



**BJP**

**Bangladesh Journal of Pharmacology**

**Research Article**

**Diuretic activity of the bark of *Ey-  
senhardtia polystachya***

## Diuretic activity of the bark of *Eysenhardtia polystachya*

Saady Saret Pablo-Pérez, María Mirian Estévez-Carmona and María Estela Meléndez-Camargo

Departamento de Farmacia, Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional (ENCB-IPN), Mexico City, 07738, Mexico.

### Article Info

Received: 2 September 2015

Accepted: 16 September 2015

Available Online: 21 January 2016

DOI: 10.3329/bjp.v11i1.24659

#### Cite this article:

Pablo-Pérez SS, Estévez-Carmona MM, Meléndez-Camargo ME. Diuretic activity of the bark of *Eysenhardtia polystachya*. Bangladesh J Pharmacol. 2016; 11: 212-17.

### Abstract

The aim of this study was to evaluate the diuretic activity of *Eysenhardtia polystachya* bark aqueous extract at different doses in a rat model. Different doses of *E. polystachya* (125, 250, 500 and 750 mg/kg body weight), furosemide (4 mg/kg) and vehicle were administered *per os* to female rats ( $n=6$  animals per group). After 6 hours in metabolic cages, the effect on urinary flow, glomerular filtration rate and electrolyte balance of sodium and potassium were assessed in all animals. *E. polystachya* at the doses of 500 and 750 mg/kg induced diuretic activity, since markedly increased ( $p<0.05$ ) the urinary flow rate, similar to that of furosemide treated group. Only the dose of 750 mg/kg produced an increment in urinary excretion of sodium but not of potassium compared with control group. These findings indicate that *E. polystachya* bark-induced diuretic activity, providing evidence for its folkloric use.

## Introduction

*Eysenhardtia polystachya* (Ort.) Sarg. is a small tree native to Mexico in the leguminosae family that grows wild and abundantly in fields (Argueta et al., 1994; Alvarez et al., 1998). Commonly, it is known as “palo azul” (blue wood) or “palo dulce” (sweet wood), because the infusion of the wood in water has a sweet flavor and displays a golden color with a bluish fluorescence (Alvarez et al., 1998). Phytochemical studies indicate that *E. polystachya* contains polyphenols, and previous chemical examination of this species led to the isolation and structural elucidation of several flavonoids (Argueta et al., 1994; Alvarez et al., 1998; Perez and García, 2014).

*E. polystachya* is widely used in folk medicine as a blood depurative, antitussive, antispasmodic, anti-diabetic, febrifuge, anti-inflammatory, anti-rheumatic and analgesic agent (Argueta et al., 1994; Perez and García, 2014). The decoction made from the fresh bark *E. polystachya* has quite a wide popular use in the traditional Mexican medicine as herbal remedy for several illnesses on the renal tract, especially as a diuretic and antimicrobial

agent for treatment of kidney and bladder infections in many areas of States of Puebla, Mexico and Hidalgo in Mexico (Argueta et al., 1994; Perez et al., 1998).

Published reports indicate that the aqueous extract of the bark of *E. polystachya* prevents the formations of kidney stones *in vivo* (Perez et al., 1998). However, a survey of the literature showed that no systematic study of its main ethnomedical use as a diuretic has been performed. With this focus, the present study was undertaken to verify the efficacy of the aqueous extract of this plant in an experimental model of diuresis in the rat.

## Materials and Methods

### Collection of the plant material

*E. polystachya* was collected with the permission of the Mexican authorities in San Pedro Tlaquilpan, Zempoala (19° 55' N, 98° 40' W), State of Hidalgo, Mexico, in February 2010. The botanical identification and



authentication of the plant samples were performed by Biologist Laura Doval Ugalde at Escuela Nacional de Ciencias Biológicas of Instituto Politécnico Nacional (ENCB-IPN). The plant samples were compared with a voucher specimen deposited in the ENCB herbarium under number 247.

