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Comparative diuretic activity of ethanolic extract of *Tragia plukenetii* and *Tragia involucrata*

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Abstract
Plants as medicines are still the mainstay of health care in several developing as well as under developing countries. People mostly rely on local herbs for food and cure of different ailments. *Tragia plukenetii* and *Tragia involucrata* (Euphorbiaceae) used in treating various ailments of human. The study aims to evaluate the diuretic effect of ethanolic extract of *Tragia plukenetii* and *Tragia involucrata* using animal models. To evaluate the diuretic activity of the selected plants, Albino rats were divided into four groups of six animals each. The control group received normal saline, the reference group received furosemide and the test groups were administered ethanolic extracts by intra-peritoneal route, respectively. Significant diuretic effects were recorded in the treated groups in a dose dependent manner. The plant extracts enhanced the excretion of sodium and potassium ions were significantly. Further studies are encouraged to isolate the pure bioactive compounds responsible for diuretic activity.

Keywords: Electrolyte, ethanolic extract, furosemide, preliminary phytochemical screening

Introduction
Diuretic are chemical agents that increase excretion of urine by the kidneys. They promote to secretion of excess water and salt that accumulates in tissues and urines, results in decrease in extracellular body fluids, so diuretics are used in management heart failure, odema associated with liver cirrhosis and kidney diseases, and hypertension [1]. Bioactive compounds from the medicinal plants are important source of mild diuretic agents [2-4]. A number of adverse effects like electrolyte imbalance, metabolic alterations associated with synthetic diuretics. There is a need to study new plant-based bioactive components that have effective diuretic activity with minimal side effects [5]. Active compounds derived from medicinal plants act as remarkable alternative with greater effectiveness and fewer side effects. In this perspective, the diuretic potential of ethanolic extract of *Tragia plukenetii* and *Tragia involucrata* (Euphorbiaceae) has been evaluated. *Tragia plukenetii* R. Smith (Tamil name: Karunkanchori) the root is diaphoretic and is given for fevers to cause perspiration [6]. The root of *T. involucrata* (Tamil name: Chenthatti) have diuretic, diaphoretic, antiperiodic, depurative and alterant activity. They are useful in pruritic skin eruptions, venereal diseases, hemorrhoids, gastropathy, guinea worms, blood impurities, dipsia, vomiting giddiness, vitiated conditions of pitta, melalgia and brachialgia [7-10]. The objective of present study is to analyse the therapeutic potential of the ethanol extract of *Tragia plukenetii* and *Tragia involucrate.*

Materials and Methods
Collection of plant samples
Mature and healthy plants of *Tragia plukenetii* and *Tragia involucrata* belonging to the family Euphorbiaceae were collected from southern Western Ghats in the district of Tirunelveli, South India. The specimens were identified, comparing the characteristics of floral and vegetative characters in the *'Flora of the Presidency of Madras'* [11]. The taxonomic features collected from the species have been confirmed with the '*Flora of Tamilnadu Carnatic'* [12].Voucher specimens are documented in the herbarium of St. Xavier's College (XCH) Herbarium, Palayamkottai, Tamil Nadu, India.

Preparation of Drug

The plant material was shade dried and pulverized. Ethanol extract of the moderate coarsely powdered material was prepared by employing Soxhlet method. The extract was concentrated and stored in brown bottles for future use.

Animals

Wister adult *albino* rats (100-150g), Guinea Pig (600-700g) and Rabbit (1-1.5kg) were procured from lab animal house, Dept of Pharmacology, Government Siddha Medical College, Palayamkottai. Tirunelveli, Tamil Nadu, India. The animals were housed in microlan boxes in a controlled environment (temperature 25 ºC and 12 hrs dark and light cycle) with standard diet and water *ad libitum*.

Diuretic activity

Diuretic activity was carried out by using *in-vivo*Lipschitz test method [12]. The rats were divided into four groups of six animals each and deprived of food and water for 18hrs. All the rats have been received priming dose of normal saline (25 ml/kgbw) orally. The extracts and Frusemide (standard) were dissolved in a normal saline. Group I served as control in which only normal saline (25 ml/kgbw) was administered through orally. Group II served as standard received frusemide (100 mg/kgbw), Group III: Ethanolic extract of *Tragia plukenetii* (100 mg/kg), Group IV: Ethanolic extract of *Tragia involucrata* (100 mg/kg). Immediately after administration, the rats (one in each cage) were placed in metabolic cages specially designed to separate urine and faces. The urine was collected in a measuring cylinder up to 6hrs. During this period no food or water was made available to animals. The volume of urine collected was measured for all groups. The parameters taken for each individual rat were body weight before and after test period. Urine volume (concentrated for water intake during test period), concentration of Na+ and K+ in urine have been calculated and also recorded.

Results and Discussion

Preliminary phytochemical Screening

The organic solvent extracts of *T. plukenetii* and *T. involucrata* were subjected to preliminary phytochemical analysis using standard chemical methods which mainly revealed the presence of phytoconstituents. In this screening process steroid, triterpenoids, sugars, alkaloids, phenols, flavonoids, saponins and tannins gave positive results and catechins and anthroquinones gave negative results (Table 1 and 2).

