DOI 10.1007/s11094-022-02758-0
Pharmaceutical Chemistry Journal, Vol. 56, No. 8, November, 2022 (Russian Original Vol. 56, No. 8, August, 2022)

EFFECT OF *Euphorbia hirta* ON URINARY FLOW IN ALBINO MALE RATS

Sivaji Asha,[1] Palaniyandi Thirunavukkarasu,[2] Gani Taju,[3] and Abdul Majeeth Mohamed Sadiq[4,*]

Original article submitted August 21, 2021.

The aim of this work was to evaluate diuretic activity of the ethanol extract of *Euphorbia hirta* leaves and elucidate the possible mechanism of its action. The diuretic activity was studied on male albino rats in comparison to furosemide as a standard drug. Two doses of *E. hirta* extract (300 and 600 mg/kg) were administered and the urinary volume and electrolyte (Na^+, K^+) concentrations were measured. Results showed that administration of the ethanol extract of *E. hirta* led to significantly increased urinary volume and excretion of urinary electrolytes such as sodium, potassium and chloride in 24 h urine compared to that for normal animals. Among the two selected doses, 600 mg/kg body weight exhibited higher diuretic activity level than 300 mg/kg dose. Although both these levels were statistically significant when compared to control in respect of all parameters, these levels were lower compared to the standard drug. Hence, the ethanol extract of *E. hirta* exhibited a dose dependent diuretic activity. Upon the isolation and identification of active compounds from *E. hirta* ethanol extract, it was found that lupeol and quercetin were the major constituents responsible for the diuretic activity in rats. The present study confirmed validity of the ethnopharmacological use of *E. hirta* as a diuretic agent under experimental conditions studied.

Keywords: *Euphorbia hirta*; diuretic activity; diuretic index; saluretic; natriuretic; urinary volume.

1. INTRODUCTION

Euphorbia hirta is an erect, small ascending annual hairy weed growing up to a height of 50 cm, with stems of reddish or purplish colour, rounded hairy, and abundant milk sap (white latex) [1]. The plant leaves are simple, elliptical, dark green on the upper and pale on lower surface, hairy on both surfaces particularly at the lower surface veins, 1 – 3 cm long with finely dentate margin. Leaves are arranged in opposite pairs on the stem. Flowers are unisexual, dense auxiliary cymes 1 mm in length at each leaf node, and purplish to greenish in color. No petals generally occur on a stalk. Fruits are three celled, yellow color, tiny keeled capsules 1 – 2 mm in diameter. Fruits contain four-sided reddish-brown, smooth young wrinkled and angular seeds. Pharmacological properties reported for *E. hirta* include anti-inflammatory, antioxidant, antitumor, antifungal, antibacterial, antidiarrheal, antimalarial, sedative, anxiolytic, analgesic, antipyretic, antiasthmatic, larvicidal, and diuretic activity [2, 3].

[1] Department of Biochemistry, D. K. M. College for Women (Autonomous), Sainathapuram, Vellore, Vellore DT, Tamil Nadu, 632001 India.
[2] Department of Biotechnology, Dr. M. G. R. Educational & Research University, Chennai-95, TN, India.
[3] Aquaculture Biotechnology Division, C. Abdul Hakeem College, Melvisharam, Vellore, Tamil Nadu, India.
[4] PG and Research Department of Biochemistry, Adhiparasakthi College of Arts and Science (Autonomous), G. B. Nagar, Kalavai, Vellore DT, Tamil Nadu, 632506 India.
[*] e-mail: mohamed68@rediffmail.com; asha.sivaji@gmail.com

2. METHODS AND MATERIALS

2.1. Chemicals and Reagents

Ethanol and other chemicals of analytical grade were obtained from Sigma Chemical Co. (United States). Furosemide standard drug was obtained from domestic national industry.

