



Pharmacognosy

Phytochemical analysis and hypotensive activity of *Ipomoea pes-caprae* on blood pressure of normotensive rats

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Abstract

Ipomoea pes-caprae (Convolvulaceae), popularly known as “salsa-da-praia”, is used in folk medicine for the treatment of several diseases, including hypertension. The aim of this research was to evaluate hypotensive activity attributed to this species and identify the bioactive compounds responsible for this effect. Hydroalcoholic extract of aerial parts of *Ipomoea pes-caprae* (PIEA) was fractionated by column chromatography with Amberlite XAD-2, thus achieving a methanolic fraction (PIEA-M). The chemical profile investigation of PIEA-M was conducted by Ultra Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS) and compound isolation through High Performance Countercurrent Chromatography (HPCCC). Hemodynamic evaluations of PIEA, PIEA-M and isolated compounds were carried out on normotensive rats. The locomotor activity of PIEA and PIEA-M were performed by Rota Rod test using Swiss mice. UPLC-MS analysis of PIEA-M led to the identification of 11 compounds corresponding to seven phenolic acids and four flavonoids. By means of HPCCC, quercetin 6''-O-acetyl-3-O- glucoside (1), a mixture of quercetin-3-O-galactoside and quercetin-3-O- glucoside (2) and quercetin 3-O-glucoside (3) were isolated. PIEA, PIEA-M and the obtained compounds (1 and 2) significantly reduced blood pressure. Also, PIEA and PIEA-M caused significant impairment on mice locomotor activity. These results indicate the potential of *Ipomoea pes-caprae* as a source of hypotensive agents suggesting that phenolic compounds present in the species are responsible for its hypotensive activity.

Key words: Convolvulaceae, hypertension, *Ipomoea pes-caprae*, phenolic compounds, rota-rod.

Resumo

Ipomoea pes-caprae (Convolvulaceae), conhecida popularmente como “salsa-da-praia”, é usada na medicina popular para o tratamento de várias doenças, incluindo a hipertensão. O objetivo desta pesquisa foi avaliar a atividade hipotensora atribuída a essa espécie e identificar os compostos bioativos responsáveis por esse efeito. O extrato hidroalcoólico das partes aéreas de *Ipomoea pes-caprae* (PIEA) foi fracionado por cromatografia em coluna com Amberlite XAD-2, obtendo-se uma fração metanólica (PIEA-M). A investigação do perfil químico de PIEA-M foi realizada por Cromatografia Líquida de Ultra-Eficiência acoplada a Espectrometria de Massas (CLUE-EM) e isolamento de compostos por Cromatografia Contracorrente de Alto Desempenho (CCCAD). Avaliações hemodinâmicas dos PIEA, PIEA-M e compostos isolados foram realizadas em ratos normotensos. A atividade locomotora de PIEA e PIEA-M foi realizada pelo teste de Rota Rod usando camundongos suíços. A análise CLUE-EM do PIEA-M levou à identificação de 11 substâncias correspondentes a sete ácidos fenólicos e quatro flavonoides. Por meio de CCCAD, a quercetina 6''-O-acetil-3-O-glicosídeo (1), uma mistura de quercetina-3-O-galactosídeo e quercetina-3-O-glicosídeo (2) e quercetina 3-O-glicosídeo (3) foram isolados. PIEA, PIEA-M e as substâncias obtidas (1 e 2) reduziram significativamente a pressão sanguínea. Além disso, o PIEA e o PIEA-M causaram um prejuízo significativo na atividade locomotora dos camundongos. Estes resultados indicam o potencial de *Ipomoea pes-caprae* como fonte de agentes hipotensores, sugerindo que os compostos fenólicos presentes na espécie são responsáveis por sua atividade hipotensora.

Palavras-chave: Convolvulaceae, hipertensão, *Ipomoea pes-caprae*, compostos fenólicos, rota-rod.

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Introduction

Natural products play an important role in the pharmaceutical industry, providing many active substances used to treat several diseases (Berlinck *et al.* 2017). In this sense, the popular knowledge significantly contributes to the development of new medicines (Viegas & Bolzani 2006). Last surveys about new drug sources, conducted between 1981 and 2014, showed most medicines derive from natural products (Newman & Cragg 2016). 122 compounds used as medicines were obtained from 94 plant species, and 80% of these plants have been used in folk medicine for the same purpose (Fabricant & Farnsworth 2001).

Hypertension is a disease affecting one billion people worldwide (World Health Organization 2013) and is the main risk-factor for emergency cardiovascular-related episodes such as stroke, peripheral arterial disease, renal and heart failures (Kannel 2000). High blood pressure complications accounts for 9.4 million deaths per year higher than cancer-related deaths per year (World Health Organization 2013, 2017). Hypertension is asymptomatic in the early stage and many people are undiagnosed. Those who are diagnosed may not be able to accomplish a successful long-term disease control. In most cases, the anti-hypertensive therapy changes, doing so that the patient inevitably needs to use two or more drugs to achieve the desirable effect (World Health Organization 2013).

