1.52	7.6±1.15
3	Group III (Cystone) 750mg/kg	3.3±1.04	15±0.14	3.1±2.08	3.3±1.15	0.8±0.14	3.1±0.14
4	Group IV *Sida acuta* (200mg/kg)	3.4±2.08	30±1.15	6.6±2.08	11.2±0.14	0.6±2.08	6.6±1.52
5	Group V *Sida acuta* (400mg/kg)	7±1.15	25.5±0.14	3.6±1.04	11.2±2.08	0.8±0.14	3.7±1.15

Values are expressed as Mean± SEM; n=6 (number of animals in each group); $p<0.001$. All comparisons are made with that of control.

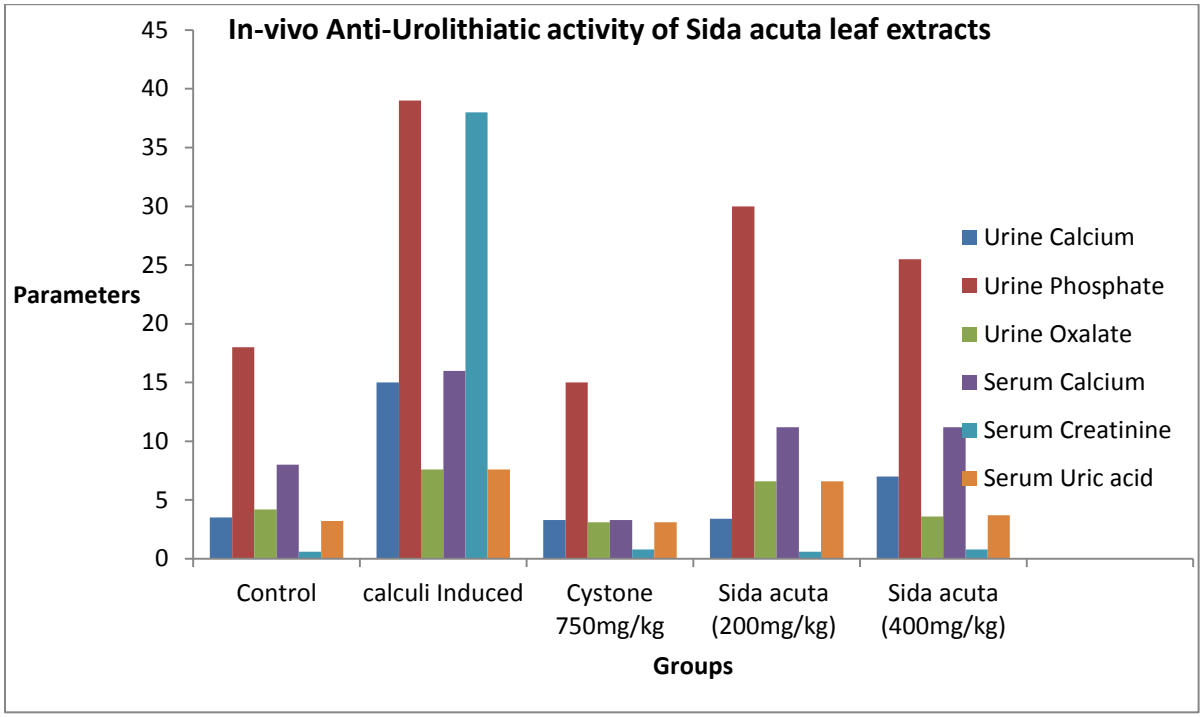

Figure 4: *In-vivo* Anti-Urolithiatic activity of *Sida acuta* leaf extracts

Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure[17]. So, diuretics play an important role in hypertensive patients. Hence any of these processes may be associated with diuretic effect of *Sida acuta* leaf extract. Further an increase in sodium, potassium, chloride and bicarbonate excretion by the extract might also be involved in diuresis. In calculi-induced rats, the elevated serum levels of creatinine, uric acid, and calcium indicate marked renal damage. These properties have been attributed to the triterpenes[18] and are present in *Sida acuta* leaf. Other possible mode of action includes excessive excretion or decrease in the concentration of urinary salts that prevent the super saturation of the crystallizing salts. Ethylene glycol-induced hyperoxaluria method was used to assess anti-urolithiatic activity in male albino rats[19]. Kidney being the principal target for Ethylene glycol induced toxicity. Its administration to rats for 28days resulted in substantial excretion of oxalate and deposition of microcrystal in kidney [20]. Upon its administration to the experimental animals (for 28 days), it is rapidly absorbed and metabolized in the liver via alcohol dehydrogenase or aldehyde dehydrogenase to glycolic acid. The glycolic acid is further oxidized to oxalic acid / oxalate by glycolate oxidase or lactate dehydrogenase thus promoting hyperoxaluria which is the main initiative factor for urolithiasis[21, 22]. The Phytochemical

screening of *Sida acuta* leaf extracts revealed the presence of tannins and flavonoids[24].These constituents may be responsible for the diuretic property of the plant Ethanolic leaf extract of *Sida acuta*, which favors the antiurolithiasis by hastening the process of dissolving or by flushing of the preformed stones. The possible mode of action of Ethanolic leaf extract of *Sida acuta* may be due to excessive secretion or decrease in the urinary concentration of the urinary salts that prevent super saturation of the crystallizing salts, based on *In-vitro* antiurolithiatic activity results.

CONCLUSION

It was already reported that tannins and flavonoids are natural products which have been shown to posses various biological properties related to Diuretic and Anti-Urolithiatic activity. All the observations provided the basis for the conclusion that the alcoholic extract of the dried leaves of *Sida acuta* is endowed with Diuretic and Anti-Urolithiatic Activity.

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