2.2. Plant Material

Herbs collection and extract preparation. *Euphorbia hirta* weed was collected, identified and authenticated. Specimen voucher is housed at the herbarium of Adhiparasakthi Agricultural College, Kalavai (Tamil Nadu) under registration No. 1380. The botanical materials were shade dried in room temperature and grounded to powder. The extraction was performed by hot percolation method using Soxhlet ap-

paratus. The solvent was eliminated at 40°C on a rotary evaporator yielding semi-solid extract.

Standardization of plant material. Plant material was standardized in terms of physico-chemical constants including ash values (total ash, acid insoluble ash, water-soluble ash), loss on drying, and fluorescent analysis by following the standard method as described in Indian Pharmacopoeia. Preliminary phytochemical investigation (carbohydrates, proteins, amino acids, alkaloids, flavonoids, saponins, tannins and phenols, steroids, terpenoids, glycosides, fat and oils, gums and mucilages) of the ethanol extract of *E. hirta* leaves was accomplished to check the existence of various phytochemical constituents by using the corresponding chemical tests.

2.3. Experimental Design

The diuretic activity of *E. hirta* ethanol extract was evaluated on albino male rats by using the method of Lipschitz, et al. [4] with some modifications of Kau, et al. [5] adopted for this test. All animals were fasted 18 h prior to experiment with free access to water. The experimental rats (weighing 160—200 g) were divided into four groups of six animals each (IAEC/APCAS/01/2013/02). Then, normal saline (0.9% NaCl at 5 mL/kg body weight (bw) dose) and drug solutions were given as oral loading to all animals as follows:

Group I: Normal – 5 mL/kg bw in saline;

Group II: Furosemide – 25 mg/kg bw in saline;

Goup III: Ethanol extract of *E. hirta* – 300 mg/kg bw in saline;

Group IV: Ethanol extract of *E. hirta* – 600mg/kg bw in saline.

All drugs, including the the vehicle, standard drug and plant extract, were administered orally. Immediately after dosing, the animals in each group were placed individually in metabolic cages to collect urine in a graduated measuring cylinder for a period of 24 h. The volume of urine excreted during 24 h from each animal in each test group (groups II, III and IV) were compared with the volume of urine produced in the control group (group I) after the administration of normal saline. In the present study, the set of parameters taken for analysis included sodium, potassium and chloride concentrations in the excreted urine.

Diuretic activity evaluation. The ratio between urinary excretion of treated group and control group was used to estimate the diuretic action of *E. hirta* ethanol extract as follows:

$$\text{Diuretic action} = \frac{\text{Urinary excretion of treatedgroup}}{\text{Urinary excretion of controlgroup}}$$

$$\text{Diuretic activity} = \frac{\text{Diuretic action of testdrug}}{\text{Diuretic action of standard drug}}$$

Analytical procedures. Using the collected urine, the electrolyte concentrations of Na^+ and K^+ were measured on a flame photometer calibrated with standard solutions of various Na^+ and K^+ concentrations [6]. Electrolyte Cl^- concentration was determined titrimetrically [7].

Isolation and structural elucidation of bioactive compounds. The major active compounds present in the ethanol extract of *E. hirta* leaves were isolated by preparative HPLC, the collected fractions were analysed by UV spectroscopy and LC-MS/DAD, and the structure confirmation was carried out using data of ^1H and ^{13}C NMR spectroscopy measurements.

2.4. Statistical Data Analysis

Results obtained for four pairs of rats, expressed as mean ± S. D. values, were processed by means of the analysis of variance (ANOVA) sofrware. Statistically significant differences between the control group and treated groups and among the selected doses were determined by Tukey test. The level of $P < 0.05$ was considered as statistically significant. Statistical analysis employed the SPSS 11.0 software.

3. RESULTS AND DISCUSSION

3.1. Study of E. hirta Extract as Diuretic Agent

Standardization of plant material, physicochemical characteristics such as ash values, extractive values, loss on drying and phytochemical analysis of extracts were carried out in the previous study [8]. Diuretics are defined as drugs that increase the urine output and urinary excretion of sodium. Under certain medical circumstances, such drugs are used to regulate the volume in addition to the composition of body fluids. Hence, these drugs are helpful in the treatment of diseases connected with the maintenance of fluids. On the opposite, countless herbal diuretics can be well thought-out as gentle and high-quality drugs, in assessment to other diuretics used at the present time.

