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Diuretic activity of ethanol extract of *Justicia Gendarussa* (Burm) f. plant leaf in rat model

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Abstract
The diuretic activity increases the rate of urine flow, sodium excretion and to maintain the volume and composition of body fluids in a various clinical disorders such as congestive heart failure (CHF), chronic renal failure, nephritis, cirrhosis, hypertension and pregnancy-induced toxaemia. Many studies regarding herbal plant used in traditional medicine as diuretics are nowadays used for treatments. The aim of the present study was to evaluate the diuretic activity of the ethanolic extract of *Justicia Gendarussa* (Burm) f. Plant leaf to provide evidence about its diuretic activity. The diuretic responses with its electrolyte excretion potency of the *J. gendarussa* (Burm) f. ethanolic extract are highly moderate in comparison to normal control rats. The *J. gendarussa* (Burm) f. ethanol extract at doses of 400 mg/kg showed a significant increase in Na^+, K^+ and Cl^- excretion. The results of urinary electrolyte excretion after treatment of *J. gendarussa* (Burm) f. ethanol extract were comparable to the furosemide group. Finally increased total urine volume and the urine concentration of Na+, K+ &Cl^- will be the evidenced for the diuretics of Ethanol extract of *Justicia Gendarussa* (Burm) f. Plant leaf and deserves further studies considering the potential for the treatment of hypertension.

Keywords: *Justicia gendarusa* (Burm) f., diuretic activity, furosemide, Na^+, K^+, and Cl^- ions

Introduction
Justicia gendarusa (Burm) f. plant belongs to the Acanthaceae family. It is an erect, branched, smooth undershrub, about 0.8 - 1.5 meters in height with long leaves (7 to 14 cm) having acute tips; small flowers and terminal pinkish spikes with purple spots. It is found throughout India and in Asian countries like Malaysia, Indonesia and Srilanka [1]. This plant used to treat fever, hemiplegia, rheumatism, arthritis, headache, earache, muscle pain, respiratory disorders, digestive disorders and for arthritics. It also possesses hepato protective property [2].
Diuretic action is nothing but to increase rate of the urine flow, adjust the volume and composition of body fluids. Diuretic action is one of the medicinal property of *Justicia gendarusa* (Burm) F. plant. Diuretic drugs are effective in the treatment of hypertension, congestive heart failure, ascites, and pulmonary edema [3]. Herbal Plants are commonly used in traditional medicine for the treatment of some renal diseases, because of their significant diuretic activity. Traditional medicinal plants were used as diuretic agents and diuretic effects were confirmed in the experimental animals [4].
The aim of the present study was to examine the effect of crude ethanolic extract of the plant (leaf) *Justicia gendarussa* (Burm) f. (EEJG) on the diuretic activity in Wistar albino rats. The use of this plant displayed both prophylactic and therapeutic effects in Albinus rats, and the activity related to a diuretic effect has been produced by the formulation [5].
In this study extracts of the medicinal plant were compared with a well-known diuretic drug furosemide using Wistar albino rats. And the urine volume, urine pH, urinary electrolyte levels, natriuretic and saliuretic effects were measured at 5 h and 24 h duration [7].

Material and Methods
Collection of plant material
The leaf of the *Justicia gendarusa* (Burm) f. (Figure 1) plant were collected from Natural Environment in Ichadi, Pudukkottai District, Tamilnadu, India and the Collected Plant was authenticated by Dr.S. Soosairaj, Assistant Professor, Department of Botany, St. Joseph's College, Tiruchirappalli District, Tamilnadu, India (specimen - SJCBOT2183).

Preparation of plant extract
The *Justicia gendarussa* (Burm) f Plant leaf was collected and washed thoroughly; shade dried and was ground into fine powder. About 50g leaf powder of *Justicia gendarussa* (Burm) f. were soaked in 500ml of ethanol and was then kept in orbital shaker for 48h at room temperature. After 48h, the mixture was filtered through a clean muslin cloth. The filtrate was again filtered by using a Whatman no. 1 filter paper and then the extracts were concentrated and dried in a rotary evaporator at 37ºC till a sticky mass was obtained. After evaporation, the dried extracts were stored at 4ºC until further use [7, 8].

