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DIURETIC ACTIVITY OF *PIPER PELTATUM* L. (PIPERACEAE) FROM ECUADOR ON *RATTUS NORVEGICUS*

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Abstract

Piper peltatum L. is a species that belongs to the Piperaceae family and it is used traditionally as diuretic medicinal plant. This study has been focused on evaluating the acute diuretic activity of *P. peltatum* from Ecuador and to determine its phenolics and flavonoids content. The extract was obtained from *P. peltatum* roots through a maceration process using ethanol 70 %. Total phenolic content was estimated by using Folin-Ciocalteu reagent and total flavonoid content was evaluated through of $AlCl_3$ complexation method. The study of the diuretic effect was performed on *Rattus norvegicus*, using extracts at doses of 25, 100 and 200 mg/kg, normal control (sodium carboxymethylcellulose, CMC, 0.5 % w/v, dose: 10 mL/kg) and standard (furosemide, dose: 10 mg/kg). Urine volume and the excretion of electrolytes increased in rats treated with *P. peltatum* ethanolic extracts, concluding that the diuretic activity of this species was statistically similar to furosemide. This activity could be related to the presence of phenolic and flavonic compounds. *Piper peltatum* is an interesting source of natural diuretic metabolites. Further detailed phytochemical, toxicity and clinical studies are required.

Keywords: *Piper peltatum, Piperaceae, diuretic activity, electrolytes, flavonoids*

Introduction

The global market for medicinal plants is continuously growing, reflecting the consumers' demand for "natural products" to promote a healthy way of living (1). Plants were once the primary source of medicines in the world. Approximately, 50% of all drugs in clinical use in the world come from natural products, from which higher plants contribute 25% of the total (2). In developing countries, around 80% of people use traditional medicine because of its affordability and cultural acceptability (2), often representing the only therapeutic system to which certain people could refer (3) (4).

The Piperaceae family is composed of about 8 genera and 3000 species. They are widespread across warm tropical and subtropical regions and are especially common in South and Central America, and central Asia (5).

The *Piper* genus is known due to the presence of aromatic oil cells in their structures. *Piper* species are used to treat gastrointestinal diseases, hypertension, women's health conditions, anti-hemorrhagic, diuretic, pains and inflammation (6) (7) (8).

Piper peltatum L., popularly known as *cordoncillo* or *santa maría,* has various traditional uses such as a diuretic, antipyretic and like an internal and external anti-inflammatory agent in some regions of the Amazon (9-11). In addition, it has also been used as an antidote against snake bites in the northwestern region of Colombia (12) (13).

P. peltatum has been tested and there are reports of its use to treat malaria, due to its anti-inflammatory and antioxidant capacity (14). Moreover, antibacterial efficacy of *P. peltatum* against *Alicyclobacillus acidoterrestris* was proved (15). In spite of the use of *P. peltatum* in traditional medicine, there are no scientific studies supporting its use as a diuretic.

Diuretics, such as thiazides and furosemide, are among the most used anti-hypertensive agents in humans. These drugs are known by their ability to reduce blood pressure in hypertension and improve the cardiovascular function in heart failure, among others (16). However, these agents are also associated with important adverse effects, such deleterious/dangerous reduction in Na+ and K+ plasmatic levels. Thus, the development of new diuretic agents with reduced adverse effects is important (16).

Plant extracts are commonly used in traditional medicine to treat some renal diseases, because of their significant diuretic activity (17). This study aimed at evaluating the acute diuretic, saliuretic, and natriuretic effects of orally administered ethanolic extract of *P. peltatum* from Ecuador in *Rattus norvegicus*.

Methods

Collection of plant material

Roots from *Piper peltatum* L. were collected from Ecuador, in Manabí province, in Carmen town (236 meters above sea level). The plant material was taxonomically identified by the botanist José Vargas at the Herbarium Misael Acosta Solís in the Universidad Técnica de Ambato (Ambato, Ecuador). Collections were approved by the Ministerio del Ambiente del Ecuador under permission MAE-DNB-CM-2018-0086.

Extraction of the plant material

Roots were selected, dried in a forced convection oven and ground in a knife mill until particle size of 2-3 mm, prior to the preparation of extracts. The dried powdered roots (100 g) were extracted through maceration with 1000 mL 70% v/v ethanol for 72 h at room temperature with occasional shaking. Then, the extracts were filtered and concentrated under reduced pressure (50°C, -0.5 bar). Finally, the remaining extract was frozen using liquid nitrogen and lyophilized.