In the traditional system of medicine, the Swahili and Sukumas people used *E. hirta* as a diuretic agent [9]. Two separate mechanisms involved in diuresis are (a) increased urine (i.e. water) volume and (b) net loss of (electrolyte) solutes (i.e. saluretic effect) [10, 11]. These processes result from the inhibition of renal tubular re-absorption of electrolytes (water) and elimination of low molecular weight organic compounds into the bloodstream. As an outcome, this suppression promotes the formation of urine [10, 12]. Johnson, et al. [13] reported that *E. hirta* extracts (water and ethanol) contained some compounds that mediated diuretic effects by increasing the rate of urine output as well as electrolyte excretion.

3.2. Effect of Plant Extract on 24 h Urinary Excretion Volume in Rats

TABLE 1 shows data on the urinary volume in (mL/100 g/24 h) units in the control, standard furosemide, and *E. hirta* ethanol extract in 300 and 600 mg/kg bw dose groups. The values of 24 h urinary excretion volume are 3.68 ± 0.48

(control), 6.02 ± 0.84 (furosemide), 4.95 ± 0.49 plant extract (300 mg/kg bw) and 5.06 ± 0.63 plant extract (600 mg/kg bw) in (mL/100 g/24 h) units. Furosemide, a reference diuretic drug, significantly ($P < 0.01$) increased the urine output as compared to the control group I with a diuretic index of 1.63. It should be pointed out that the outcome of a single dose of the reference diuretic drug was more rapid and successful than that of the plant extract. The effect of oral administration of the ethanol extract of *E. hirta* in two different doses was comparable with that in the control group. The 24 h cumulative urinary excretion was found to be significantly greater compared to the control after treatment. The administration of test drug (*E. hirta* extract) at 300 mg/kg dose resulted in significant increase ($P < 0.05$) in the urine volume and a diuretic index of 1.34 as compared to the control. The results demonstrated that 600 mg/kg dose of *E. hirta* produced significantly ($P < 0.05$) increased urine volume and a diuretic index of 1.37 compared to control group. Thus, the *E. hirta* leaves extract exhibited a dose-dependent diuretic activity compared to control group. The most apparent and very significant effect of the extract was obtained at a dose of 600mg/kg as seen in results of the 24 h period study**.**

TABLE 1. Effect of Oral Administration of Ethanol Extract of *E. hirta* Leaves on Urinary Volume* in Albino Male Rats

Treatment group	Urinary volume (mL/100 g/24 h)	Diuretic index (24 h period)	Diuretic activity (24 h period)
Control	3.68 ± 0.48	–	-
Furosemide	6.02 ± 0.84*	1.63	-
E. hirta extract (300 mg/kg)	4.95 ± 0.49**	1.34	0.82
E. hirta extract (600 mg/kg)	5.06 ± 0.63**†	1.37	0.84

* Values are expressed as mean ± S. D. (ANOVA one-way analysis): * $p < 0.01$ compared to the control group which received saline; ** $p < 0.05$ compared to the control group which received saline; † $p < 0.05$ compared to group III which received 300 mg/kg of extract.

3.3. Effect of Plant Extract on 24 h Urinary Electrolyte Excretion

TABLE 2 shows data on the electrolyte sodium excretion (mEq/100 g/24 h) in urine samples of the *E. hirta* extract treated, furosemide treated, and control rats. The results for sodium are 2.70 ± 0.49 (normal), 5.12 ± 0.70 (furosemide), 3.94 ± 0.33 (plant extract 300mg/kg bw) and 4.23 ± 0.73 (plant extract 600 mg/kg bw) mEq/100 g/24 h, respectively. From these results, it was found that the extract in both doses produced significant increase ($p < 0.05$ and $p < 0.01$ for 300 and 600 mg/kg dose, respectively) in sodium excretion compared to the control group. The standard drug group also showed significant ($p < 0.001$) increase in the urinary sodium level as compared to the control group. Thus, the plant extract caused a dose depended urinary excretion of sodium electrolyte in rats, i.e., 600 mg/kg ($p < 0.01$) dose induced a higher activity of sodium than did 300 mg/kg dose of extract.