Phytochemical analysis
Various compounds like flavonoids, triterpenes, tannins, alkaloids, and fatty acids possessing wide range of bioactivities were isolated from leaves of the plant using standard protocol [9-11].

Test for carbohydrates
To about 1 ml ethanol extract of the plant about 5 ml of Benedict's reagent was added and was boiled for 5 minutes. Appearance of bluish green showed the presence of carbohydrates.

Test for Glycosides
About 1 ml concentrated sulphuric acid was added to 1 ml ethanol extract of the plant. Fehling's solution was added to this test solution. A black red precipitate was formed indicating the presence of glycosides.

Test for Alkaloids
2 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids.

Test for Flavonoid
2 ml of test solution in alcohol was added with a bit of magnesium and one or two drops of concentrated HCl and heated. Formation of red or orange colour indicates the presence of flavonoids.

Test for Tannins
2 ml of test solution was added with H_2O and lead acetate. Formation of white precipitate indicates the presence of tannins.

Test for Saponins
2 ml of test solution was added with H_2O and shake well. Formation of foamy lather indicates the presence of saponins.

Test for Steroids
2 ml of test solution and minimum quantity of chloroform was added to 3-4 drops of acetic anhydride and one drop of conc. H_2SO_4. Formation of purple colour which later changes blue or green indicates the presence of steroids.

Test for Triterpenoids
2 ml of test solution was added with a piece of tin and a drop of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids.

Animals
Nine months male young adult albino Wistar rats (No 16), weighing 150 - 200 g were used. All animal experiments and maintenance were carried out according to the ethical guidelines suggested by the Institutional Animal Ethics Committee. Animals were housed in polypropylene cages under controlled conditions of12 h light/dark cycle at 27 ± 2°C. All the rats received standard pellet diet (Sai Enterprises, Chennai).

Diuretic activity
This method described by Lipschitz was employed for the assessment of the diuretic activity of *J. gendarussa* (Burm) f. ethanolic extract [12]. The animals were fasted for 18 h prior to experiment allowing only water during the fasting period. Twenty-four healthy Wistar albino male rats were divided into four groups consisting of four rats in each group. Group 1 vehicle group received saline orally at the dose of 10 ml/kg. Group 2 is the Standard group which was given furosemide orally at the dose of 100 mg in 10 ml saline /kg. Group 3 is Test group which received *J. gendarussa* (Burm) f. ethanolic extract orally at the dose of 400 mg in saline/kg respectively.

After 1 h of respective treatments, the animals were kept in metabolic cages individually for the collection of urine. After the dose of administration, the urine was collected at 5 and 24h. Diuretic assay parameters such as pH, total urine volume and urinary excretion of Na^+, K^+ and Cl^- were measured in collected urine. Electrolyte (Na^+, K^+ and Cl^-) concentrations were estimated and expressed as mmol/L [13]. The total difference in the collected urine volume of the respective test groups were compared with the standard and control group. Furthermore, the ratio of urine volume of the test group and the control group was calculated as a diuretic index. The urinary Na^+, K^+ and Cl^- content of the respective test groups are also compared with that of control and standard group [14].

Statistical analysis
The data is expressed as mean ± SD using SPSS v24.0 data analysis. Statistical significance between means was analysed by one-way analysis of variance (ANOVA) and a *P* value <0.05 was considered as statistically significant.

Results and Discussion
Qualitative phytochemical analysis
Qualitative estimation of phytochemicals of the ethanolic extract of the *Justicia gendarussa* (Burm) plant leaves were tabulated in Table 1. It shows the presence of Carbohydrates, Glycosides, Alkaloids, Flavonoids, Tannins, phenols, Saponins and Steroids. The presence of chemical constituents makes us understand that the plant could be useful for pharmacognostic studies and standardization of herbal drugs which are used currently in folk medicine. They also provide therapeutic diagnostic tools for scientists who wish to evaluate herbal medicines obtained from indigenous resources.