Preliminary phytochemical screening

The main phytochemical groups were identified carrying out the tests: Sudan, catechins, resins, Fehling, Baljet, Liebermann-Buchard, FeCl3, foam, Borntrager, Shinoda, ferric hydroxamate,

Dragendorff, Wagner, Mayer, mucilage and bitter principles (19).

Total flavonoid determination

The total flavonoids were measured through a colorimetric assay modified by Boukhris et al. (2013). 1 mL aliquot of the diluted sample or standard solution of quercetin (20, 40, 60, 80 and 100 mg/L) was added to a 10 mL volumetric flask containing 4 mL of H_2O. At zero time, 0.3 mL of $NaNO_2$ (5%, w/w) was poured into the flask. After 5 min, 0.3 mL $AlCl_3$ (10% w/w) was added. At 6 min, 2 mL of NaOH (1 M) was added to the mixture. Immediately, the reaction flask was diluted with 2.4 mL of H_2O. The absorbance of the mixture was determined at 510 nm and compared to a water control. Total flavonoids were expressed in mg quercetin equivalents (QE)/100 g of hydroalcoholic extract (20) (21).

Total phenolics determination

Folin-Ciocalteau method was used in accordance with Waterman and Mole (22). 200 mL of deionized water were mixed with 50 g of Na_2WO_4, 6.13 g of $H_3PMo_{12}O_{40}$, 25 mL of concentrated HCl, and 12.5 mL of 85% o-H_3PO_4, and the solution was refluxed for 10h. A few drops of Br_2(liq) was added, and the final volume was adjusted to 250 mL. Sample (200 µL) was vortexed with 10 mL of distilled water and 1 mL of Folin-Ciocalteu reagent. After 1 min and before 8 min, 3.75 mL of a 20 g/100 mL Na_2CO_3 solution was added, and time was recorded as time zero. The volume was made up to 20 mL with distilled water, and the solution was vortexed three to four times during the next 2 h. After exactly 2 h, the absorbance was recorded at 760 nm. Total phenols were expressed as mg gallic acid equivalents (GAE)/100 g of hydroalcoholic extract, using a calibration curve of a freshly prepared gallic acid solution (21) (22).

Animals

Eight-week-old albino female rats (*Rattus norvegicus*) weighing 160–240 g were used in this study. They were kept under a 12 h light and 12 h dark cycle. The animals were fed with a basic diet for rodents and water *ad libitum* (21).

Diuretic activity

The method described by Lipschitz with few modifications was employed to evaluate the diuretic activity of hydroalcoholic extract of *P. peltatum* (23). Experimental animals were fasted for 12 h prior to the experiment allowing them to drink only water during the fasting period. Isotonic saline solution (NaCl 0.9% p/v) was administrated 25 minutes before the treatment administration to impose a uniform hydric load (24) (21).

Twenty five healthy albino rats were selected and divided randomly into groups of 5 animals.

Group 1: Normal Control (sodium carboxymethylcellulose, CMC, 0.5% w/v, dose: 10 mL/kg). Group 2: Standard (furosemide, dose: 10 mg/kg). From Group 3 to Group 5 served as test groups and received *P. peltatum* extract orally at the dose of 25, 100 and 200 mg/kg, respectively. After 1 h of treatments, the animals were kept in metabolic cages individually for the collection of urine. The urine was collected for 6 h after the dose was administered. The urine samples were filtered and finally stored at -20 °C for electrolyte analyses (21).

The diuretic action and diuretic activity were derived from the ratio of urine volume in test group and in control and standard groups, respectively. (25).

The sum of Na^+ and Cl^- urinary excretion was calculated as a parameter of saliuretic activity. The ratio Na^+/K^+ was calculated for natriuretic activity. The ratio Cl/(Na+K) was calculated to estimate carbonic anhydrase inhibition (21).

Data Analysis

All values were expressed by means of values ±SD (standard deviation) and those data were analyzed by applying an analysis of variance (ANOVA) followed by Dunnett test to compare treatments v.s. standard. The results were considered statistically significant if $P<0.05$.

Results

Phytochemical screening

The roots of *Piper peltatum* presented primary and secondary metabolites: fats, lactones, coumarins, triterpenes, steroids, reducing sugars, alkaloids, catechins, phenols, tannins, flavonoids and saponins (see table 1.)

Total flavonoids and total phenolics determination

Table 2 shows the total phenolics content (TPC) and total flavonoids (TFC) content of the hydroalcoholic extract of *P. peltatum* expressed in terms of quercetin equivalents and gallic acid equivalents, respectively.

The TPC was calculated using the linear regression equation based on the calibration curve of gallic acid: A=0.0014C−0.0144; R^2 =0.999. Where A is absorbance and C is amount of gallic acid in µg/mL.