#### *Ethnobotanical survey*

A survey was conducted around the municipality of San Pedro Tlaquilpan, Zempoala, State of Hidalgo using a questionnaire to interview individuals with knowledge on the subject matter as herb seller, traditional medical healers and consumers about the part of the plant and the usefulness of *E. polystachya* in ethnomedicine (Bahmani et al., 2014). A specimen of the plant was shown to people at the time of the interview to avoid confusion.

#### *Preparation of the aqueous extract*

The bark was air-dried at room temperature in the shade, and then was ground in a mill (FITZ® MILL model D Comminutor, Industrial Drive Elmhorst, USA). The *E. polystachya* bark aqueous extract was freshly prepared just before administration by decoction as follows. A part of the bark of *E. polystachya* was boiled at 100°C in water for 5 min and subsequently filtered. All doses of extract used were calculated respect to the weight of starting dry material.

#### *Phytochemical screening*

The secondary metabolites of aqueous extract were detected by standard color and precipitation phytochemical tests. Briefly, Dragendorff, Mayer, Sonnenschein and Wagner reactions were used to identify alkaloids. Fehling and Benedict reactions for reductor sugars. Erlich reaction and observation under UV light of alkalized extracts for coumarins. Kedde, Legal and Baljet reactions for cardiac glycosides. A frothing test was done for saponins. The tannins were identified by FeCl<sub>3</sub> test and jelly reagent. Liebermann-Buchard reaction for triterpenoids and steroids. Bornträger reaction for quinones and Shinoda reaction for flavonoids were used, among others (Meléndez-Camargo et al., 2014; Saleem et al., 2015).

#### *Experimental animals*

Adult female Wistar rats (180-220 g) and female NIH mice (20-30 g) were used. They were housed and maintained in the animal house at room temperature (22-24°C) and 50-55% relative humidity, with day/night cycles of 12 × 12 hours. The animals were fed a standard rodent diet with water *ad libitum*. Care and handling of animals followed internationally accepted procedures according to the Institute for Laboratory Animal Research's Guide for Care and Use of Laboratory Animals.

#### *Reagents and drug*

Reagent-grade reagents were used. Furosemide (Lasix®) was used as reference diuretic drug.

#### *Diuretic activity of aqueous extract of E. polystachya*

##### Effect of aqueous extract on urinary flow rate

The experiments were carried out in un-anesthetized rats. The rats were formed into six random treatment groups. Control group received water (1 mL/kg body weight (bw)), furosemide-treated group (4 mg/kg) and four groups treated with the extract at the doses of 125, 250, 500 and 750 mg/kg, respectively. The doses of extract used were calculated respect to the weight of starting dry material. In all groups, the administration was *per os* and there were at least six animals per group.

At the beginning of the experiment, the urinary bladder was emptied by gentle compression of the abdomen, as previously described (Meléndez et al., 2004), subsequently, the treatments were administered. The animals were kept during 6 hours in individual metabolic cages in a quiet and warm environment under fluid and food deprivation. At the end of this period, the sample of urine was obtained; also by abdominal compression to ensure complete emptiness of the bladder; urine volume was measured. The animals were decapitated and blood samples were obtained.

##### Effects on electrolyte balance

Sodium and potassium concentrations were measured in the urine and plasma samples (Flame photometer Corning 400, Corning Medical and Scientific, England) from all rats. Subsequently, urinary excretions and clearances of sodium and potassium were calculated (Meléndez et al., 2004).

##### Effect on glomerular filtration rate

Glomerular filtration rate was estimated by the clearance of endogenous creatinine. Plasma and urinary creatinine were determined by the method of Jaffe alkaline picrate modified by Meléndez et al., 2004. Creatinine clearance and fractional excretion of sodium and potassium were calculated using the conventional equations (1 and 2):

$$C_x = (U_x * V / P_x) \text{ --- Equation 1}$$

$$FE_x = (C_x / C_{creat}) * 100 \text{ --- Equation 2}$$

Where, C<sub>x</sub> and C<sub>creat</sub> are the clearance (mL/min) of substance x and creatinine, respectively. U<sub>x</sub> and P<sub>x</sub> are the concentrations (μEq/L) of substance x in urine and plasma, respectively, and V is the urinary flow (mL/min). FE<sub>x</sub> is the fractional excretion (%) of the substance x