Ipomoea pes-caprae (L.) R. Br. (Convolvulaceae) is used in folk medicine for the treatment of hypertension. This species is popularly known as “salsa-da-praia” and is usually found in coastal dunes of tropical and subtropical regions (Lorenzi & Matos 2002; Barni *et al.* 2009; Miryeganeh *et al.* 2014). In Brazil, this plant is spread throughout the coastal region. Besides hypertension, ethnopharmacological surveys describe several medicinal utilizations of this plant such as: analgesic, anti-inflammatory, diuretic, and healing cramps, ulcers, dermatitis caused by jellyfish venom, arthritis and rheumatism (Wasuwat 1970; Souza *et al.* 2000; Lorenzi & Matos 2002; Pereda-Miranda *et al.* 2005; Barni *et al.* 2009).

Phytochemical profile of *Ipomoea pes-caprae* is characterized by the presence of glycosidic resins, coumarins, steroids, flavonoids and triterpenes (Krogh *et al.* 1999; Pereda-Miranda *et al.* 2005; Teramachi *et al.* 2005; Escobedo-Martínez *et al.* 2010; Yu *et al.* 2011; Banerjee *et*

al. 2013; Sharmin *et al.* 2013). *Ipomoea* flavonoids are mainly quercetin derivatives which are known for possessing anti-hypertensive activity (Krogh *et al.* 1999; Loizzo *et al.* 2008; Barni *et al.* 2009; Larson *et al.* 2010). Considering the utilization of this plant in folk medicine as antihypertensive and the need of new anti-hypertensive drugs, the aim of the present study was to evaluate the hemodynamic effects of *Ipomoea pes-caprae* hydroalcoholic extract, fractions and isolated substances.

Materials and Methods

General experimental procedure

UPLC-ESI-MS analyses were performed on a Shimadzu LC-20A UPLC instrument (Shimadzu, Kyoto, Japan) equipped with a degasser, a binary pump, an automatic sampler, a column oven and a diode array detector (DAD). The system was coupled to a high resolution mass spectrometer equipped with an electrospray ion source (ESI) and quadrupole time-of-flight mass analyzer (Q-TOF) (Bruker Daltonics, Bremen, Germany). Chromatograms were recorded and processed by LC Solution 1.25 by Shimadzu and the LC/MS system was controlled by HyStar 3.2 software (Bruker Daltonics, Bremen, Germany).

The High Performance Countercurrent Chromatography (HPCCC) was acquired on a Dynamic Extractions Spectrum HPCCC (Berkshire, UK) equipped with a 142 mL multilayer coil columns (1.6 mm i.d.). The β -value ranges from 0.52 to 0.86 and revolution speed can be set up to 1,600 rpm. The system comprises two Knauer Smartline 100 V5010 pumps, a Knauer Smartline 2500 V7604 UV absorbance detector, a Büchi C-660 fraction collector, a LabTech Smart H-150–1500 chiller and a HiChrom 5 mL manual injection valve. The solvent system comprising chloroform: butanol: methanol: water (7:3:3:4; v/v) was chosen by the shake-flask method as described by Berthod & Carda-Broch (2004).

The NMR spectra were recorded on a Bruker DPX-500 operating at 500 MHz to ^1H and 125 MHz to ^{13}C . Dissolution of the samples was made with Deuterated dimethyl sulfoxide (DMSO- d_6 purity > 99.9%), contains 0.1% v/v TMS.

The column chromatography (at atmospheric pressure) was conducted with XAD-2 (tyrene-divinylbenzene copolymer) in a glass column with 90 × 3 cm (length × internal diameter). The TLC analyses were performed by using Merck GF₂₅₄ silica gel 60 revealed by different chemical and physical methods.

The hemodynamic parameters were measured using Bioamp7 equipment (ADInstruments, Australia) and GraphLab software (version 7.0; AD Instruments). Evaluation of motor coordination was conducted with a Rota Rod instrument (EFF 411, Insight®).

Chemicals

HPLC-grade formic acid, acetonitrile and methanol were purchased from TEDIA. Water was purified using a Sartorius Arium® mini system. Deuterated dimethyl sulfoxide (DMSO-d₆ purity > 99%) was purchased from Sigma-Aldrich. Other chemical reagents were purchased from SYNTH. Isoflurane and diazepam (5 mg·mL⁻¹) were purchased from Cristália, heparin (5,000 UI·mL⁻¹) from Blaú-Cristal Pharma and sodium chloride solutions (NaCl 0.9%) was obtained from Sanobiol.

Animals

In vivo tests were performed on male, albino, normotensive Wistar rats (*Rattus norvegicus*) weighing between 200–300 g and male, albino Swiss mice (*Mus musculus*) weighing between 25 and 30 g. Animals were kept in plastic cages with controlled temperature (22 ± 2 °C) and with light and dark light cycles of 12 hours with water and food *ad libitum*. The present study was approved by UENF's Ethics Committee for Animal Use (CEUA) registered under protocol number 294, and approved on December 15th of 2015.

