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Diuretic activity of *Smilax canariensis*, an endemic Canary Island species

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ABSTRACT

Ethnopharmacological relevance: Smilax canariensis is an endemic species of the Canary Islands, popularly known as "Zarzaparrilla sin espinas". This species has wide use in folk-medicine practice on the islands, especially as diuretic. So the aim of our study is to evaluate the diuretic activity of an aqueous and a methanol extract of this species.
Material and methods: Three infusions doses (250, 500 and 750 mg/kg) and two methanol extract doses (100 and 200 mg/kg) were orally administered to laboratory rats. Water excretion rate, pH, density, conductivity, and content of Na^+ and K^+ were measured in the urine of saline-loaded rats.
Results: Water excretion rates were significantly increased in a dose-dependent manner by both hot water infusions and the alcohol extract. The electrolytic excretion was also dose-dependent, although potassium excretion was markedly reduced when using the alcohol extract compared with that observed for the infusion.
Conclusions: Smilax canariensis presents a notable diuretic effect which appeared to be related both to its potassium content and to the presence of polar organic compounds. The present results provide a quantitative basis explaining the traditional folk-medicine use of *Smilax canariensis* as a diuretic agent by the Canary Island population.

1. Introduction

Smilax (Liliaceae) plants are widely distributed in tropical and temperate regions throughout the world, especially in East Asia and North America. Many of them have been long used as medicinal herbs.

Smilax canariensis Willd. is an endemic species of the Canary Islands. It is popularly known as "Zarzaparrilla sin espinas" and it grows sporadically on various central islands, where humid soils support vegetative cover (Darias et al., 1986; Kunkel, 1992; Pérez-Paz and Hernández, 1999).

This species has wide use in folk-medicine practice on the islands due to the wide variety of medicinal properties attributed to it. The parts used as medicine are the rhizome, leaves and stem that habitually are employed as a hot water infusion administered orally. Traditionally this plant has been used as diuretic, laxative, depurative and hypoglycaemic (Jaén, 1984, 1989; Darias et al., 1986, 1989, 2001; Pérez-Paz and Hernández, 1999).

Until the present, no studies had been carried out on the chemical composition of this species. However, many *Smilax* species from other parts of the world such as *Smilax china* have been submitted to numerous chemical and biological studies due to their interesting properties. These species have been shown to contain steroidal saponins and phenolic compounds (Kubo et al., 1992; Sashida et al., 1992; Bernardo et al., 1996; Shu et al., 2002; Li et al., 2006; Sautour et al., 2006; Xu et al., 2006) and have demonstrated interesting anti-inflammatory, anti-conceptive, cytotoxic, antioxidant, immunomodulatory, anticancer, hypoglycaemic, uricosuric and antifungal activities (Giachetti et al., 1988; Suh et al., 1996; Fukunaga et al., 1997; Lee et al., 2001; Jiang and Xu, 2003; Cox et al., 2005; Kuo et al., 2005; Sautour et al., 2005; Thabrew et al., 2005; Xu et al., 2005; Chu and Ng, 2006; Huang et al., 2006; Wang et al., 2006; Li et al., 2007).

No formal studies have been made previously on the biological activities and medicinal properties of *Smilax canariensis*. The present study represents the first research into the diuretic effects of a water infusion and methanol extract of this plant employing laboratory mice and rats as test animals.

2. Materials and methods

2.1. Plant material

Smilax canariensis was harvested from La Palma Island in a place called Las Nieves, Canary Islands (Spain) at 265 m altitude above sea level, in June 2003, and labeled Exp. NE. UTM E228098-N3177053.

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It was identified by Dr. Pedro Pérez de Paz, Department of Plant Biology, University of La Laguna (Tenerife, Spain), where voucher specimens have been deposited (TFC 44393).

2.2. Extract preparation

Rhizomes, leaves and stems (20:40:40) of *Smilax canariensis* were air-dried in an oven at 40 °C for 4 days and then the dry plant was cut and ground to a powder by mechanical milling. Three infusions doses, at 250, 500 and 750 mg/kg body weight (bw) with respect to dry initial plant material, were freshly prepared in distilled boiling water just prior to administration, by mean traditional method applied in Canaries (aqueous extract yield 15.2%).

In a second test procedure, the dried powdered plant material was submitted to a continuous extraction in a soxhlet extractor for 5 days using 100% methanol as a solvent. The solvent was then eliminated by vacuum distillation in a rotary vacuum evaporator (Buchler Corp.), representing a yield of 17.17% of the dry material extracted. The methanol residue obtained was dissolved in distilled water just before administration, and administered at doses of 100 and 200 mg/kg bw in a volume of 5 ml/kg bw.

2.3. Animals

Male albino Sprague-Dawley rats (180–210 g) and male and female albino Swiss mice (20–24 g) obtained from the Central Animal House, University of La Laguna, were used for the experiments, according to the guidelines of the European Community Council Directive 86/609, for the handling and use of laboratory animals.

