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Tragia spp: Assessment of Diuretic Activity and Standardization of the Whole Plant

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Abstract

Background: Due to several toxic effects of synthetic diuretics drugs, people relay of medicinal plants as a source of diuretics. Hence, the efficacy of *Tragia* spp. which belonging to plant family Euphorbiaceae is an important medicinal plant used as a diuretic in Sri Lanka.

Objective: The aim of the present study was to investigate diuretic activity and standardize whole plant of *Tragia* spp.

Materials and methods: Plant extract (doses of 550, 1100, 1650, 2200 mg/kg), vehicle (2 ml of distilled water), reference drug (furosemide at 13 mg/kg) were given orally to separate groups of Wistar male rats (N = 6). In order to assess the diuretic activity, urine output was measured hourly for five hours. Na+ and K+ content, specific gravity, pH of urine measured in all groups. Whole plant of *Tragia* spp. was standardized in terms of physico-chemical parameters, phytochemical screening and antioxidant activity.

Results: Results revealed that urine output of rats were significantly ($P<0.05$) increased when increasing the dose of *Tragia* spp. extract. Diuretic activity was initiated in the first hour and declined with time. Diuresis was maximum at the 1 h and at a dose of 2200 mg/kg urine output was significantly ($P<0.05$) higher than the reference drug. *Tragia* spp. extract also triggered a clear increase in Na+ and K+ levels and decreased pH levels in urine. Physico-chemical parameters, phytochemical screening and antioxidant activity were determined in order to standard *Tragia* spp.

Conclusion: *Tragia* spp. exhibit marked diuretic activity and its mechanism of action is similar to a loop diuretic drug. Further, establishment of standardization parameters of *Tragia* spp. will help in correct identification and prevent adulteration.

Keywords: *Tragia* spp.; Diuresis; Phytochemicals; Standardization

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Introduction

Medicinal plants play an important role for the treatment of various diseases such as diabetes, inflammation, gastric ulcers, fever, urinary disorders, etc. Even at present a major amount of synthetic drugs in the world are derived from plant materials. In WHO's essential medicine list, 11% out of the total 252 drugs are derived exclusively from plant origin [1]. At present herbal remedies are becoming more popularized because it is a well known fact that natural herbs are safer than synthetic medications.

In Sri Lanka, *Tragia* spp. is used as a substitute for *Tragia involucrata* L. Both plants are called "Welkahambiliya" in Sinhala, and "Indian stinging nettle" in English. *Tragia* spp. and *T. involucrata* are extensively used as medicinal plants in Sri Lankan medicinal systems including Ayurveda, Siddha, Unani and traditional treatments. Both *Tragia* species and *T. involucrata* have morphological similarities and differences. *T. involucrata* is a vine which grows on a support whereas *Tragia* spp. grows as a shrub and then the apical bud tends to vine around a support. *Tragia* spp. leaves are linear than that of *T. involucrata*. Both plants are perennial, hispid herbs, with scattered stinging

hair which irritates the skin and cause itching, inflammation, and oedema[2]. *Tragia* spp. and *T. involucrata* are mainly found in North and Southern province of Sri Lanka [2]. *Tragia* spp. is also found as a weed in coconut plantations in North Western province of Sri Lanka. Whole plants of both *Tragia* spp. and *T. involucrata* are extensively used in Sri Lankan medical systems as a remedy forurinary disorders, loss of appetite, cough, asthma, fever, and cardiac diseases.

Recently, we have investigated the diuretic effect of *T. involucrata* using the hot water extract whole plants in rats [3]. In practice, *Tragia* spp. is used as a substitute for *T. involucrata,* hence, it is worth to investigate the diuretic activity of *Tragia* spp in order to validate and compare the efficacy of diuretic activity between *Tragia* spp. and *T. involucrata.* Therefore, the aim of this study was to (a) investigate the diuretic activity of hot water extract made out of *Tragia* spp. whole plants in rats and (b) standardize whole plant of *Tragia* spp in terms of physico-chemical parameters, phytochemical screening and antioxidant activity.