Plant material

Aerial parts (leaves and stems) of *Ipomoea pes-caprae* were collected in Grussaí beach (São João da Barra, Rio de Janeiro, Brazil) in July 2016, under the geographical coordinates 21°41'37.2"S and 41°01'29.9"W. A voucher specimen (H9793) was deposited at UENF's herbarium. SisGen number: AC4650E.

Extraction and fractionation

The aerial parts (100 g) were extracted with 350 mL of ethanol: water (1:1, v/v) in orbital shaker with controlled rotation (135 rpm) and temperature (40 °C). The solvent was changed every 24 hours for 10 days. The obtained solutions were filtered, concentrated in a rotary evaporator under reduced pressure, and subsequently lyophilized to obtain a completely dried extract (PIEA, 30 g). An aliquot of this extract (20 g) was fractionated through XAD-2 Amberlite® column eluted with distilled water

and, then, eluted with methanol, thus obtaining a methanolic fraction (PIEA-M - 6.82 g).

UPLC-ESI-MS and MS/MS analyses

The PIEA-M was analyzed by UPLC-ESI-MS in order to determine the phytochemical profile and identify phenolic compounds. The separation was performed on a C-18 Zorbax column (1.7 µm, 2.1 × 75 mm, Phenomenex). The mobile phase consisted of ultra pure water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The following gradient elution program was determined after several optimization procedures: 0–2 min 2.5% B, 2–25 min 2.5 to 9.5% B, 25–55 min maintained at 9.5% B, 55–60 min 9.5 to 9.6% B, 60–85 min 9.6 to 11% B, 85–110 min 11 to 15% B, 110–140 min 15 to 25% B, 140–145 min 25 to 100% B, 145–150 min kept at 100% B, 150–155 min from 100 to 2.5% B, 155–160 min 2.5% B. The flow rate was maintained at 0.25 mL·min⁻¹, the column temperature was adjusted to 40 °C, and the injection volume was of 10 µL. The UV/DAD detector was monitored at 220 nm (for phenolic acids and flavan-3-ol) and 345 nm (for flavonoids). The column effluent was introduced into the mass spectrometer at 0.25 mL·min⁻¹.

The nebulizer gas was high-purity nitrogen (N₂), produced online by a Peak Scientific NM32LA nitrogen generator. Analysis parameters were set using negative ionization mode with spectra acquired over a mass range from *m/z* 50 to 1,400. Optimum ESI-MS parameters were: capillary voltage, +3.5 kV; drying gas temperature, 210 °C; drying gas flow, 10 L·min⁻¹; nebulizing gas pressure, 72.5 psi; collision RF, 200 Vpp; transfer time 120 µs; and pre-pulse storage, 3 µs. In addition, automatic MS/MS experiments were performed adjusting the collision-energy values as follows: *m/z* 500, 30 eV; *m/z* 1,000, 50 eV; *m/z* 1,400, 70 eV; and using nitrogen as collision gas. MS data were processed through Data Analysis 4.0 software (Bruker Daltonics, Bremen, Germany). External instrument calibration was performed using a Cole Palmer syringe pump (Vernon Hills, IL, USA) directly connected to the interface, eluting a sodium formate (NaCHO₂) solution cluster containing 5 mM sodium hydroxide (NaOH) and 0.2% formic acid in water:isopropanol 1:1 (v/v).

HPCCC separation procedure

The PIEA-M was subjected to HPCCC using a solvent system comprising chloroform: butanol: methanol: water (7:3:3:4; v/v). The

HPCCC column was entirely filled with the stationary phase (upper phase). The lower phase (mobile phase) was pumped at a flow rate of 1.5 mL·min⁻¹ from the head-to-tail direction while the column was rotated at 1,100 rpm. After reaching hydrodynamic equilibrium, stationary phase's retention was measured (76.7%) and 457.9 mg of sample was injected and the eluate collected in 2.5 mL fractions. These fractions were pooled according to similarity of their chromatographic profiles by TLC in 35 fractions. Fractions 10 (PIEA-M-1-10-Compound 1), 18 (PIEA-M-1-18-Compounds 2a and 2b) and 19 (PIEA-M-1-19 Compound 3, identical to 2b) were evaluated by NMR experiments.

