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# Evidences of antihypertensive potential of extract from *Solanum capsicoides* All. in spontaneously hypertensive rats

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ABSTRACT

*Background:* *Solanum capsicoides* All. is morphologically similar to *Solanum sisymbriifolium* Lam. which is used in folk medicine in South America for antihypertensive and diuretics purposes. This similarity has led to species identification errors, which therefore may result in errors by patients.
*Purpose:* To evaluate the antihypertensive and diuretics potential of the methanol extract from *Solanum capsicoides* All. (MeOH–Sc), *in vitro* and *in vivo*, in spontaneously hypertensive rats (SHR).
*Methods:* Initial experiments were performed in rat mesenteric artery to evaluate the *in vitro* vascular effect of MeOH–Sc and its fractions, in addition to the mechanisms involved during the observed effect. Mean arterial pressure (MAP) and heart rate (HR) were recorded in non-anesthetised hypertensive and normotensive rats. In another set of experiments, MeOH–Sc was administered for 21 consecutive days. Daily body weight measurements were conducted and MAP, HR and urinary volume were measured every 5 days. The mesenteric artery from treated animals was tested for phenylephrine and sodium nitroprussiate (SNP) sensitivity.
*Results:* Initially, MeOH–Sc and fractions relaxed phenylephrine-induced contractions in mesenteric artery rings. The vasorelaxant effect was not changed in the presence of a blocker of eNOS (L-NAME) in rings with an intact endothelium. In denuded-endothelium rings, the vasorelaxant response was significantly reduced in the presence of a cAMP inhibitor (SQ 22536 10 μM) in SHR but not in Wistar Kyoto rats (WKY). However, in the presence of a cGMP inhibitor (ODQ 10 μM), a curve shift to the right was observed in WKY animals, but not in SHR. Intravenous bolus injections of MeOH–Sc into non-anesthetised SHR and WKY, induced hypotension that was associated with an increase in HR. A significant antihypertensive effect was observed in animals that received MeOH–Sc orally for 21 days, which also prevented the development of cardiac hypertrophy. Urine volume from animals treated with MeOH–Sc significantly increased. Finally, MeOH–Sc induced beneficial changes in vascular responsiveness.
*Conclusion:* MeOH–Sc has a potential antihypertensive effect in SHR.


## Introduction

Hypertension is one of the most common disorders in the world, affecting 1 billion individuals, and contributing to approximately 7.1 million deaths per year (Prado et al. 2007). Despite its high prevalence, control of the disease is far from adequate. Data from 2005 to 2008, show that only 46 to 51% of hypertensive individuals actively control blood pressure, defined as a level below 140/90 mmHg (Chobanian et al. 2003; James et al. 2014).

Due to the global impact of hypertension, investigation into new therapeutic alternatives, including the use of natural substances for drug development, merit discovery, evaluation and

*Abbreviations:* MeOH–Sc, methanol extract from *Solanum capsicoides* All; MAP, mean arterial pressure; HR, heart rate; SNP, sodium nitroprussiate; SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto; HPLC, high-performance liquid chromatography; CC, column chromatography; ACN, acetonitrile; DMSO, dimethyl sulphoxide; Phe, L-phenylephrine chloride; Ach, acetylcholine chloride; $E_{max}$, maximum effect; $EC_{50}$, concentration required to relax the induced tone by 50%; Hex–Sc, hexane fraction from *S. capsicoides*; Cl–Sc, chloroform fraction from *S. capsicoides*; Eta–Sc, ethyl acetate fraction.

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global distribution, especially to patient populations in developing countries. Fortunately, the importance of phytotherapy has been established in pharmacological and clinical studies for the primarily healthcare and as recognised tool for modern medicine (Nguelefack et al. 2009).

In the northeast of Brazil, 80 species of *Solanum* genus are widely distributed throughout the region and are used in folk medicine (Agra 1999). Among the species of this genus is *Solanum capsicoides* All. (Solanaceae), commonly known as "Gogoia", that can be found in the north-central coast of Santa Catarina State, in Brazil (Batista-Franklim 2008; Falkenberg 1999; Silva et al. 2007). Currently, there is no reported use of this species in traditional medicine. However, this species is visibly similar to *Solanum sisymbriifolium* Lam. (Agra et al. 2009; Lorenzi 2000), which is a shrub used in folk medicine in several South America countries for antihypertensive and diuretics purposes (Gonzales-Torrez 1992; Ibarrola 2000). However, the morphological similarity has led to errors in species identification, even among recognised researchers in this area (Ferreira et al 2013), thus complicating and misleading the consuming population.

Some biological cardiovascular activities have been described for *S. sisymbriifolium* Lam., including the hypotensive effect of crude root extract in both normotensive and hypertensive rats, surgically induced for unilateral nephrectomy and administration of ethyl deoxycorticosterona. Furthermore, a potent hypotensive action of the butanolic fraction and the B3 subfraction have already been observed, which were determined by hypotensive activity guided fractionation (Ibarrola 1996, 2000, 2006).

Given the common use of *S. sisymbriifolium* as an antihypertensive and a diuretic agent, which can also be attributed to the *S. capsicoides*, the aim of this study was to evaluate the antihypertensive potential of methanol extract from *S. capsicoides* All. (MeOH–Sc), *in vitro* and *in vivo*, in spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto (WKY). In addition, the HPLC profile was determined.