#### *Acute toxicity study*

An acute toxicity study was performed in mice. Mice were fasted overnight with water given *ad libitum* and randomly assigned into five treatment groups. Four animals were used for complete evaluation at each dose

level. The aqueous extract at doses from 2, 4, 8 and 16 g/kg were administered *per os*. Water was administered to the control group. All deviations in general behavior associated with the administration of *E. polystachya* were monitored and recorded continuously for the first 3 hours after the administration. For the next 14 days, the number of dead animals was also recorded. During this period, the animals had access to food and water *ad libitum* (Hilaly et al., 2004; Mukinda and Eagles, 2010).

### Statistical analysis

All data are expressed as means  $\pm$  SEM (standard error of the mean). To perform statistical analyses, Sigma Plot® 11.0 software was used. Statistical analyses were performed with one-way analysis of variance (ANOVA). The statistical test Student-Newman-Keuls (S-N-K) was used for *post-hoc* comparisons. Significant differences were set at p values less than 0.05.

## Results

One hundred questionnaires were applied which showed that it was generally called 'palo dulce' (sweet wood), 'palo azul' (blue wood) and 'hierba de la víbora'. *E. polystachya* is specially used to treat several diseases as kidney pain (42%), urolithiasis (31%), diuretic (15%), anuria or oliguria (7%) and renal infection (5%). On the other hand, its use during inflammatory processes also was mentioned.

*E. polystachya* is prepared as an infusion, decoction or maceration, using one or two small pieces of the bark in one liter of water. Many of interviewed people indica-

ted that the main way of use of plant was by the decoction of the bark for enhancing the urinary volume, and it was taken orally as tea until the symptoms disappear.

Phytochemical screening of the extract revealed the presence of medicinal active constituents as anthraquinones, cardiac glycosides, coumarins, reductor sugars, saponins and tannins. Flavonoids were the major constituents. However, the extract was devoid of alkaloids, cyanogenic glycosides and sesquiterpenlactones.

Rats treated with extract (500 and 750 mg/kg) exhibited an increase ( $p < 0.05$ ) in urinary flow rate, similar to that of furosemide (Figure 1).

The increment of the urinary excretion of sodium was depended on the doses of the extract, but only the increments in 750 mg/kg of extract and furosemide were different respect to the control group (Figure 2). *E. polystachya* did not increase the urinary excretion of potassium, in contrast to furosemide (Table I).

The basal values of plasma electrolytes were similar in all groups (data not shown). Sodium clearance increased ( $p < 0.05$ ) in the extract (500 and 750 mg/kg) and furosemide groups respect to control group. The increments were dose-dependent. However, the extract did not increase the potassium clearance, in contrast to furosemide (Table I).

The glomerular filtration rate remained unchanged in all treated groups. Increments in fractional excretion of sodium and potassium were shown in the furosemide group, but not in the groups treated with the extract (Table II).