Spectral data of isolated compounds

Compound 1: ¹H NMR (500 MHz, DMSO-d₆): 7.53 (m, H-6'), 7.53 (m, H-2'), 6.84 (d, *J* = 9.1 Hz, H-5'), 6.42 (d, *J* = 1.9 Hz, H-8), 6.21 (d, *J* = 1.9 Hz, H-6), 5.38 (d, *J* = 7.4 Hz, H-1''), 4.13 (dd, *J* = 11.8, 2.0 Hz, H-6''a), 3.94 (dd, *J* = 11.5, 6.0 Hz, H-6''b), 1.73 (s, 3H, CH₃COO-). ¹³C NMR (125 MHz, DMSO-d₆): 157.0 (C-2), 133.6 (C-3), 177.8 (C-4), 161.7 (C-5), 99.1 (C-6), 164.6 (C-7), 94.0 (C-8), 156.8 (C-9), 104.3 (C-10), 121.5 (C-1'), 116.6 (C-2'), 145.3 (C-3'), 149.0 (C-4'), 115.6 (C-5'), 122.0 (C-6'), 101.4 (C-1''), 74.4 (C-2''), 76.7 (C-3''), 70.2 (C-4''), 74.1 (C-5''), 63.2 (C-6''), 170.3 (-OCOCH₃), 20.6 (-OCOCH₃). This fraction was identified as quercetin 6''-O-acetyl-3-O-glucoside (Deepralard *et al.* 2009).

Compound 2: Compound 2a: ¹H NMR (500 MHz, DMSO-d₆): 7.67 (d, *J* = 8.2 Hz, H-6'), 6.86 (m, H-2'), 7.67 (d, *J* = 8.2 Hz, H-5'), 6.41 (s, H-8), 6.21 (s, H-6), 5.38 (d, *J* = 7.4 Hz, H-1''), 3.34 (m, H-6''a), 3.50 (m, H-6''b). ¹³C NMR (125 MHz, DMSO-d₆): 156.8 (C-2), 133.8 (C-3), 177.9 (C-4), 161.7 (C-5), 99.2 (C-6), 164.7 (C-7), 94.0 (C-8), 156.8 (C-9), 104.4 (C-10), 121.6 (C-1'), 115.7 (C-2'), 145.3 (C-3'), 148.9 (C-4'), 116.7 (C-5'), 122.1 (C-6'), 102.3 (C-1''), 71.7 (C-2''), 73.7 (C-3''), 68.4 (C-4''), 76.3 (C-5''), 60.6 (C-6''). Compound 2b: ¹H NMR (500 MHz, DMSO-d₆): 7.67 (d, *J* = 8.2 Hz, H-6'), 6.86 (m, H-2'), 7.58 (m, H-5'), 6.41 (s, H-8),), 6.21 (s, H-6), 5.47 (d, *J* = 7.5 Hz, H-1''), 3.36 (m, H-6''a), 3.65 (m, H-6''b). ¹³C NMR (125 MHz, DMSO-d₆): 156.7 (C-2), 134.0 (C-3), 178.0 (C-4), 161.7 (C-5), 99.2 (C-6), 164.7 (C-7), 94.0 (C-8), 156.3 (C-9), 104.4 (C-10), 121.6 (C-1'), 115.7 (C-2'), 145.3 (C-3'), 148.9 (C-4'), 116.4 (C-5'), 122.5 (C-6'), 101.4 (C-1''), 74.5 (C-2''), 77.0 (C-3''), 70.4 (C-4''), 78.0 (C-5''), 60.8

(C-6''). This fraction was identified as a mixture of quercetin-3-O-galactoside (Compound 2a) and quercetin-3-O-glucoside (Compound 2b) (Agrawal 1989).

Hemodynamic evaluation

Normotensive Wistar rats were anesthetized by inhalation with isoflurane. The left carotid artery of the animals was cannulated to allow the measurement of systolic, diastolic and mean blood pressure. The right jugular vein was also cannulated to allow intravenous sample administration. Cannulas were heparinized with a sodium heparin solution in 0.9% sodium chloride solution in order to avoid blood clotting. The samples (PIEA, PIEA-M, compounds 1 and 2) were administered at 30 mg·kg⁻¹ diluted in DMSO with a volume of 0.1 mL per animal. Prior to the tests, 100 µL of DMSO alone was injected as a control of hemodynamic conditions to eliminate the hypotensive effects of DMSO on the results.

Rota-Rod test

Swiss mice were previously tested on the rotating bar. Those who fell two or more times in a three-minute period were discarded. After animals selection, samples (PIEA and PIEA-M) and diazepam positive control were administered intraperitoneally at 30 mg·kg⁻¹ concentration in 0.1 mL of DMSO. Each individual was placed with all four legs on a rotating bar already in motion (20 rpm). The mice were observed at the times of 15, 30, 60 and 120 min after sample administration and remained on the rotating bar for three minutes. The number of falls during the three-minute interval was counted.

Statistical analysis

Results of hemodynamic evaluation were tabulated by LabChart 7 and statistically analyzed through Graphpad Prism 5 software. All obtained data were corrected by Bartlett test. Variables obtained were statistically compared by means of parametric tests, among moments within the groups and among groups by analysis of variance (ANOVA) followed by Newman Keuls multiple comparison test. Student's t-test was also used. For the Rota-Rod test, the results were also statistically analyzed through Graphpad Prism 5 and analysis the variance was performed, followed by Newman Keuls test and Bonferroni means comparison. A 95% confidence interval was established (*p* < 0.05).