Materials and Methods

Plant material

Whole plants of *Tragia* spp. were collected at flowering stage during the period of March to June, 2015 from North and Western provinces of Sri Lanka. The plant was authenticated by the Botany Section of the Bandaranaike Memorial Ayruveda Research Institute, Navinna, Sri Lanka and a voucher specimen was deposited at Institute of Indigenous Medicine, University of Colombo, Rajagirya, Sri Lanka (TS-1). The plants were cleaned, cut into small pieces, washed with tap water and shade dried for 7 days. Then the plants were ground into powder, stored in air tight polythene containers and kept in a refrigerator at 4°C. Standardization of *Tragia* spp. whole plant

Physicochemical parameters

Ash values (including total ash, acid insoluble and water soluble ash) and extractable matter (including water, methanol, ethanol, ethyl acetate, and dicholoromethane) were determined for *Tragia* spp. using the dry powder of whole plants by following the guidelines of World Health Organization (WHO) [4].

Phytochemical screening

Presence or absence of phytochemicals such as steroids, polyphenols, flavonoids, alkaloids, saponins and tannins were carried out for *Tragia* spp. [5] using hot water extract.

Quantitative determination of total polyphenolic content and total flavonoid content: Folin – Ciocalteumethod [6] was applied for the quantification of total phenolic content and expressed the results as gallic acid equivalents (mg gallic acid/g extract). Total flavonoid content was quantified using a method described by Meda and co-workers [7] and expressed the results as quercetin equivalents (mg quercetin/g extract).

Animals: As the experimental model healthy adult male Wistar rats (weighing 200–225 g) were used and ethical clearance was obtained from the Ethical Review Committee of Faculty of Medicine, General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka. (Project No - RP/2013/12). Rats were housed in rat cages under standardized animal house conditions (including 12 h dark/light cycles with room temperature: 25 ± 3°C) and fed with standard rat feed and water ad libitum. Rats were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment.

Preparation of *Tragia* spp. hot water extraction: *Tragia* spp (60 g) was boiled over 4 h in 1.92 L of distilled water (DW) to get the final volume reduced up to 240 ml. Then the extract was filtered and filtrate was concentrated under vacuum, freeze dried (yield 11.6% dry weight basis) and stored at 4°C until use.

Administration of extract

Doses of hot water extract of *Tragia* spp. at 550, 1100, 1650, 2200 mg/kg of were administered orally by gastric gavage to separate groups of rats. Each dose was dissolved 2 ml of DW. The lowest dose (550 mg/kg) of hot water extract of *Tragia* spp. corresponds to the normal therapeutic dose administered to adult humans as calculated on the basis of relative surface areas of humans and rats [8].

Investigation of diuretic activity hot water extract of *Tragia* spp. whole plant

Healthy male Wistar rats were fasted for 18 h and separated into 6 groups and treated as follows:

Group 1 (6 rats/group): 550 mg/kg of hot water extract of *Tragia* spp

Group 2 (6 rats/group): 1100 mg/kg of hot water extract of *Tragia* spp

Group 3 (6 rats/group): 1650 mg/kg of hot water extract of *Tragia* spp

Group 4 (6 rats/group): 2200 mg/kg of hot water extract of *Tragia* spp

Group 5 (6 rats/group): 2 ml of distilled water (as the control group)

Group 6 (6 rats/group): 13 mg/kg of furosemide (as the reference group).

In brief, urinary bladders of rats were emptied by gentle compression of the pelvic area and by pulling of their tails. One hour prior to the treatment either with extract or vehicle or reference drug, all animals received physiological saline (NaCl 0.9%) at an oral dose of 5 mL/100 g body weight to impose a uniform water and salt load [9].