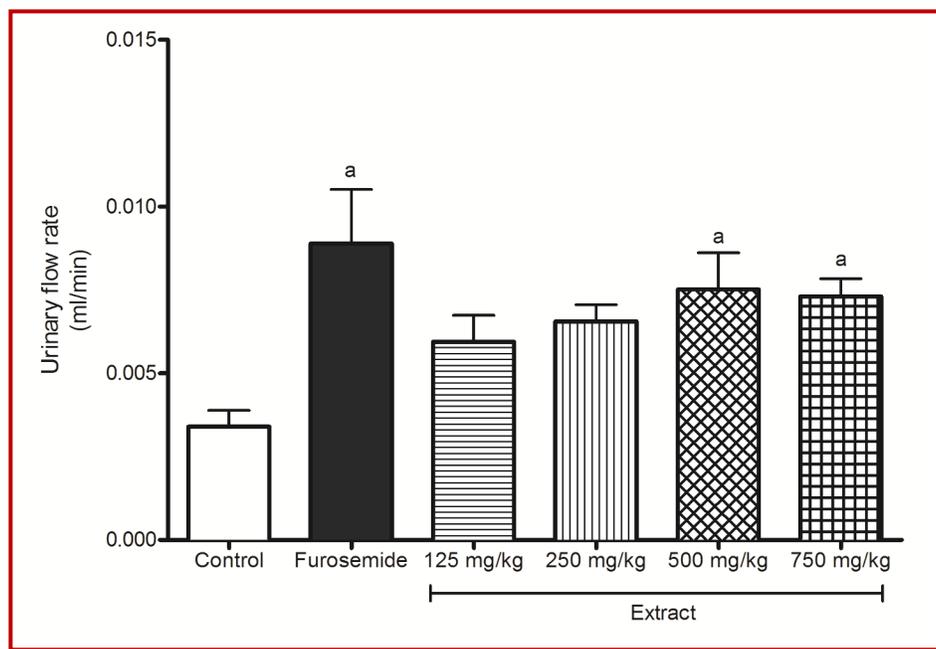


Figure 1: Effect of aqueous extract of *E. polystachya* on urinary flow rate in rats. Mean  $\pm$  SEM of six animals per group;  $^*p < 0.05$  compared with control group

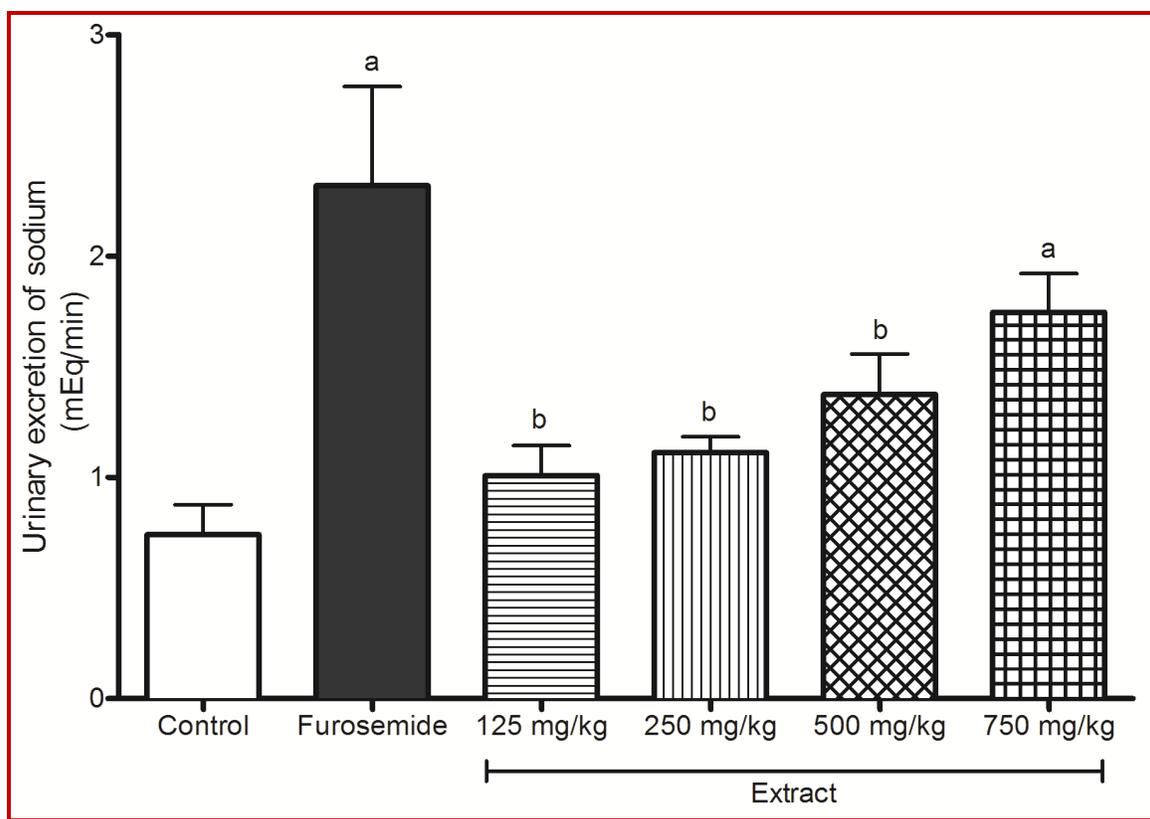


Figure 2: Effect of aqueous extract of *E. polystachya* on urinary excretion of sodium in rats. Mean  $\pm$  SEM of six animals per group; <sup>a</sup> $p < 0.05$  compared with control group; <sup>b</sup> $p < 0.05$  compared with furosemide group