Results and Discussion

Fractionation, identification and isolation

The fractionation of the hydroalcoholic extract using XAD-2 was performed to concentrate free sugars in the aqueous fraction and phenolic compounds

in methanolic fraction. Table 1 summarizes the identified compounds in methanolic fraction by means of molecular formula and MS/MS fragments with their relative abundance. The compounds were identified (Fig. 1) by comparison of fragmentation profiles with literature data and identified as phenolic acids and heterosyl flavonols (Fig. 2).

Table 1 – Phenolic compounds identified in the methanolic fraction of *Ipomoea pes-caprae* by Ultra Performance Liquid Chromatography coupled with a diode array detector and Mass Spectrometry. Mass Spectrometer equipped with an electrospray ion source (ESI) and quadrupole time-of-flight mass analyzer (Q-TOF). Analytical conditions of UPLC: same conditions of Figure 1. Analytical conditions of ESI-MS: ionization mode: negative; spectra acquired over a mass range from m/z 50 to 1400; capillary voltage: +3.5 kV; drying gas temperature: 210 °C; drying gas flow: 10 L·min⁻¹; nebulizing gas pressure: 72.5 psi; collision RF: 200 Vpp; transfer time: 120 μs; pre-pulse storage: 3 μs; collision-energy values: m/z 500, 30 eV; m/z 1000, 50 eV; m/z 1400, 70 eV; collision gas: nitrogen.

Peak	RT (min)	λ_{\max} (nm)	[M – H] ⁻	Theoretical mass (m/z)	Error (ppm)	Molecular formula	MS/MS (% abundance)	Compound	References
1	7.5	216; 324	353.0897	353.0878	5.38	C ₁₆ H ₁₈ O ₉	191 (100), 179 (36), 135 (65)	3- <i>O</i> -Caffeoylquinic acid	Jaiswal <i>et al.</i> 2014
2	11.9	217; 322	179.0335	179.0349	-7.82	C ₉ H ₈ O ₄	135 (100)	Caffeic acid	Santos <i>et al.</i> 2014
3	13.7	217; 324	353.0899	353.0878	5.95	C ₁₆ H ₁₈ O ₉	191 (100)	5- <i>O</i> -Caffeoylquinic acid	Jaiswal <i>et al.</i> 2014
4	15.6	217; 325	353.0898	353.0878	5.66	C ₁₆ H ₁₈ O ₉	191 (100), 135 (76), 173 (61), 179 (57)	4- <i>O</i> -Caffeoylquinic acid	Ncube <i>et al.</i> 2014
5	37.7	254; 351	463.0881	463.0882	-0.22	C ₂₁ H ₂₀ O ₁₂	300 (100)	quercetin 3- <i>O</i> -galactoside	Santos <i>et al.</i> 2014; Zhao <i>et al.</i> 2017
6	41.5	254; 359	463.0887	463.0882	1.08	C ₂₁ H ₂₀ O ₁₂	300 (100)	quercetin 3- <i>O</i> -glucoside	Santos <i>et al.</i> 2014; Zhao <i>et al.</i> 2017
7	59.8	212; 324	515.1202	515.1195	1.36	C ₂₅ H ₂₄ O ₁₂	179 (100), 173 (95), 353 (50), 191 (48), 335 (25), 161 (20), 135 (13)	3,4-di- <i>O</i> -caffeoylquinic acid	Heyman <i>et al.</i> 2015
8	63.2	214; 326	515.1201	515.1195	1.16	C ₂₅ H ₂₄ O ₁₂	191 (100), 179 (72), 353 (29), 173 (5)	3,5- <i>O</i> -Dicaffeoylquinic acid	Clifford <i>et al.</i> 2005.
9	68.9	254; 347	505.0992	505.0988	0.79	C ₂₃ H ₂₂ O ₁₃	300 (100)	quercetin 3- <i>O</i> -acetylgalactoside	An <i>et al.</i> 2013; Zhao <i>et al.</i> 2017.
10	70.8	254; 349	505.0987	505.0988	-0.20	C ₂₃ H ₂₂ O ₁₃	300 (100)	quercetin 3- <i>O</i> -acetylglucoside	An <i>et al.</i> 2013; Zhao <i>et al.</i> 2017.
11	90.6	212; 326	515.1194	515.1195	-0.19	C ₂₅ H ₂₄ O ₁₂	173 (100), 179 (93), 353 (65), 191 (33), 135 (11)	4,5- <i>O</i> -Dicaffeoylquinic acid	Clifford <i>et al.</i> 2005.