After that, each rat was individually placed in a metabolic cage and measured the urine output for 5 h at hourly intervals. In addition, the colour of urine was noted and determined the pH (Consort C 533, multi parameter analyzer), specific gravity and Na^+ and K^+ levels using Atomic Absorption Spectrophotometry - Flame Photometry (Schimedzu, Japan).

Statistical Analysis

Results were expressed as mean ± standard error of mean (S.E.M). Statistical analysis of the data was performed with one way analysis of variance (ANOVA) (IBM SPSS statistics 22) and significant differences were indicated by P values lower than 0.05.

Discussion

According to the estimation of WHO, 80% of world population still relies on herbal medicines [10]. Medicinal plants have widespread acceptability for various disorders such as diabetes, inflammation, fever, cough, urinary disorders, etc. [11]. They are thought to be considered as safe and therefore people consume the herbal drugs without prescription. However, some medicinal plants may cause toxic effects, some may not be effective and some may interact with other drugs [12]. Therefore, standardization of medicinal plants is very important.

Table 1 illustrates the physico-chemical parameters of *Tragia* spp. Further ash values including total ash and soluble ash content were markedly higher in *Tragia* spp. compared to that of *T. involucrata* which has been evaluated in a previous study [3]. The extractive value is an important parameter of medicinal plants, as it gives an idea about the nature of chemical constituents present in the plant material. Results of the present study revealed that extractive values of water extracts (both hot and cold) are higher when compared to hot and cold solvent extractive values. Therefore, water soluble extractive values are higher than any other solvents including methanol, ethanol, ethyl acetate or dichoromethane. In medicinal preparations, *Tragia* spp. is mostly given in the form of decoction in which the medium of extraction is water. In addition, both hot ethanolic extractable matter and hot methanolic extractable matter were significantly ($P<0.05$) higher in *Tragia* spp. compared to that of *T. involucrata.* Similar to *T. involucrate* [3], when decreasing the polarity from water to dichloromethane there was a reduction in respective extractive values **(Table 1).**

The active ingredients of medicinal plants and herbal drugs are due to the naturally occurring phytochemical constituents present in the plants, which are helpful in preventing and curing diseases [13]. Qualitative phytochemical screening of *Tragia* spp. shows that the plant possesses high amounts of flavonoids, terpenoids, and tannins. Flavonoids which are excellent antioxidants [13] seem to be present in high amounts in more polar solvents like water and methanol extracts. Previous studies have shown that tannins posseses activities such as lowering of serum lipid levels, anti-microbial activity, and also accelerates blood clotting [14]. Terpenoids exhibit anti-inflammatory, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities [13]. The total polyphenolic content and total flavonoid content of *Tragia* spp. extract was 18.8 ± 0.30 mg gallic acid equivalents/g extract and 13.6 ± 0.45 mg quercetin equivalents/g extract respectively.

Recently, high blood pressure has become a major threat to the health of human being. Reported data of Global Health Observatory (GHO) raised that blood pressure is estimated to cause 7.5 million deaths, which is about 12.8% of the total deaths [15]. The world was estimated to have close to 1 billion people with hypertension in the year 2000, and predicted an increase to 1.56 billion by 2025 [16]. Due to potential side effects of diuretics [17], the world population is looking for healthier alternatives for modern diuretics. As a natural alternative therapy people are looking for medicinal herbs to be used as significant source of diuretics. From ancient time many mono and poly-herbal preparations have been used in the form of decoction, tincture, tablets and capsules as diuretics **(Table 2)** [18]**.**

Tragia spp. is a well known medicinal herb used in Sri Lankan herbal medicines as a diuretic [19]. The present investigation was performed to scientifically validate the diuretic action of the whole plant of *Tragia* spp. using healthy Wistar rats. The hot water extract of *Tragia* spp. showed a significant dose-dependent diuretic activity as well as natriuretic and kaleuretic activity in urine excretion **(Table 3).** The diuretic activity of the extract initiates quickly, within 1 hour shows the maximum activity, but gradually decreases with time **(Figure 1).** The maximum urine output was observed at a dose of 2200 mg/kg hence the dose response showed a linear relationship (**Table 3**).