Table I

Effect of *E. polystachya* aqueous extract on urinary excretion and clearances of sodium and potassium

Treatment group	Urinary excretion of potassium (mEq/min)	Sodium clearance ( $\mu$ L/min)	Potassium clearance ( $\mu$ L/min)
Control (vehicle)	0.6 $\pm$ 0.1	5.2 $\pm$ 1.0	138.3 $\pm$ 22.3
Furosemide (4 mg/kg)	1.6 $\pm$ 0.4 <sup>a</sup>	14.8 $\pm$ 2.5 <sup>a</sup>	396.9 $\pm$ 67.8 <sup>a</sup>
Extract (125 mg/kg)	0.8 $\pm$ 0.1 <sup>b</sup>	6.9 $\pm$ 0.8 <sup>b</sup>	212.2 $\pm$ 37.7 <sup>b</sup>
Extract (250 mg/kg)	0.8 $\pm$ 0.1 <sup>b</sup>	8.4 $\pm$ 1.3 <sup>b</sup>	191.9 $\pm$ 27.3 <sup>b</sup>
Extract (500 mg/kg)	0.9 $\pm$ 0.2	8.6 $\pm$ 1.8 <sup>a</sup>	252.1 $\pm$ 46.2
Extract (750 mg/kg)	0.9 $\pm$ 0.2	10.2 $\pm$ 2.2 <sup>a</sup>	259.1 $\pm$ 56.2

Data are mean  $\pm$  SEM of six animals in all groups. <sup>a</sup> $p < 0.05$  compared with control group, <sup>b</sup> $p < 0.05$  compared with furosemide treated group

None of the doses of aqueous extract produced mortality. Moreover, any signs of toxicity were observed after oral administration. Because no deaths were observed at the dose levels used, the LD<sub>50</sub> was assumed greater than 16 g/kg.

## Discussion

The present results reveal that *E. polystachya* aqueous extract (500 and 750 mg/kg) induced an important diuretic action because increments in the urinary flow and urinary excretion of sodium similar to furosemide

were shown. This effect on urinary sodium excretion is in proportion to the ability of the plant to excrete water, which suggests that the diuretic effect of *E. polystachya* is saluretic type in contrast to aquaretic type typical of most phytodiuretic agents (Mekonnen et al., 2010).

Only extract at a dose of 750 mg/kg induced an increment in sodium clearance similar to furosemide-treated group. However, in contrast to furosemide, the extract does not affect the potassium clearance, the fractional excretion of sodium and potassium. This suggesting that the extract is acting as potassium-sparing diuretic and this effect should be viewed as a

Table II

Effect of aqueous extract of *E. polystachya* on glomerular filtration rate, fractional excretion of sodium and potassium

Treatment group	Glomerular filtration rate (mL/min)	Fractional excretion of sodium (%)	Fractional excretion of potassium (%)
Control (vehicle)	1.1 ± 0.1	0.5 ± 0.1	13.4 ± 2.2
Furosemide (4 mg/kg)	1.3 ± 0.1	1.1 ± 0.2 <sup>a</sup>	29.0 ± 3.1 <sup>a</sup>
Extract (125 mg/kg)	1.6 ± 0.3	0.4 ± 0.1 <sup>b</sup>	13.4 ± 2.3 <sup>b</sup>
Extract (250 mg/kg)	1.3 ± 0.2	0.7 ± 0.1 <sup>b</sup>	16.1 ± 3.2 <sup>b</sup>
Extract (500 mg/kg)	1.5 ± 0.2	0.6 ± 0.1 <sup>b</sup>	17.2 ± 2.4 <sup>b</sup>
Extract (750 mg/kg)	1.2 ± 0.2	0.9 ± 0.3 <sup>b</sup>	22.3 ± 4.8

