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EVALUATION OF DIURETIC AND *IN-VITRO* ANTI-UROLITHIATIC ACTIVITIES OF ETHANOLIC LEAF EXTRACT *OF GOMPHERNA SERRATA*

M.N.Palaksha[1*], V.Sudheer[2], Y.Satyaambika[2], V.Alekhya[2], Y.Srilakshmi[2], Y.Nagalakshmi[2] And Y.Sitasrilakshmi[2]

1. Department of Pharmacology, Aditya College of Pharmacy, Surampalem, E.G.Dist-533437.

2. Student, Aditya college of Pharmacy, Surampalem, E.G.Dist-533437.

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For Correspondence:

M.N.Palaksha
Department of Pharmacology, Aditya College of Pharmacy, Surampalem, E.G.Dist-533437

ABSTRACT

The present study was undertaken to investigate the Diuretic and antiurolithiatic activities of Ethanolic leaf extract of *Gompherna serrata* 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. Ethanolic extract showed inhibition at 0 min 22.56, 36.25 and 41.68%), and maximum inhibition of the crystallization of calcium oxalate at 10 min (45.24, 56.93 and 70.72%). A significant diuretic and *in-vitro* urolithiatic effect was observed from the experimental animals treated with extract of *Gompherna serrata* individually compared to the control. The results obtained suggest potential usefulness of extract of *Gompherna serrata* leaf as an antiurolithiatic agent.

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].

The genus Gomphrena (Fam: Amaranthaceae) comprises approximately 120 species found in the Americas, Australia, and Indo-Malaysia, only a few species are found in forest[4]. A number of Brazilian Gomphrena species are employed in the treatment of bronchial asthma, diarrhea, and fever, and as an analgesic, tonic, or carminative. This species show antimalarial and diuretic activities[5]. There is little phytochemical and pharmacological screening report on this genus[6]. The aim of the present work is to evaluate the Diuretic and Anti-Urolithiatic Activity of *Gompherna serrata* leaf extract.

MATERIALS AND METHODS:

Collection of plant parts:

The whole plant of *Gompherna serrata* was collected from the surroundings of Surampalem, East Godavari dist, Andhrapradesh. 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 *Gompherna serrata* 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.

Phytochemical screening:

The freshly prepared extracts of *Gompherna serrata* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was performed using standard procedures[7, 8].

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*[9], and Kavimani *et al*[10], 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 *Gompherna serrata* 200mg/kg
Group-IV (Test-2)	Treated with Ethanolic leaf extract *Gompherna serrata* 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[11]. The volume of urine collected was recorded after 5 hrs and urine was subjected to determine the sodium, potassium ions by flame photometry[12], chlorides and bicarbonates by titrimetric analysis[13], after 24 hrs the Saluretic, Natriuretic, diuretic indices were also calculated.

***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[14].

Preparation of synthetic urine: Synthetic urine was prepared by dissolving 3.8gm of potassium chloride, 8.5gm of sodium chloride,1.5gm of calcium 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[15].

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[16]. The % of inhibition was calculated using the following formula:

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

RESULTS:

1. Phytochemical screening:

Preliminary phytochemical screening of the extract of *Melochia corchorifolia* revealed the presence of various bioactive components of which flavonoids and tannins were the most prominent and the result of phytochemical test are summarized in (Given in Table No.1).

Table No.1: Phytochemical screening of Ethanolic leaf extract of Gompherna serrata:

Chemical constituents	Chemical tests	Ethanolic extract
Alkaloids	Dragendroff's test	+
	Mayers's test	+
	Hager's test	+
Steroids	Libermann-Burchards test	+
carbohydrates	Molisch's test	+
	Fehling's test	+
Reducing sugars	Bendict's test	+
Saponins	Froth formation test	+
Glycosides	Keller-killiani test	+

+=Presence

2. Diuretic activity:

The results obtained in diuretic activity were shown in Table No 2&3. Table no.1 and fig no. 1 shows urine volume (mL/5hrs) and excretion of electrolytes sodium, potassium, chlorides and bicarbonate ions in urine.

TABLE NO.2: DIURETIC EFFECT OF *GOMPHERNA SERRATA* LEAF EXTRACT.

Group	Volume of urine (mL/Hr) after 24hrs	Na^+ µmoles/Kg	K^+ µmoles/Kg	Cl^- µmoles/Kg	HCO_3^- µmoles/Kg
Group I (Control)	0.16±0.04	173.33±0.35	111.48±0.48	98.66±0.59	9.97±0.17
Group II (Standard)	0.72±0.01**	212.14±0.65*	134.34±0.20*	142.52±0.39**	25.36±0.33**
Group III (*Gompherna serrata*- 200mg/kg)	0.30±0.03	170.23±0.03	114.93±0.45	130.98±0.33*	24.24±0.53*
Group IV *(Gompherna serrata*- 400mg/kg)	0.50±0.06*	186.21±0.52	118.76±0.53	148.92±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 no.2 and fig no.2 shows the saluretic, natriuretic and diuretic indices of ethanol extract of leaf *Gompherna serrata*. From the results it can be observed that ethanolic extract of leaf *Gompherna serrata* 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 NO. 3: COMPARISON OF SALURETIC, NATRIURETIC, AND DIURETIC INDICES OF *GOMPHERNA SERRATA* LEAF EXTRACT.

Sl.no.	Group	Saluretic Index [Na^+ + Cl^-]	Natriuretic Index [Na^+/ K^+]	Diuretic Index
1	GroupI (Control)	271.02	1.41	-
2	GroupII (Standard)	383.66	1.62	4.9
3	GroupIII *(GS* -200mg/kg)	322.21	1.43	2.0
4	GroupIV *(GS* -400mg/kg)	354.13	1.52	3.4

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

3. Anti-Urolithiatic Activity:

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 41.68%), and maximum inhibition of the crystallization of calcium oxalate at 10 min (45.24, 56.93 and 70.72%). The results of in *In-vitro* Antiurolithiatic activity of *Gompherna serrata* leaf extract are given in Table 4

TABLE NO.4: *IN-VITRO* ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *GOMPHERNA SERRATA*

	100 µG\ML	**300 µG\ML**	**500 µG\ML**
0 min	22.56	36.25	41.68
2 min	28.64	37.25	49.62
4 min	37.14	42.14	60.25
6 min	38.56	46.23	62.25
8 min	43.12	51.23	65.50
10 min	45.24	56.93	70.72

DISCUSSION:

The Phytochemical screening of *Gompherna serrata* leaf extract revealed the presence of steroids, saponins , glycosides etc., 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 *Gompherna serrata* leaf extract. Further an increase in sodium, potassium, chloride and bicarbonate excretion by the extract might also be involved in diuresis.

Gompherna serrata leaf extract at a concentration of (100, 300 and 500µg/ml) was subjected to *in-vitro* anti-urolithiatic activity and the leaf extracts have shown dose and time dependent % of inhibition 45.24, 56.93 and 70.72 at 600seconds (10 min). The possible mode of action of Ethanolic leaf extract of *Gompherna serrata* 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. These properties have been attributed to the steroids[18] and are present in *Gompherna serrata* leaf.

The Phytochemicals present in the *Gompherna serrata* leaf extract may be responsible for the diuretic property of the plant Ethanolic leaf extract of *Gompherna serrata,* which favours the antiurolithiasis by hastening the process of dissolving or by flushing of the preformed stones.

CONCLUSION:

It was already reported that are natural products like steroids, saponins , glycosides 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 *Gompherna serrata* is endowed with Diuretic and Anti-Urolithiatic Activity.

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