Seven phenolic acids derived from hydroxycinnamic acid were found and identified by UV and MS/MS fragmentation data. Peaks 1, 2, 3, 4, 7, 8 and 11 showed the UV spectra characteristic of hydroxycinnamic acids by means of two absorption bands at 212–217 nm and 322–326 nm. Peak 3 was identified as 5-*O*-caffeoylquinic acid (m/z 353), characterized by a unique m/z 191 fragment, indicating loss of the caffeoyl group, which yields a quinic acid residue (m/z 191). Peak 1 refers to 3-*O*-caffeoylquinic acid isomer, which exhibits the same m/z 191 fragment besides others: m/z 179 corresponding to the formed caffeic acid given the loss of quinic acid; and m/z 135, indicating loss of CO₂ of caffeic acid. Peak 4 was identified as 4-*O*-caffeoylquinic acid isomer which exhibits the highest number of fragments: m/z 191, m/z 179, m/z 135 and m/z 173, which is relative to the loss of H₂O from quinic acid. Peak 2 (m/z 179) was identified as caffeic acid. Peaks 7, 8 and 11 are related to three different isomers of dicaffeoylquinic acid (m/z 515). The difference between these isomers is the relative abundance of their fragments. Peak 7 was identified as 3,4-di-*O*-caffeoylquinic acid. This molecular ion resulted in the following fragments: m/z 179, formed by loss of a quinic acid and a caffeoyl group; m/z 191, caused by loss of two caffeoyl groups; m/z 353, corresponding to the caffeoylquinic acid formed

by loss of one caffeoyl group; m/z 335, formed by loss of H₂O of caffeoylquinic acid; m/z 161, relative to the loss of H₂O of caffeic acid. Peak 8 refers to 3,5-di-*O*-caffeoylquinic acid isomer with fragments m/z 191, m/z 179, m/z 353 and m/z 173. Peak 11 was identified as 4,5-di-*O*-caffeoylquinic acid, characterized by the fragments m/z 173, m/z 179, m/z 353, m/z 191 and m/z 135.

Peaks 5, 6, 9 and 10 showed the UV spectra characteristic of flavonols-3-*O*-hexoses on account of two absorption maxima at 247–349 and at 254 nm. Peaks 5 and 6 were identified as quercetin 3-*O*-hexose (m/z 463), which was characterized by a unique m/z 300 fragment indicating loss of one hexose. Peaks 9 and 10 (m/z 505) showed the same fragmentation pattern of m/z 300, for which they were attributed to quercetin-3-*O*-acetylhexose. Peak 10's attribution was only possible due to quercetin-6''-*O*-acetyl-3-*O*-glucoside isolation by countercurrent chromatography and its analysis by UPLC MS-MS. As peak 9 has the same molecular mass and fragmentation as peak 10, it was identified as quercetin-6''-*O*-acetyl-3-*O*-galactoside. Fractions 18 and 19 obtained from countercurrent separation of methanolic fraction were submitted to NMR analysis. Fraction 18 was identified as a mixture of quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside, whereas fraction 19 as quercetin 3-*O*-glucoside. Quercetin 3-*O*-glucoside isolation

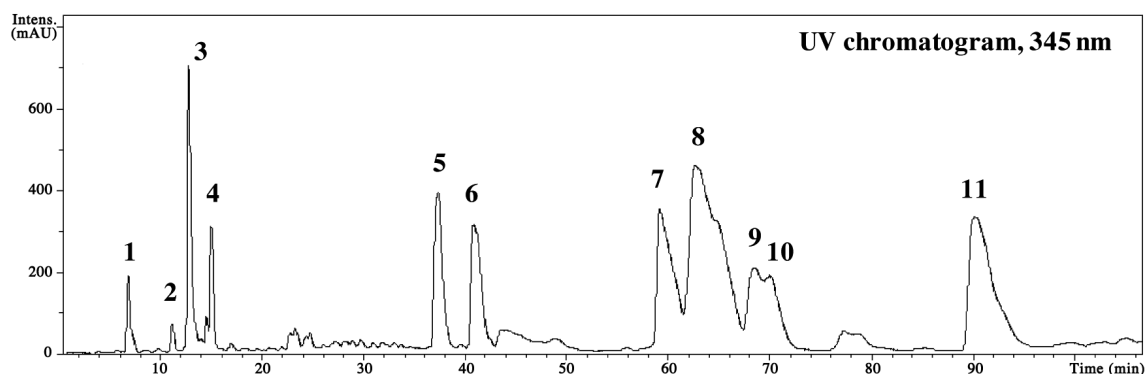


Figure 1 – UPLC-UV chromatogram at λ 345 nm of the methanolic fraction from *Ipomoea pes-caprae*. Analytical conditions: stationary phase: C-18 Zorbax column (1.7 μ m, 2.1 \times 75 mm, Phenomenex); temperature: 40 °C; mobile phase: A = ultra pure water containing 0.1% formic acid, B = acetonitrile containing 0.1% formic acid; flow rate: 0.25 mL·min⁻¹; detection: 345 nm; injection volume: 10 μ L; solvent composition during analysis: 0–2 min 2.5% B, 2–25 min 2.5 to 9.5% B, 25–55 min maintained at 9.5% B, 55–60 min 9.5 to 9.6% B, 60–85 min 9.6 to 11% B, 85–110 min 11 to 15% B, 110–140 min 15 to 25% B, 140–145 min 25 to 100% B, 145–150 min kept at 100% B, 150–155 min from 100 to 2.5% B, 155–160 min 2.5% B. Peak numbering indicates the substances identified in the UPLC-MS analysis: 3-*O*-Caffeoylquinic acid (1), Caffeic acid (2), 5-*O*-Caffeoylquinic acid (3), 4-*O*-Caffeoylquinic acid (4), quercetin 3-*O*-galactoside (5), quercetin 3-*O*-glucoside (6), 3,4-di-*O*-Caffeoylquinic acid (7), 3,5-*O*-Dicaffeoylquinic acid (8), quercetin 3-*O*-acetyl-galactoside (9), quercetin 3-*O*-acetylglucoside (10), 4,5-*O*-Dicaffeoylquinic acid (11).