Table 1: Values of the Physicochemical parameters of *Tragia spp.*

Total ash content	11.0% ± 0.03
Water soluble ash content	3.7% ± 0.05
Acid insoluble ash content	0.7% ± 0.04
Cold water extractive value	33.3%
Hot water extractive value	31.4%
Cold methanol extractive value	10.4%
Hot methanol extractive value	13.2%
Cold ethanol extractive value	4.8%
Hot ethanol extractive value	9.4%
Cold ethyl acetate extractive value	2.9%
Hot ethyl acetate extractive value	4.2%
Cold dichloromethane extractive value	2.7%
Hot dichloromethane extractive value	2.6%

Table 2: Results of phytochemical screening of *Tragia spp.*

	Phytochemicals	Hot water extract
1	*Alkaloids*	–
2	*Coumarins*	+
3	*Flavonoids* · 10% Lead acetate test · Dil. NH_3 and Conc. H_2SO_4	+++ +
4	*Glycosides*	–
5	*Cardiac Glycosides* Keller-Kiliani test	–
6	Steroid Glycosides Liebermann's test	–
7	*Saponins* Froth test	+
8	*Tannins* Feric chloride test	+++
9	*Terpenoids* Salkowski test	+

Indicates (-): absence, (+): presence in low concentration, (++): presence in moderate concentration, (+++): presence in high concentration

Table 3: Total urine volume, diuretic action (*Urinary excretion of test group/urinary excretion of control group)*, diuretic activity (*Diuretic action of extract/Diuretic action of reference drug),* electrolyte levels, pH, and specific gravity of urine in healthy Wistar rats treated with different doses of hot water extract of whole plant of *Tragia spp*, vehicle and reference drug.

Group	Total urine volume	Diuretic action	Diuretic activity	Na+ ppm	K+ ppm	Natiuretic index Na+/k+	pH	Specific gravity
Control	0.72 ± 0.09	-	-	1537.3 ± 129.5	2461.7 ± 51.2	0.62	6.3	1.0009
Reference drug (Furosemide 13 mg/kg)	4.02 ± 0.62*	5.58	-	5672.0 ± 96.0	1922.3 ± 79.0	2.01	5.27	0.9694
550 mg/kg	0.87 ± 0.20*	1.21	0.22	6836.3 ± 82.5	5698.7 ± 151.8	0.77	5.84	1.0045
1100 mg/kg	2.08 ± 0.51*	2.89	0.52	5619.0 ± 121.8	4690.0 ± 81.8	0.82	6.26	1.0000
1650 mg/kg	2.98 ± 0.28*	4.14	0.74	4555.3 ± 121.6	3896.7 ± 88.5	1.26	5.95	0.9976
2200 mg/kg	4.01 ± 0.24*	5.56	1.00	4010 ± 89.3	1752.3 ± 152.6	1.28	6.17	0.9957

Values are expressed as mean ± S.E.M., n=6
*Significant when compared to the control; $P \leq 0.05$.