Data are mean ± SEM of six animals in all groups. <sup>a</sup>p<0.05 compared with control group, <sup>b</sup>p<0.05 compared with furosemide treated group

favourable feature of the extract in regards to electrolyte excretion (Gasparotto et al., 2011), for example the extract exhibited advantageous effect with regard to hypokalemia, a potential adverse effect of furosemide (Mekonnen et al., 2010; Sadki et al., 2010).

The glomerular filtration rate was not modified in all treated groups; but this variable is modified in conventional treatment with high doses of furosemide as a side effect (Meléndez et al., 2004). Since *E. polystachya* can excrete sodium and water is possible to suggest that the extract exerted its diuretic effect by inhibiting tubular reabsorption of water and electrolytes.

The diuretic capability of plants has been described in previous studies with other medicinal plants (Benjumea et al., 2005; Lahlou et al., 2007; Martín-Herrera et al., 2007; Adam et al., 2009; Amuthan et al., 2012). However, the mechanism of action of these has not been elucidated because of aqueous or organic extracts with biological activity, are a mixture of compounds may be working by synergism and some hypotheses have been formulated in order to explain medical properties of plants.

Several authors have reported a relationship between the presence of polar secondary metabolites in plants and its diuretic activity. For example, the presence of polar organic compounds as flavonoid glycosides and saponosids in plants can produce diuresis because they are more likely to have contact with renal tissues (Lahlou et al., 2007; Adam et al., 2009; Amuthan et al., 2012; Meléndez-Camargo et al., 2014). Natural compounds in medicinal plants might stimulate the regional blood flow and/or promote an initial vasodilatation as do the osmotic diuretics and xanthenes (Osswald and Schnermann, 2011; Saleem et al., 2015), but might also produce inhibition of renal tubular reabsorption of water and anions (Abdala et al., 2012; Gasparotto et al., 2011); the result in these cases is diuresis. In addition, the electrolytes present in plants can induce a diuretic activity (Adam et al., 2009).

So, one can suppose that the phenolic compounds such

as anthraquinones, coumarins, saponins, tannins and flavonoids detected in the extract might be responsible, at least in part, for the observed diuretic effect and that they may act individually or synergistically.

Despite the widespread use of herbal medicines, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies (Hilaly et al., 2004; Mukinda and Eagles, 2010). The present investigation shows that the *E. polystachya* does not produce death, hence it can be concluded that *E. polystachya* is not toxic orally.

## Conclusion

The bark of *E. polystachya* induces an increase in urinary excretion of water and sodium, similar to furosemide, but with the advantage of a potassium-saving effect. These findings support the claims of the diuretic efficacy of the bark of *E. polystachya* in the Mexican Traditional Medicine.

## Acknowledgement

This work was partially supported by the Secretaria de Investigación y Posgrado, IPN. The authors are deeply grateful to Lic. Margarito Salazar-Rodríguez and M. en C. Anuar Salazar-Gómez for their support during the investigation. SSPP has scholarship from CONACyT, México (237225).

## Conflict of Interest

The authors do not declare any conflicts of interest.

## References

- Abdala S, Martín-Herrera D, Benjumea D, Gutiérrez, SD. Diuretic activity of some *Smilax canariensis* fractions. J Ethnopharmacol. 2012; 140: 277-81.
- Adam Y, Somchit MN, Sulaiman MR, Nasaruddin AA, Zuraini