TABLE 2 also shows the effect of plant extract orally administered at different doses on 24 h urinary potassium level in rats. The higher dose (600 mg/kg) produced moderate increase in the urinary excretion of potassium (1.18 ± 0.36 vs. 1.04 ± 0.19 mEq/100 g/24 h, $p < 0.001$) as compared to control rats. The lower dose (300 mg/kg) had only slight effect on the urinary excretion of potassium (1.13 ± 0.27 vs. 1.04 ± 0.19 mEq/100 g/24 h, $p < 0.01$) as compared to the control group. However, the dose of 600 mg/kg ($p < 0.05$) caused a dose dependent increase in potassium excretion compared to the 300 mg/kg dose of plant extract. The standard drug furosemide at 24 h increased the urinary excretion of potassium in urine (1.32 ± 0.36 vs. control 1.04 ± 0.19 mEq/100 g/24 h, $p < 0.001$) as compared to the control.

In addition, Table 2 shows the level of chloride ion excretion in urine of the normal, furosemide and extract treated groups. As was the case with a dose of 600mg/kg, there was significant increase in chloride excretion with urine after ex-

TABLE 2. Effects of Oral Administration of Ethanol Extract of *E. hirta* Leaves on Urinary Electrolyte Excretion in Albino Male Rats

Treatment group	Urine electrolyte concentration (mEq/100 g/24 h)			Saluretic index			Na^+/K^+
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	
Group I	2.70 ± 0.49	1.04 ± 0.19	2.93 ± 0.61	–	-	-	2.62
Group II	5.12 ± 0.70***	1.32 ± 0.36***	5.31 ± 0.80***	1.89	1.28	1.81	3.87
Group III	3.94 ± 0.33**	1.13 ± 0.27*	4.16 ± 0.49**	1.45	1.09	1.41	3.48
Group IV	4.23 ± 0.73*††	1.18 ± 0.36***†	5.01 ± 0.35***†	1.56	1.14	1.70	3.58

Values are expressed as mean ± S.D for six rats: $^{***}p < 0.001$, $^{**}p < 0.05$, $^{*}p < 0.01$ compared to the control group using ANOVA one-way analysis and Tukey's *post hoc* test; $^{\dagger}p < 0.05$ compared to Group III which received 300 mg/kg of extract; $^{\dagger\dagger}p < 0.01$ compared to Group III which received 300 mg/kg bw dose. Group I (control), Group II (furosemide treated), Group III (ethanol extract of *E. hirta,* 300 mg/kg bw), and Group IV (ethanol extract of *E. hirta,* 600 mg/kg bw); Saluretic index = mEq. extract group/mEq. control group.

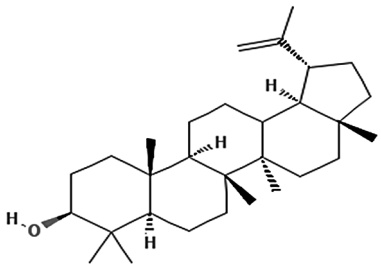

Fig. 1. Lupeol structure.

tract administration (5.01 ± 0.35 mEq/100 g/24 h vs. 2.93 ± 0.61 mEq/100 g/ 24 h, $p < 0.001$) compared to the control chloride excretion. Also, there was a significant change ($p < 0.05$) noticed in urine electrolyte chloride concentration after *E. hirta* treatment at a dose of 300 mg/kg (4.16 ± 0.49 vs. control 2.93 ± 0.61 mEq/100 g/24 h). These results revealed that 600 mg/kg dose of *E. hirta* extract was more potent ($p < 0.05$) for chloride excretion in a dose dependent manner. The standard drug furosemide also caused increased urinary chloride excretion at 24 h as compared to the control group I. Thus, with regard to the urinary electrolyte excretion, *E. hirta* extract increases this in a dose dependent manner.