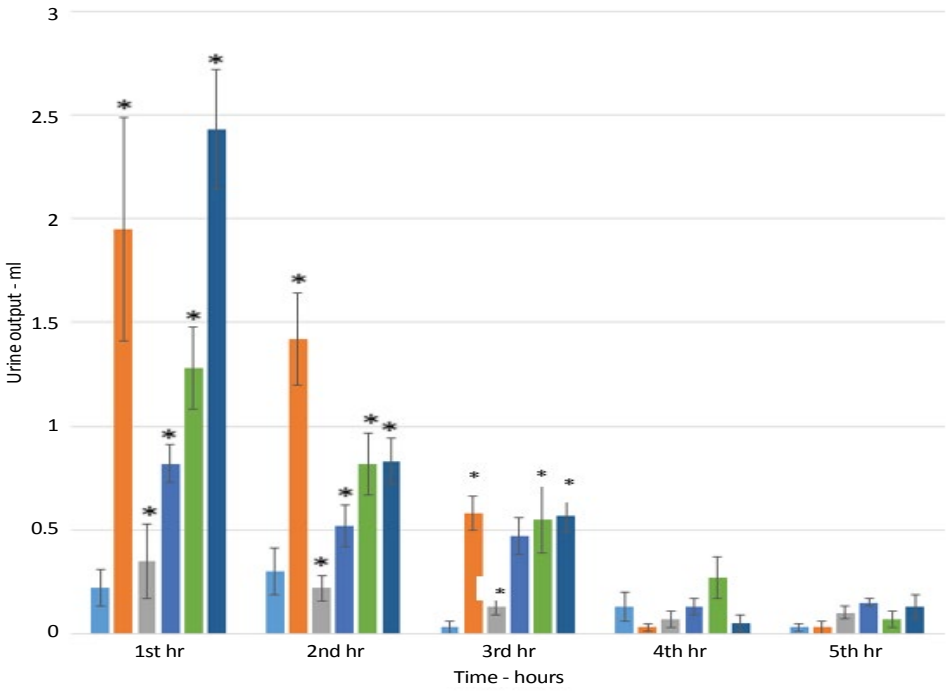

Figure 1 Time course of diuresis in rats treated with (550, 1100, 1650, 2200 mg/kg) different doses of hot-water extract *Tragia spp.* whole plant, vehicle and reference drug. Values are expressed as mean ± S.E.M., n=6. *Significant when compared to the control; $P \leq 0.05$.

Since there is a dose-dependent activity, it is evident that the diuresis causing effect is intrinsic and may not have been as a result from nonspecific action [3]. The urine output of the rats treated with *Tragia* spp. extract in the 1st hour was significantly (P<0.05) higher than that of furosemide, the reference drug. In addition, the total urine output after 5 h was almost the same in rats treated with either furosemide, the reference drug or hot water extract of *Tragia* spp. **(Table 3).**

According to a previous study, hot water extract of *T. involucrata* showed a maximum diuretic activity of 0.76 [3] whereas *Tragia* spp. showed a maximum diuretic activity of 1.00 **(Table 3)**. This indicates that hot water extract of *Tragia* spp. whole plant is more potent than the hot water extract of *T. involucrata* whole plant. Similar to *T. involucrate* [3], the hot water extract of *Tragia* spp. also showed kaleuretic and nateuretic activity with slight acidification of urine **(Table 3)** which are the features of loop diuretics. According to literature, loop diuretics cause kaleuretic and natriuretic effects from the inhibition of of K^+ reabsorption along the thick ascending limb of Henle's loop and from increased Na^+ delivery to the distal tubule [20]. In addition, loop diuretics also cause acidification in urine [21].

The diuresis activity of *Tragia* spp. may be contributed towards the high concentration of flavonoids **(Table 2)**. Previous studies have shown that flavonoids can cause diuretic action [22,23]. Since the diuresis action of *Tragia* spp. is similar to that of a loop diuretic, it can be worth mentioning that the hot water extract may be used as a natural therapeutic agent for hypertension and pulmonary oedema since loop diuretics are clinically used in

these conditions [24]. Further studies have to be performed to evaluate the diuresis activity of hot water extract of *Tragia* spp. on humans to scientifically validate the safety and the efficacy of the drug towards dieresis. Therefore, majority of people who still rely on herbal medicines can be benefited by this herbal medicine which shows great potential towards diuresis in rat model.

Conclusions

The diuretic activity of hot water extract of *Tragia* spp. was evaluated for the first time using the whole plant. Results revealed that *Tragia* spp. exhibits significant diuretic activity and acts similar to a loop diuretic. In addition, present study will be helpful in the correct identification and the authentication of this medicinal plant so as to prevent adulteration.

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