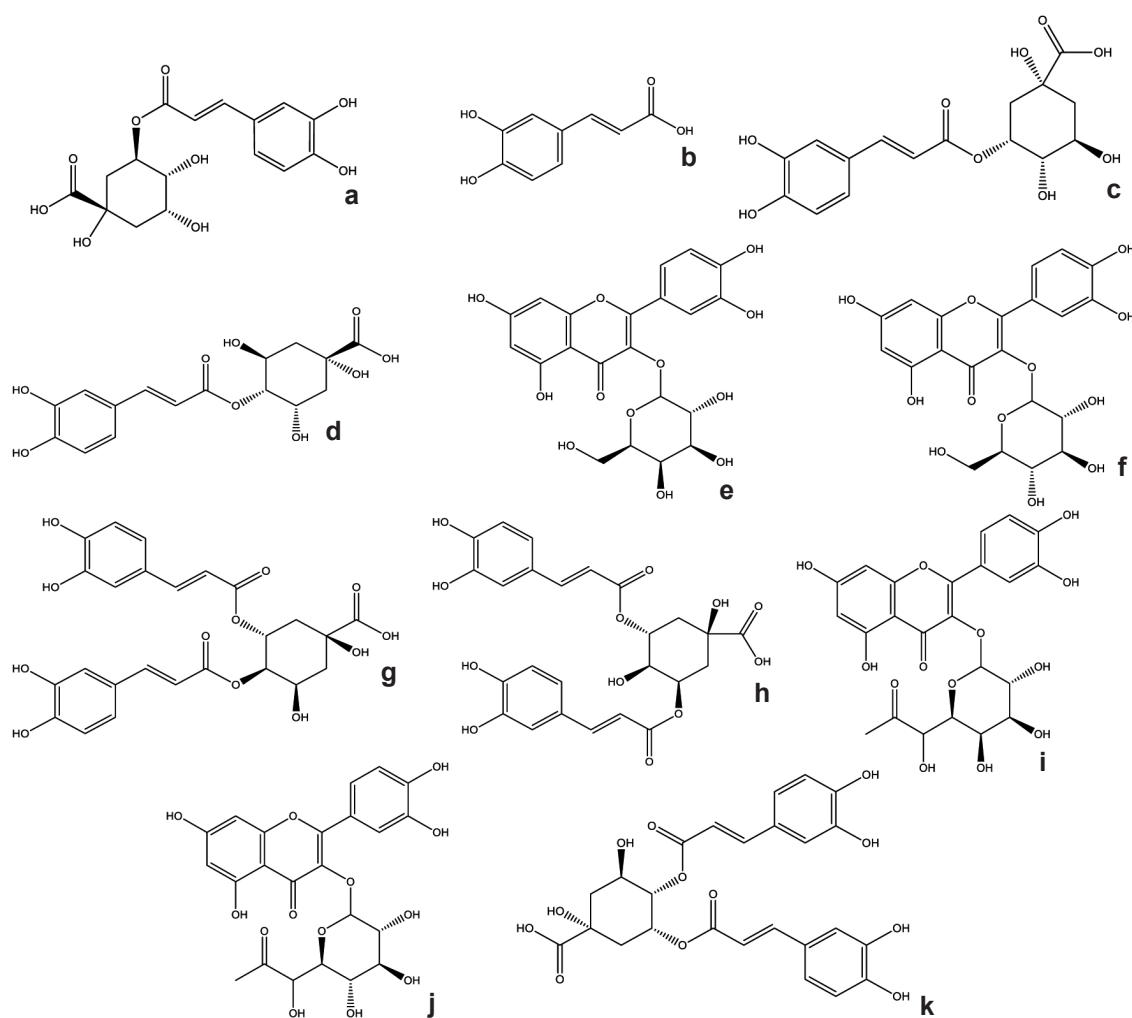


Figure 2 – a. k. Substances identified in the methanolic fraction from *Ipomoea pes-caprae* – a. 3-*O*-Caffeoylquinic acid; b. Caffeic acid; c. 5-*O*-Caffeoylquinic acid; d. 4-*O*-Caffeoylquinic acid; e. quercetin 3-*O*-galactoside; f. quercetin 3-*O*-glucoside; g. 3,4-di-*O*-Caffeoylquinic acid; h. 3,5-*O*-Dicaffeoylquinic acid; i. quercetin 3-*O*-acetylgalactoside; j. quercetin 3-*O*-acetylglucoside; k. 4,5-*O*-Dicaffeoylquinic acid.

and UPLC-MS-MS analysis confirmed the hexoxide type of peaks 5 and 6 (Tab. 1).