With regard to saluresis and natriuresis, higher indices were observed for 600 mg/kg dose of *E. hirta* ethanol extract treated animals (Na^+, 1.56; Cl^-, 1.70; Na^+/K^+, 3.58) compared to control group. At the same time, 300 mg/kg dose of *E. hirta* ethanol extract produced moderate saluresis and natriuresis condition (Na^+, 1.45; Cl^-, 1.41; Na^+/K^+, 3.48) with inferior indices compared to 600 mg/kg dose of *E. hirta* extract, hence, a dose dependent activity was manifested. Furosemide exhibited higher values of saluresis and natriuresis (Na^+, 1.89; Cl^-, 1.81; Na^+/K^+, 3.87) compared to control and extract treated animals. Hence, the obtained data seem to show that 600 mg/kg dose of *E. hirta* ethanol extract is more potassium sparing compared to furosemide as a standard reference diuretic drug.

The present study examined diuretic potential of *E. hirta* leaf extract in rats. Similarly to the way of extract administration to humans in traditional medicine, oral treatment was used in this work. The mechanism of diuretic action induced by the orally administered ethanol plant extract was investigated in a normal adult male albino rats and also compared with a reference drug, furosemide [14, 15] and control group. Furosemide is quite protective but, in spite of this, it is able to cause ototoxicity, hypokalemia, hypomagnesemia, metabolic alkalosis and hyperuricemia [16]. Furosemide, a sulphonamyl derivative, is a highly effective diuretic acting on the medullary ascending limb of Henle's loop, distal tubule and collecting duct.

In this work, a dose dependent significant increase in the urine output and electrolyte excretion was observed in the rats treated with ethanol extract of *E. hirta* [17]. The plant extract exert its diuretic activity by inhibiting tubular re -absorption of water and its associated anions [18, 19]. With respect to the results of, from the existing diuretic activity of *E. hirta* seemed to be mediated through a change in potassium transport. Thus, we can say that plant extracts may inhibit potassium absorption or stimulate potassium secretion, or both, leading, to more potassium retention in the lumen of the kidney tubules and osmotic water flow [20].

The acute treatments of rats with the plant extract derive a significant diuretic activity in a dose-dependent manner. Results showed that the higher dose 600 mg/kg of *E. hirta* possesses a strong diuretic activity when given orally at a single dose. Stimulation of diuretic activity by plant extracts continued for at least 24 h after their administration to animals. Concerning electrolyte excretion, we observed significant increase in urinary excretion of Na^+ and higher K^+ concentrations under the action of plant extract in the present study.

Like the ethanol extract of *E. hirta* plant, the standard diuretic drug furosemide also showed obvious changes in the urinary volume and excretion of sodium, potassium and chloride in urine [21 – 23]. These results suggest a similar mechanism of action in both cases. Resembling other loop diuretics, furosemide inhibit the luminal Na^+, K^+, $2Cl^-$ symporter located in the thick ascending limb of Henle's loop. The loop diuretics inhibit the Na^+, K^+, $2Cl^-$ transporter and thus reduce the reabsorption of NaCl in the kidney and to reduce the lumen-positive potential that derives from K^+ recycling.