- A, Bustamam AA, Zakaria, ZA. Diuretic properties of *Orthosiphon stamineum Benth.* J Ethnopharmacol. 2009; 124: 154-58.
- Alvarez L, Rios MY, Esquivel C, Chávez MI, Delgado G, Aguilar MI, Villarreal ML, Navarro V. Cytotoxic isoflavans from *Eysenhardtia polystachya*. J Nat Prod. 1998; 61: 767-70.
- Amuthan A, Chogtu B, Bairy KL, Sudhakar, Prakash M. Evaluation of diuretic activity of *Amaranthus spinosus* Linn. aqueous extract in Wistar rats. J Ethnopharmacol. 2012; 140: 424-27.
- Argueta VA, Cano ALM, Rodarte ME. Atlas de las Plantas de la Medicina Tradicional Mexicana. Tomo II. Mexico City, Mexico, Instituto Nacional Indigenista, 1994.
- Bahmani M, Zargaran A, Rafieian-Kopaei M, Saki K. Ethnobotanical study of medicinal plants used in the management of diabetes mellitus in the Urmia, Northwest Iran. Asian Pac J Trop Med. 2014; 7S1: S348-54.
- Benjumea D, Abdala S, Hernandez-Luis F, Pérez-Paz P, Martín-Herrera D. Diuretic activity of *Artemisia thuscula*, an endemic canary species. J Ethnopharmacol. 2005; 100: 205-9.
- Gasparotto JA, Gasparotto FM, Boffo MA, Lourenço ELB, Stefanello MÉA, Salvador MJ, da Silva-Santos JE, Andrade MMC, Kassuya CAL. Diuretic and potassium-sparing effect of isoquercitrin-an active flavonoid of *Tropaeolum majus* L. J Ethnopharmacol. 2011; 134: 210-15.
- Hilaly JE, Israili ZH, Lyoussi B. Acute and chronic toxicological studies of *Ajuga iva* in experimental animals. J Ethnopharmacol. 2004; 91: 43-50.
- Lahlou S, Tahraoui A, Israili Z, Lyoussi B. Diuretic activity of aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. J Ethnopharmacol. 2007; 110: 458-63.
- Martín-Herrera D, Abdala S, Benjumea D, Pérez-Paz P. Diuretic activity of *Withania aristata*: An endemic Canary Island species. J Ethnopharmacol. 2007; 113: 487-91.
- Mekonnen T, Urga K, Engidawork E. Evaluation of the diuretic and analgesic activities of the rhizomes of *Rumex abyssinicus* Jacq in mice. J Ethnopharmacol. 2010; 127: 433-39.
- Meléndez CME, Berdeja B, Miranda G. Diuretic effect of the aqueous extract of *Bidens odorata* in the rat. J Ethnopharmacol. 2004; 95: 363-66.
- Meléndez-Camargo ME, Contreras-León I, Silva-Torres R. Diuretic effect of alkaloids fraction extracted from *Selaginella lepidophylla* (Hook. et Grev.) Spring. BLACPMA. 2014; 13: 92-99.
- Mukinda JT, Eagles PFK. Acute and sub-chronic oral toxicity profiles of the aqueous extract of *Polygala fruticosa* in female mice and rats. J Ethnopharmacol. 2010; 128: 236-40.
- Osswald H, Schnermann J. Methylxanthines and the kidney. Handb Exp Pharmacol. 2011; 200: 391-412.
- Perez GRM, García BE. Evaluation of antidiabetic, antioxidant and antiglycating activities of the *Eysenhardtia polystachya*. Pharmacogn Mag. 2014; 10: S404-18.
- Perez GRM, Vargas SR, Perez GS, Zavala SM. Antirolithiatic activity of *Eysenhardtia polystachya* aqueous extract on rats. Phytother Res. 1998; 12: 144-45.
- Sadki C, Hacht B, Souliman A, Atmani F. Acute diuretic activity of aqueous *Erica multiflora* flowers and *Cynodon dactylon* rhizomes extracts in rats. J Ethnopharmacol. 2010; 128: 352-56.
- Saleem H, Ahmad I, Gill MSA. Phytochemical screening and diuretic activity of *Euphorbia granulata*. Bangladesh J Pharmacol. 2015; 10: 584-87.

**Author Info**

María Estela Meléndez-Camargo (Principal contact)  
e-mail: emelendezc@hotmail.com

## **Your feedback about this paper**

1. Number of times you have read this paper

2. Quality of paper

3. Your comments