## Materials and methods

### *Plant material*

*S. capsicoides* All aerial parts was collected at Morro da Fumaça (latitude: 28° 39' 8"; Longitude: 49° 12' 38), Santa Catarina, Brazil. The preparation of extracts and fractions was based on previous work by Berté and co-workers (2014). The air-dried powdered aerial parts (1.15 kg) of *S. capsicoides* were exhaustively extracted with methanol (2 × 5 L) for 7 days, yielding the crude methanolic extract (102.56 g; 8.9%). Briefly, the MeOH residue was suspended in a solution of MeOH/$H_2O$ (9:1) (0.5 L) and partitioned successively with *n*-hexane, chloroform, and ethyl acetate (3 × 0.2 L each), furnishing 7.93, 7.21, and 4.09 g, respectively. The plant name was confirmed with www.theplantlist.org on February 13, 2015.

### *Phytochemical characterization*

High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-20 AC chromatographer equipped with a quaternary pump, scan spectrum photo diode array automatic detector and SIL-20A injector. The LC Solution® software was used to record the chromatograms and measure peak areas. Scan range detection was performed between the wavelength range of 190–400 nm. The mobile phase HPLC grade components acetonitrile (ACN) and phosphoric acid acidificated water (pH 2.5) were vacuum filtered through 47 mm diameter and 0.45 mm porosity regenerated cellulose membrane and ultrasound degassed. For the analysis 2 mg of extract and 0.1 mg of standards, components were dissolved in 1 ml of acetonitrile (HPLC grade). A 5 μm C18 Phenomenex column (250 × 4,5 mm) was used and the elution was carried out with gradient solvent systems with ACN (A) and phosforic acid acidificated water (B) at a flow rate of 0.8 ml/min, at 35 °C starting with 5% A increasing to 100% A in a 95 mins run.

### *Method validation*

The HPLC method for determination of cilistadiol was validated according to ICH guidelines (ICH 2005). Linearity was determined by injecting seven solutions of cilistadiol in triplicate 1–100 μg/ml. Precision was determined by analysis of six solutions of extract at concentration of 1 mg/ml. The limits of detection (LOD) and quantification (LOQ) were determined by the signal–noise relationship.

### *Total phenols determination*

The concentration of total phenolic content was determined spectrophotometrically, using the Folin–Ciocalteu total phenols procedure, described by Souza et al. 2007, with minor modifications. Gallic acid standard solutions were prepared at 50, 100, 250, 500, 750, and 1000 μg/ml. Methanolic extract (1 g) was solubilised in methanol (10 ml). Test extract solution (25 μl) appropriately diluted or the gallic acid standard (25 μl) was transferred to 10 ml test tubes. Folin–Ciocalteu reagent was added to each test tube and vortex mixed. After 1 min, 0.5 ml of 15.0% (w/v) $Na_2CO_3$ in water was added and the obtained solution was mixed and maintained at room temperature. After 30 min, the absorbance was determined at $\lambda = 750$ nm, using a Shimadzu UV 1601 spectrophotometer. The concentration of total phenolic compounds in the extract was determined comparing the absorbance of the extract samples to that of gallic acid standard solutions. Extract sample was analysed in triplicate and total phenolic content (TPC) was expressed as gallic acid equivalents (GAE) in mg per g dry extract.

### *Animals*

Spontaneously hypertensive rats (SHR) and their normotensive controls, Wistar Kyoto rats (WKY), aged 12–16 weeks (250–300 g), were used in this study. For sub-chronic experiments, animals at 9 weeks of age were used. The animals were housed in cages at a temperature of 24 ± 1° C, exposed to a 12 h light/12 h dark cycle and free access to food and water. The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals, adopted by the US National Institutes of Health.

### *Drugs and solutions*

The drugs used in this study were Cremophor EL, dimethyl sulphoxide (DMSO), L-phenylephrine chloride (Phe), $N^{\omega}$-Nitro-L-arginine methyl ester (L-NAME), acetylcholine chloride (Ach), 1H-[1,2,4]oxadiazolo[4,3-a]-quinoxalin-1-one (ODQ), 9-(terahydro-2-furanyl)-9H-purin-6-amine (SQ 22536), ketamine, xylazine, heparin and captopril (Sigma–Aldrich Chemical Co., St. Louis, MO, USA). Furosemide was obtained from ACHÉ (Guarulhos, SP, Brazil). All compounds were dissolved in distilled water. The composition of Tyrode's solution used was as follows (mM): NaCl, 158.3; KCl, 4.0; $CaCl_2$, 2.0; $MgCl_2$, 1.05; $NaH_2PO_4$, 0.42; $NaHCO_3$, 10.0, and glucose, 5.6.

For the preparation of MeOH–Sc and the *n*-hexane fraction, both were solubilised in Cremophor, and their effective concentration never exceeded 0.01%. Chloroform and ethyl acetate fractions were solubilised in DMSO (dimethyl sulphoxide—stock solution). For the preparation of vehicle solutions, Cremophor (0.003 g/ml of solution) was solubilised in NaCl 0.9% (*in vivo* experiments) or