In the adopted model, the elimination of urinary electrolytes Na^+, K^+ and Cl^- after administration of the ethanol extract, the results showed enhanced natriuretic and kaliuretic effect with raising doses [23]. In this study, marked natriuresis was evident possibly because of the inhibition of Na^+ reabsorption in the nephron[24], thereby increasing the urinary output. Calculation of the saluretics index showed that the maximum dosage of *E. hirta* increased the elimination of Na^+, K^+ and Cl^- in urine with respect to control group. The Na^+/K^+ ratio also describes the diuretic action of plant extracts in a dose dependent manner. These features proved that the extract of *E. hirta* leaves acts in a similar way as furosemide acts. Because of the similar diuretic and saluretic index of the ethanol extract of *E. hirta* and furosemide, it is likely stated that active components of the *E. hirta* leaves exhibit a furosemide-like action as compared to control.

On the other hand, the fact that increased concentration of *E. hirta* extract raises water excretion and ionic excretion and also increases probability of the proposed mechanism of diuretic action. It is possible that *E. hirta* at high concentration (600 mg/kg) increases its diuretic activity by inhibiting tubular reabsorption of water and sodium.

In short, we ascertain that *E. hirta* leaves extract exhibits a notable diuretic effect in various doses relative to furosemide. Furthermore, *E. hirta* extract is very safe without toxicity and with K^+-saving effect at a concentration of 300 and 600 mg/kg. These characteristics strongly suggest that *E. hirta* leaves extract is acting as a loop diuretic. The

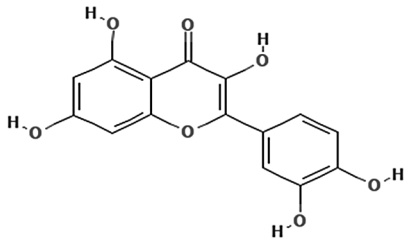

Fig. 2. Quercetin structure.

$Na^{+}/K^{+}/Cl^{-}$ co-transport system in the thick ascending loop of nephron is inhibited by loop diuretics and in doing so it increases both natriuresis and kaliuresis [26, 20].

The reported diuretic effect of *E. hirta* leaves extract agrees with previous findings reported by many other authors [17, 25, 27 – 31]. Further studies were concerned with isolation and structural elucidation of bioactive compounds in the ethanol extract by chromatography and spectroscopic analysis. Results obtained from this analysis showed that lupeol (Fig. 1) and quercetin (Fig. 2) are probably the major constituents present in the *E. hirta* leaves extract which may be responsible for diuresis. Detailed mechanisms of action of these compounds are yet to be elucidated.