2.4. Drugs

Hydrochlorothiazide (HCTZ; Sigma Chemical Co.) was used as a reference diuretic drug.

2.5. Acute toxicity test

Groups of 10 mice, 5 male and 5 female weighing 20–24 g were used for administration of the infusion and MeOH extract of *Smilax canariensis*. The animals had free access to standard commercial diet and water *ad libitum* in a 12-h light/12-h dark cycle at 22 °C. Increasing doses of the infusion up to 2.5 g/kg bw (0.4 ml/20 g bw) and MeOH extract up to 1 g/kg bw, respectively, were administered orally by means of a gastric catheter. Food was withdrawn 16 h before the start of the experiment. The mice were observed for symptoms of toxicity for 15 days in terms of weight loss, and autonomic and neurobehavioral alterations. On the 15th day, the animals were sacrificed and their vital organs were individually observed for overt pathology.

2.6. Diuretic activity

Diuretic activity was determined following the methods of Kau et al. (1984), with minor modifications. Male rats were divided into seven groups of eight animals each, in laboratory cages. They were fed laboratory diet *ad libitum* and allowed free access to drinking water. They were exposed to a 12-h light/12-h dark cycle at 22 °C. Eighteen hours before testing, the animals were fasted overnight, with free access to tap water only. Then all animals were given an oral loading of normal saline (5% bw). Subsequently, three groups of rats were orally administered 5 ml/kg bw of each infusion dose of *Smilax canariensis,* two groups of rats were orally administered 5 ml/kg bw of the methanol extract at 100 and 200 mg/kg of weight, respectively, and other two groups of rats were orally administered 5 ml/kg bw of HCTZ at 10 and 25 mg/kg, respectively. Control rats received the same amount of deionised water (5 ml/kg bw). Immediately after administration, the rats were paired and placed in metabolism cages. Urine was collected in a graduated cylinder and its volume was recorded at 2 h intervals for 8 h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 g bw. Electrolyte (Na^+, K^+) concentrations, pH, density and conductivity were estimated from a pooled urine sample of each pair of rats at the end of the experiment (8 h) and expressed as meq./100 g bw.

2.7. Analytical procedures

Na^+ and K^+ concentrations were measured using a Jenway Corp. model PFP7 flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na^+ and K^+. pH and conductivity were directly determined on fresh urine samples using a HI-8424 Hanna Instruments pH-meter and a LF-320 WTF conductivity meter, respectively. Density estimation was made by weighing with a Mettler AE163 (±0.1 mg) analytical balance on urine volume measured with a Nichiryo micropipette.

2.8. Statistical analyses

Results are expressed as the mean values ± SEM (standard error of mean). The statistical evaluation was carried out by analysis of variance (ANOVA) followed by Studentïs *t*-test for multiple comparisons. When comparing with control groups, values of *P* less than 0.05 were considered significant.

Table 1
Effect of oral administration of the infusion and the methanol extract of *Smilax canariensis* on urinary volume and electrolyte excretion

Group	*n*	Urine volume (ml/100 g/8 h)	Diuretic index[a]	Na^+ (meq./100 g/8 h)	K^+ (meq./100 g/8 h)	Saluretic index[b]		Na/K
						Na	K	
Control	16	4.37 ± 0.17	–	0.53 ± 0.02	0.19 ± 0.02			2.79
HCTZ (10 mg/Kg)	4	6.14 ± 0.23**	1.39	0.70 ± 0.07**	0.29 ± 0.07***	1.32	1.53	2.41
HCTZ (25 mg/Kg)	4	6.07 ± 0.07**	1.40	0.69 ± 0.04**	0.27 ± 0.01***	1.30	1.42	2.56
Smilax canariensis (250 mg/kg)	4	4.97 ± 0.83	1.14	0.50 ± 0.06	0.19 ± 0.06	0.94	1.00	2.63
Smilax canariensis (500 mg/kg)	4	5.37 ± 0.82	1.23	0.52 ± 0.09	0.24 ± 0.02	0.98	1.26	2.16
Smilax canariensis (750 mg/kg)	4	5.77 ± 0.35*	1.32	0.58 ± 0.04*	0.29 ± 0.02***	1.09	1.53	2.00
Smilax canariensis (MeOH, 100 mg/Kg)	4	5.29 ± 0.41	1.21	0.60 ± 0.03	0.16 ± 0.02	1.13	0.84	3.75
Smilax canariensis (MeOH, 200 mg/Kg)	4	6.21 ± 0.28**	1.42	0.72 ± 0.02**	0.18 ± 0.02	1.36	0.95	4.00

The results show the mean values and standard errors; *n* = number of pairs used in each group. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared with the control group (Studentïs unpaired *t*-test).

[a] Diuretic index = volume problem group/volume control group.

[b] Saluretic index = meq. problem group/meq. control group.