The TFC were determined using the linear regression equation based on the calibration curve of quercetin: A=0.0512C+0.0286; R^2=0.999. Where A is absorbance and C is amount of quercetin in µg/mL.

Hydroalcoholic extract of *P. peltatum* presented 274.86 ± 3,27 mg of GAE / g of extract (TPC) and 86.62 ± 13.67 mg of QE / g of extract (TFC).

Diuretic activity

Table 3 shows the result of diuretic effect of *P. peltatum* expressed in urine volume excretion, diuretic action and diuretic activity. Both the standard treatment (furosemide) and hydroalcoholic extracts of *P. peltatum* in different concentracions have a diuretic effect in *Rattus novergicus.* There was a statistical similarity between the treatment with furosemide and with the extracts of the studied species, which has shown an equivalent effectiveness of the diuretic conventional drug and *P. peltatum*.

There was no significant difference between the extracts of *P. peltatum* at 25 mg/kg, 100 mg/kg and 200 mg/kg. However, the treatment with 100 mg/kg has shown a more effective diuretic activity.

The electrolyte excretion potency of *P. peltatum* extracts were high or moderate in comparison to normal control rats, depending on the concentration and the ion studied. *Piper peltatum* extracts showed an increase in Na^+, K^+ and Cl^- excretion, except for the concentration of 25 mg/kg in the sodium analysis. There was a greater excretion of all electrolytes at a concentration of 100 mg/kg of *P. peltatum* extract and at this concentration, Na^+ and Cl^- excretion were comparable to the furosemide group. Nevertheless, the K^+ excretion was higher in all extract doses compared to the group treated with furosemide (Table 4).

Table 6 shows the saliuretic activity, natriuretic activity and carbonic anhydrase inhibition. Furosemide group (10 mg/kg) and extract of *P. peltatum* at 100 mg/kg and 200 mg/kg showed high saliuretic activity compared to normal control. Extracts of *P. peltatum* showed low natriuretic effect and didn't show any carbonic anhydrase inhibition.

Discussion

Piper peltatum L. collected in the coast of Ecuador showed the presence of various secondary metabolites, especially alkaloids and phenolic compounds such as flavonoids. The same species from the Peruvian Amazon showed similarity to those from Ecuador in its phytochemical composition; however, it did not present saponins or catechins and showed an abundant amount of resins (26). The difference of content of secondary metabolites is influenced by the genotype of the plant (the species and the variety), the environmental characteristics (solar radiation and water availability), the growth rate, maturity, nutrients of soil, predation and diseases (27) (28).

Piper peltatum extracts from Ecuador showed a significant diuretic *action* at all dose levels proved (25, 100 and 200 mg/kg) over a period of 6 h,

especially at 100 mg/kg. Previous studies have demonstrated that there are several compounds which could be responsible for the plants diuretic effects such as saponins, organic acids and flavonoids (29). Gasparotto Junior et al. (2012) showed that *Tropaeolum majus* has a diuretic effect, which was associated to flavonoid isoquercitrin (16). The diuretic effect of *Piper* species investigated can be related to the presence of naturally bioactive compounds such as flavonols and other phenolic compounds (30).

The diuretic effect of plant metabolites may be produced by stimulating regional blood flow or initial vasodilatation (31) or by producing inhibition of tubular reabsorption of water and anions (32), with the result in both cases being diuresis (16).

The diuretic activity of *P. peltatum* was better compared to *Piper guinense*, which had a significant increase in urine volume at 1000 and 500 mg/kg (3.50 ± 0.14 ml and 2.95 ± 0.07 ml), respectively. On the other hand, *Piper guinense* enhanced urinary excretion of sodium, chloride and potassium as *P. peltatum* (33).

After 6 hours of treatment, *Piper amalago* extract showed an approximate urine volume of 2.5 ml at a concentration of 125 mg / kg, so that diuretic activity of the *P. peltatum* extract at 100 mg / kg was greater (3.8 ± 1.11 ml). In addition, *P. amalago* exhibited higher levels of Na^+ and K^+ excretion than *P. peltatum*, especially in sodium electrolyte. It was strikingly determined, in both species, that at concentrations greater than 125 mg / kg, the volume of urine decreased (30).

Piper peltatum has shown a significant saliuretic effect but not a natriuretic one. This may be due to the fact that most diuretics, except potassium-sparing drugs, have caused an increase in the excretion of sodium and potassium ions, because of the inhibition of sodium reabsorption in the proximal segment of the nephron by increasing the secretion of K^+ in the tubular lumen at the distal segment, in a flow-dependent manner (21).