Table 1: Phytochemical compounds of the *Justicia gendarussa* (Burm) plant

S. No	Phytoconstituents	Ethanol
1	Carbohydrates	++
2	Glycosides	++
3	Alkaloids	++
4	Flavonoids	+
5	Tannins	+
6	Phenols	++
7	Saponins	+
8	Steroids	++
9	Terpenoids	++

Diuretic activity of *J. gendarussa* (burm) f. plants ethanolic extract
The Diuretic effect of *J. gendarussa* (Burm) f. ethanolic extract on urine volume, pH is present in Table 2. Experimental animal's urine was slightly alkaline in nature. Ethanolic extract of *J. gendarussa* (Burm) f. plant at the dose of 400 mg/kg showed significantly ($p < 0.05$) increase in the urine volume than the normal control and the effects were significantly less in the standard group during both 5h and 24 h. The diuretic activity of a drug was considered none if it is less than 0.72, little if it is between 0.72 and 1.00, moderate if it is within 1.00–1.50 and good if it is above 1.50. In this respect, *J. gendarussa* (Burm) f. exhibits good diuretic activity.

Table 2: Diuretic Effect of Ethanolic Extract of *J. gendarussa* (Burm) f. on urine volume in albino rats at 5 and 24 h interval

Groups and Dosage	After 5 hrs drug administration			After 24 hrs drug administration			pH
	Urine Volume (ml)	Diuretic Action	Diuretic Activity	Urine Volume (ml)	Diuretic Action	Diuretic Activity	
Control (Saline 10ml/kg p.o)	1.27 ± 0.21[d]	1.00	-	1.64 ± 0.16[d]	1.00	-	7.12±0.15[d]
Furosemide (100 mg/kg p.o)	4.2 ± 0.2[a]	3.31	1.00	5.3 ± 0.25[a]	3.24	1.00	7.89±0.06[a]
EEJG (400mg / kg p.o)	3.37 ± 0.31[b]	2.66	0.81	4.37 ± 0.27[b]	2.67	0.83	7.58±0.06[b]

EEJG - Ethanolic Extract of *J. gendarussa* (Burm) f. The values expressed as a mean ± SEM of 4 rats (n=4). [a, b, c, d] Significant change at P < 0.05 with respect to control rats. (p.o.) = per oral

The diuretic responses with its electrolyte excretion potency of the *J. gendarussa* (Burm) f. ethanolic extract were highly moderate in comparison to normal control rats. The *J. gendarussa* (Burm) f. ethanol extract at doses of 400 mg/kg showed a significant increase in Na^+, K^+ and Cl^- excretion. The results of urinary electrolyte excretion after treatment of *J. gendarussa* (Burm) f. ethanolic extract were comparable to the furosemide group (Table 3).

Table 3: Effect of ethanolic extract of *J. gendarussa* (Burm) f. on urinary electrolyte excretion of albino rats at 5 h and 24 h of urine sample

Groups and Dosage	After 5 hrs of Drug administration						After 24 hrs of Drug administration					
	Urine Na^+ (mmol/l)	Urine K^+ (mmol/l)	Urine Cl^- (mmol/l)	Na^+ index	K^+ index	Cl^- index	Urine Na^+ (mmol/l)	Urine K^+ (mmol/l)	Urine Cl^- (mmol/l)	Na^+ index	K^+ index	Cl^- index
Control (Saline10 ml/kg p.o)	53.97±1.51[d]	41.55 ± 0.69[c]	101.97±2.86[d]	1.00	1.00	1.00	54.67±1.57[d]	45.35 ± 0.77[c]	105.17±2.93[d]	1.00	1.00	1.00
Furesemide (100 mg/kg p.o)	181.57±0.81[a]	54.08 ±1.06[a]	136.94±1.65[a]	3.37	1.31	1.35	193.77±2.6[a]	67.54 ±1.16[a]	166.9±1.5[a]	3.55	1.49	1.59
EEJG (400mg/kg p.o)	154.37±1.06[b]	46.75 ± 1.37[b]	126.7±2.73[b]	2.87	1.12	1.25	176.34±1.53[b]	58.44 ± 1.31[b]	139.34±1.53[b]	3.23	1.29	1.33

EEJG - Ethanolic Extract of *J. gendarussa* (Burm) f. The values expressed as mean ± SEM (n=4). [a, d,c,d] Significant change at P <0.05 with respect to control rats. (p.o.) = per oral