4. CONCLUSION

Results observed for *E. hirta* extract in a 600 mg/kg dose suggest that the active components like lupeol and quercetin in the ethanol extract of *E. hirta* leaves had diuretic spectrum similar to that of the reference drug furosemide. For 300 mg/kg dose, there is increased urine output and enhanced Na^{+}, K^{+}, Cl^{-} levels compared to control group, but it showed lower capacity compared to 600 mg/kg. In the present study, it is reliably established that *E. hirta* leaves extract exhibits very potent diuretic activity. In particular, 600 mg/kg dose of extract showed an almost level of diuretic activity, hence it is taken for further studies. It is strongly regimented from the present diuretic activity that *E. hirta* has significant diuretic and contractile effects which makes it useful in the propulsion of urinary stones.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. E. M. Lind and A. C. Tallantire, *Some Common Flowering Plants of Uganda*, Oxford University Press: Nairobi (1971), p. 182.
2. M. C. Lanhers, J. Fleurentin, and P. Cabalion, *J. Ethnopharmacol.,* **29**, 189 – 198 (1990).
3. M. C. Lanhers, J. Fleurentin, P. Dorfman, et al., *Planta Med.,* **57**, 225 – 231 (1991).
4. W. L. Lipschitz, Z. Hadidian, and A. Kerpcsar, *J. Pharmacol. Exp. Ther.,* **79**, 97 – 110 (1943).
5. S. T. Kau, J. R. Keddi, and D. Andrews, *J. Pharmacol. Methods,* **11**, 67 – 75 (1984).
6. P. B. Godkar and D. B. Godkar, *Textbook of Medical Laboratory Technology* (2nd edn.), Bhalani Publishing Home: Mumbai (2003), pp. 409 – 418.
7. O. Schales and S. S. Schales, *J. Biol. Chem.,* **140**, 879 – 883 (1941).
8. S. Asha, P. Thirunavukkarasu, and A. Mohamad Sadiq. *World J. Pharm. Sci.,* **3**(6), 1104 – 1112 (2015).
9. J. M. Watt and M. G. Breyer-Brandwijk, The Medicinal and Poisonous Plants of Southern and Eastern Africa. E. S. Livingstone Ltd., London (1962).
10. G. De Stevens, Diuretics: Chemistry and Pharmacology, Academic Press: New York (1963), pp. 52 – 58.
11. E. K. Jackson, Drugs affecting renal and cardiovascular function. Pergamon Press: New York (1996), pp. 685 – 713.
12. J. C. Milton, M. Shelton, and H. Brainerd, Hand book of medical treatment, Lange Medical Publication–Blackwell Scientific Publication: Oxford (1970), pp. 220 – 229.
13. P. B. Johnson, E. Abdurahman, A. T. Emmanuel, and I. M. Hussaini, *J. Ethnopharmacol.,* **65**(1), 63 – 9 (1999).
14. J. Diezi, Diurétiques, in: M. Schorderet, *Pharmacologie, des Concepts Foundamentaux aux Applications Therapeutiques*. Frison-Roche: Paris (1992), pp. 151 – 167.
15. F. G. Smith and A. M. Strack, *Am. J. Physiol.,* **269**, 149 – 152 (1995).
16. C. Presne, M. Monge, J. Mansour, et al., *Nephrol. Ther.*, **3**, 392 – 423 (2004).
17. J. R. Montejano-Rodriguez, G. Almaguer-Vargas, J. A. Gayosso-De Lucio, et al., *J. Pharm. Res.,* **6**, 709 – 713 (2013).
18. C. V. Pantoja, L. C. Chiang, B. C. Norris, et al., *J. Ethnopharmacol.,* **31**, 325 – 331 (1991).
19. L. H. Bevevino and M. M. Aires, *J. Ethnopharmacol.,* **43**, 203 – 207 (1994).
20. S. I. Kreydiyyeh and J. Usta, *J. Ethnopharmacol.,* **79**, 353 – 357 (2002).
21. B. D. Rose, Diuretics, in: *Kidney Int*., Vol. 39 (1991), pp. 336 – 352.
22. T. Shinkawa, F. Yamaski, T. Notsu, et al., *Eur. J. Pharmacol.,* **238**, 317 – 325 (1993).
23. E. K. Jackson, *The Pharmacological Basis of Therapeutics*, McGraw- Hill: New York (2001), pp. 775 – 788.
24. J. Leuschner, *Arzneim. Forsch*., **45**, 165 – 168 (1995).
25. W. D. Ratnasooriya, K. P. Pieris, U. Samaratunga, and J. R. Jayakody, *J. Ethnopharmacol.,* **91**, 317 – 20 (2004)
26. H. P. Rang, M. M. Dale, J. M. Ritter, and P. K. Moore, *Pharmacology* (5th edn.), Churchill Livingstone: Edinburgh–New York (2003), pp. 352 – 366.
27. A. Cáceres, L. M. Girón, and A. M. Martínez, *J. Ethnopharmacol.,* **19**(3), 233 – 245 (1987).
28. F. B. O. Mojiminiyi et al, *J. Med. Med. Sci.,* **2**, 77 – 80 (2000).
29. O. M. Arafat, S. Y. Tham, A. Sadikun, I. Zhari, et al., *J. Ethnopharmacol.,* **118**, 354 – 360 (2008)
30. A. Herrera-Arellano, S. Flores-Romero, M. A. Chavez-Soto, and J. Tortoriello, *Phytomedicine,* **11**, 375 – 382 (2004).
31. C. Sadki, B. Hacht, A. Souliman, and F. Atmani, *J. Ethnopharmacol.,* **128**, 352 – 356 (2010).