Loop diuretics take action mainly by blocking luminal Na^+:K^+:$2Cl^-$ transporter in the thick ascending limb of the loop of Henle. Loop diuretics, as furosemide, induce several effects such as metabolic alkalosis, ototoxicity, hyperuricemia, hypomagnesaemia, allergic reactions, etc. Thus, our findings suggest that ethanolic extract of *P. peltatum* have some action on Loop of Henle (30).

The present research supports the ethnopharmacological use of *P. peltatum* as a diuretic agent, although further studies are necessary to identify the active chemical compounds and the mechanisms involved in the biological activity.

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Table 1. Phytochemical screening of *P. peltatum* roots extract.

Name of the test	Phytochemical identified	*Piper peltatum* L. (roots)		
		Ethereal Extract	Alcoholic Extract	Aqueous Extract
Sudan	Fat	+		
Baljet	Lactones / Coumarins	++	++	
Dragendorff	Alkaloids	-	+	+
Wagner	Alkaloids	-	+	++
Mayer	Alkaloids	-	-	+++
Liebermann – Burchard	Terpenes – Steroids	+	-	
Catechins	Cathechins		+	
Resins	Resins		-	
Fehling	Reducing sugars		+	+
Ferric chloride	Phenols		+	+
Foam	Saponins		-	+
Borntrager	Quinones		-	
Shinoda	Flavonoids		+	+
Anthocyanidins	Anthocyanidins		-	
Mucilage	Mucilages			-

+ : present, - : absent.

Table 2. Total phenolics and flavonoids content of *P. peltatum* roots extract

Extract	Total phenolics content mg of GAE / g of extract	Total flavonoid content mg of QE / g of extract
Roots of *Piper peltatum* L.	274.86 ± 3.27	86.62 ± 13.67

Content expressed in terms of quercetin equivalents and gallic acid equivalents, respectively. Values are expressed as mean±SD, n=3.

Table 3. Urine volume, diuretic action and diuretic activity of *P. peltatum* roots extract at 25, 100 and 200 mg/kg, after 6 hours of treatment administration.

Treatment	Urine volumen (mL)	Diuretic action	Diuretic activity
CMC 0.5% (10 mL/kg)	1.00 ± 0.00	1	-
Furosemide (10 mg/kg)	4.53 ± 2.53	4.53	1
***P. peltatum* roots extract (25 mg/kg)**	1.7 ± 0.83 *	1.7	0.38
***P. peltatum* roots extract (100 mg/kg)**	3.8 ± 1.11 *	3.8	0.84
***P. peltatum* roots extract (200 mg/kg)**	2.8 ± 0.35 *	2.8	0.62

*Urine volume values are mean±SD, n=5. * No significant difference at P<0.05 respect to rats treated with furosemide.*

Table 4. Effect of *P. peltatum* roots extract on urinary electrolyte excretion after 6 hours of treatment administration.

Treatment	Urinary Na^+ (mmol/L)	Urinary K^+ (mmol/L)	Urinary Cl^- (mmol/L)
CMC 0.5% (10 mL/kg)	110.33 ± 17.04	51.5 ± 3.65	76.57 ± 22.78
Furosemide (10 mg/kg)	132.27 ± 16.24	33.07 ± 3.70	127.07 ± 21.36
***P. peltatum* roots extract (25 mg/kg)**	82.47 ± 13.66	66.93 ± 26.28	80.37 ± 16.51
***P. peltatum* roots extract (100 mg/kg)**	137.73 ± 14.89	125.6 ± 20.44	113.6 ± 19.70
***P. peltatum* roots extract (200 mg/kg)**	115.63 ± 10.43	88.87 ± 15.01	102.47 ± 1.35

Table 5. Effect of *P. peltatum* roots extract on natriuretic effect, saliuretic effect and carbonic anhydrase inhibition (CAI) after 6 hours of treatment administration.

Treatment	Saliuretic effect ($Na^+ + Cl^-$)	Natriuretic effect (Na^+/K^+)	CAI ($Cl/[Na^+ + K^+]$)
CMC 0.5% (10 mL/kg)	186.90 ± 27.32	2.14 ± 0.20	0.48 ± 0.17
Furosemide (10 mg/kg)	259.33 ± 37.60	4.05 ± 0.81	0.76 ± 0.07
***P. peltatum* roots extract (25 mg/kg)**	162.83 ± 26.79	1.49 ± 1.01	0.54 ± 0.15
***P. peltatum* roots extract (100 mg/kg)**	251.33 ± 30.20	1.11 ± 0.12	0.44 ± 0.09
***P. peltatum* roots extract (200 mg/kg)**	218.10 ± 11.06	1.33 ± 0.30	0.50 ± 0.02