Table 4: Effect of *J. gendarussa* (Burm) f. on Natriuretic Effect, Saluretic Effect and Carbonic Anhydrase Inhibition of Wistar albino rats at 5 and 24 h of urine sample collection

Groups (P.o.)	After 5 hrs of Drug administration						After 24 hrs of Drug administration					
	Saluretic Effect (Na^++ Cl^-)	Natriuretic Effect (Na^+/ K^+)	CAI[Cl^-/(Na^++K^+)]	Saluretic Index	Natriuretic Index	CAI Index	Saluretic Effect (Na^++ Cl^-)	Natriuretic Effect (Na^+/ K^+)	CAI [Cl^-/(Na^++K^+)]	Saluretic Index	Natriuretic Index	CAI Index
I	155.94	1.3	1.06	1.00	1.00	1.00	159.84	1.21	1.06	1.00	1.00	1.00
II	318.51	3.36	0.59	2.05	2.59	0.56	360.67	2.87	0.64	2.26	2.38	0.61
III	281.07	3.31	0.63	1.81	2.55	0.6	315.68	3.02	0.6	1.97	2.5	0.57

EEJG - Ethanolic Extract of *J. gendarussa* (Burm) f. The values expressed as mean ± SD (n=4). CAI - Carbonic Anhydrase Inhibition (p.o.) = per oral

Natriuretic, Saliuretic activity and carbonic anhydrase (Table 4) inhibition after administration of the *J. gendarussa* (Burm) f. ethanol extract, is comparable with the values obtained for standard drug Furosemide. The *J. gendarussa* (Burm) f. ethanolic extract showed a significant increase in saliuretic and natriuretic activities when compared to the control group. The natriuretic ratio values >2.0 indicate favourable natriuretic activity [15]. *J. gendarussa* (Burm) f. ethanol extract dose 400 mg/kg, showed significant increase in natriuretic ratio 3.31, which demonstrates the natriuretic activity of *J. gendarussa* (Burm) f. ethanol extract.

A reduction of the CAI ratio values <0.8 could indicate a strong CAI activity [15]. *J. gendarussa* (Burm) f. ethanolic extract at 400 mg/kg 100mg/kg doses showed a CAI < 0.8, indicating a significant diuretic activity. CAI activity of 400 mg/kg and 100mg/kg dosage was comparable to furosemide group.

The diuretic effect of ethanolic extract of *J. gendarussa* (Burm) f. was evaluated in Wistar albino rats. The results indicate that plant leaves significantly increased urine output in a dose-dependent manner over a period of 5 and 24 h. The diuretic activity of the plants is normally attributed to the presence of some compounds like flavonoids and tannins which is responsible for the process. The activity of these compounds was found to be similar to the reference drug furosemide. The mechanism of action of furosemide is that it induces a loss of water through the inhibition of NaCl reabsorption [16]. These results agree with the results of the previous study, which stated that this reinforces the plant *J. gendarussa* (Burm) f. having diuretic activities, which may be advantageous in Urolithiatic condition. The increase of urine output will dilute the concentration of urinary electrolytes, which in turn could reduce the chances of precipitation of calcium and phosphorus [17]. May be the presence of this plant phytochemicals such as sterols, tannins, flavonoids, alkaloids and/or nitrogenous bases that may have either diuretic effects or stone dissolving effects and may possess antioxidant potential which has been that established in previous studies [18, 19].

Therefore, remarkable diuretic activity was observed among rats treated with furosemide and ethanolic extract of *J. gendarussa* (Burm) f. Doses. The diuretic activities of the extracts were found to be highly potent when compared to the normal control group. However, significant differences in urinary excretion followed by diuretic action and diuretic activity were also observed. This study suggests the ethanolic extract of *J. gendarussa* (Burm) f. have effective and dose responsive of diuretic activity [20].

Conclusion

The present study is an evidence for the diuretic activity of ethanolic extract of *J. gendarussa* (Burm) f. against renal issues like calcium oxalate stones inhibition, formation or protection of renal epithelial cells. The use of this plant shows a promising results and a rapid recovery gives hope for future research and might be effective in renal patients. The exact mechanism of interaction remains unknown and clinical studies are required for further confirmation.

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Conflict of Interest
The author(s) declare that they have no conflict of interest.

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