Table 1: Preliminary phytochemical Screening of ***Tragia plukenetti***

Solvent	Steroids	Triterpenoids	Reducing Sugars	Sugars	Alkaloids	Phenols	Flavonoids	Catachins	Saponins	Tanins	Anthroquinones	Amino acids
Petroleum ether	+	+	_	_	+	+	_	_	+	_	_	_
Chloroform	+	+	-	+	+	_	+	_	_	_	_	+
Benzene	+	+	+	_	_	+	_	_	_	_	_	+
Ethanol	+	+	_	+	+	+	+	_	+	+	_	_

Table 2: Preliminary phytochemical Screening of ***Tragia involucrate***

Solvent	Steroids	Triterpenoids	Reducing Sugars	Sugars	Alkaloids	Phenols	Flavonoids	Catachins	Saponins	Tanins	Anthroquinones	Amino acids
Petroleum ether	+	+	_	_	_	+	_	_	+	_	_	_
Chloroform	+	+	_	+	+	_	_	_	+	+	_	+
Benzene	+	+	_	_	_	+	_	_	+	_	_	_
Ethanol	+	+	+	+	+	+	+	_	+	+	_	+

Diuretic activity

Effect on urine volume

The results of diuretic activity of the extract obtained from the urine sample of rats are shown in Table 3. The ethanolic extracts of *T. plukenetii* and *T. involucrata* increased urine volume significantly (p<0.05) at 100 mg/kgbws during the 5 h of the test. The ethanolic extract of *Tragia plukenetii* ***(***9.43±0.033 ml/kg) and *Tragia involucrata* (11.85±0.042 ml/kg) on significantly increased urinary output to that of the control (2.75±0.075 ml/kg).

Effect on urinary electrolyte excretion

The effect of single doses of furosemide (100 mg/kg BW) and the ethanolic plant extract of *Tragia plukenetii* and *Tragia involucrata* on electrolyte (Na+, K+) excretion in the 5 h urine in presented in Table 3. The plant extracts enhanced the excretion of sodium and potassium ions were significantly (p<0.001). Among two species of *Tragia*, *T. involucrata* has more secretion of sodium and potassium ions than *T. plukenetii*. The diuretic activity of the plant may be due to the presence of phytophenol compounds. Therefore, the whole part of the plant possesses diuretic activity was justified.

Table 3: Effect of Ethanolic extract of *Tragia plukenetii* and *Tragia involucrata*on Urine volume and Na^+, K^+ concentration in rats.

Group	Treatment	Dose	Urine volume (ml/kg)	Concentration of excreted ions		Na^+/K^+ Ratio
				Na^+(mEq/l)	K^+(mEq/L)	
I	Saline	25 ml/kg	2.75±0.075	62.43±0.043	50.62±0.010	1.234
II	Frusemide	100 mg/kg	13.65±0.086*	154.25±0.170	135.51±0.052*	1.139**
III	*Tragia plukenetii*	100 mg/kg	9.43±0.033*	123.44±0.065	138.50±0.028*	0.695**
III	*Tragia involucrata*	100 mg/kg	11.85±0.042*	137.47±0.078	149.63±0.030*	0.890**

Results are expressed as mean ± standard error n= 6 in each group.

* Significantly difference compared to control group at p<0.05.

** Significant difference compared to control group at p<0.001

In the present study, preliminary phytochemical screening of the ethanol extract of *Tragia plukenetii* and *Tragia involucrata* revealed the presence of alkaloids, flavonoids, polyphenols, and tannin. Rizvi *et al*. [14] and Sood *et al*. [15]. Showed that alkaloids, flavonoids, polyphenols, and tannin are responsible for diuretic activity by exerting favorable effects on physiological processes of the kidney such as by increasing potassium sparing capacity, binding with adenosine A1 receptor associated with diuretic activity or possibly by inhibiting tubular reabsorption of water and accompanying anion. Diuretic activity associated with quinone like alkaloid and other phytochemicals like steroids, tannins, phenolic compounds, terpenoids and flavonoids [16]. Tannin and flavonoid combination with alkaloid produce apparent diuretic activity [17]. Leo Stanley *et al*. [18] reported quercetin in the *Tragia plukenetii*. The flavonoids like quercetin, ferulic acid, and gallic acid possess diuretic effect [19]. The presence of polar compounds like flavonoids and terpenoids are responsible for diuretic activity [20]. Presence of saponin cause significant diuretic activity [21, 22, 23]. Presence of nitrates and essential oil possess diuretic effect [24].

The possible mechanism of action of phytochemical constituents that are responsible for diuretic action may be, Saponins due to local irritation of kidney epithelium [25], Alkaloids due to vasodilatory action on renal blood vessels [26]. Glycosides due to increased blood flow to the kidneys from enhanced cardiac contractility [27]. These herbs usually contain monosaccharides, flavonoids, volatile oils, saponins, terpenes, or tannins, which increase urine volume by promoting kidney blood flow and raising the glomerular filtration rate. Nevertheless, unlike synthetic diuretics, they do not reduce the resorption of Na^+ and Cl^- in the renal tubules [28].

Panda *et al*. [29] identified quercetin and rutin in the ethyl acetate extract of *T. involucrata*. Rutin is a flavonoid that can be found in several plant species used in the treatment of urinary diseases [30, 31]. It can decrease the levels of oxalate and calcium in the kidneys and urine. Oxalate and calcium are the main components of urinary stones. This effect is thought to be due to the inhibition of oxalate synthesis and the increase in calcium sequestration by nitric oxide [32]. Moreover, Kappel *et al*. [33] have shown, that rutin increases the uptake of calcium into skeletal muscles, which is mediated through mitogen-activated kinase (MEK) and protein kinase A (PKA) signaling pathways.

Conclusion

Ayurveda Pharmacopoeia [34] reported that in traditional system of medicine *Tragia* shows diuretic activity, Root of *Tragia involucrata* all urinary problems [35] and dysuria [36]. The present study validates the traditional claim of the selected plant.

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