Hemodynamic evaluation

Intravenous infusion of the extract, the methanolic fraction, the fraction 10 (PIEA-M-1-10-Compound 1) and the fraction 18 (PIEA-M-1-18-Compounds 2a and 2b) were performed in normotensive rats to investigate changes in arterial blood pressure. Although fraction 18 contained a quercetin-3-*O*-glycoside mixture, this fraction was used in hemodynamic test because these flavonols have the same aglycone and fraction 19 had not sufficient quantity to be used for testing.

The effective dose (30 mg.kg⁻¹) was chosen for being the middle value in the concentration logarithmic graph (from 1 mg.kg⁻¹ to 300 mg.kg⁻¹ of animal). This concentration allowed the evaluation whether the plant extract has any activity without sacrificing many rats.

After extract administration, all parameters analyzed were significantly reduced when compared to initial pressure and control (DMSO) in normotensive rats (Fig. 3a-c). A comparison between the initial and the extract infusion pressure showed systolic blood pressure (SBP) decreased by 67.7 mmHg, diastolic blood pressure (DBP) decreased by 69.6 mmHg and mean

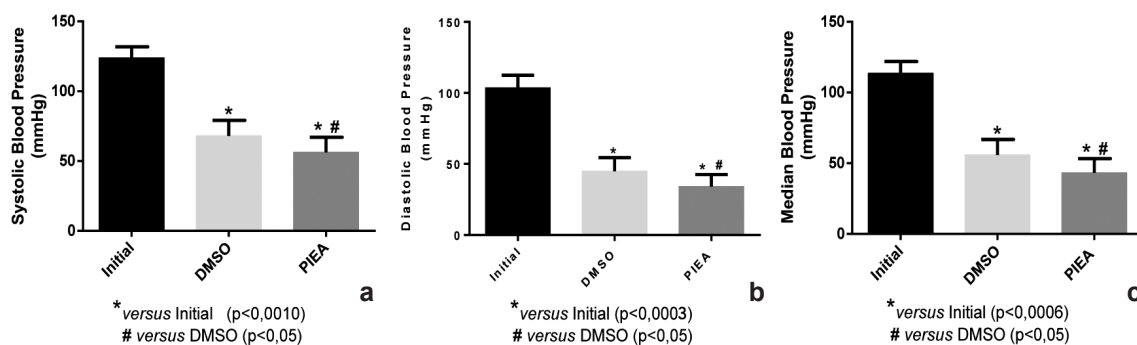


Figure 3 – a-c. Effects of *Ipomoea pes-caprae* extract (30 mg.kg⁻¹) on blood pressure of normotensive Wistar rats – a. systolic blood pressure (SBP); b. diastolic blood pressure (DBP); c. mean arterial pressure (MBP). Values are expressed as mean of five experiments.

arterial pressure (MBP) decreased by 70.4 mmHg. The control (DMSO) and the initial pressure comparison showed a lower reduction in which SBP decreased by 55.9 mmHg, DBP decreased by 58.6 mmHg and MBP decreased by 57.8 mmHg. The reduction induced after application of DMSO was already expected once this solvent has hypotensive effect caused by histamine release (Santis & Prata 2009). The differences between the changes in blood pressure caused by DMSO and by the extract indicates a statistically significant ($p < 0.05$) hypotensive effect of the hydroalcoholic extract. The chemical profile obtained for the extract suggests its effect could be caused by phenolic compounds such as flavonoids, which are recognized for their cardiovascular and vasodilatory activities. For instance, quercetin is a flavonoid which reduces high blood pressure. In an *in vivo* test with hypertensive mice using 10 mg.kg⁻¹ of quercetin daily for five weeks, has shown a reduction of systolic, diastolic and

medium blood pressure (Duarte *et al.* 2001; Guerrero *et al.* 2002; Oboh *et al.* 2012). Besides, phenolic compounds are responsible for the hypotensive activity of other *Ipomoea* species. For example, a polyphenol rich extract of *Ipomoea reniformis* exhibiting hypotensive activity (Jabeen & Aslam 2013) and also *Ipomoea batata* with verified hypotensive effect, in part due to its inhibitory activity of angiotensin converting enzyme of its caffeoylquinic acids (Ishiguro *et al.* 2007).

After administration of methanolic fraction, a significant reduction of all analyzed parameters was verified in comparison with initial pressure and control (Fig. 4a-c). Compared with initial pressure, the methanolic fraction infusion reduced SBP by 74.5 mmHg, DBP decreased by 72.9 mmHg and MBP decreased by 73.6 mmHg. DMSO (control) caused a lower reduction than the sample, suggesting the hypotensive effects of the tested sample. DMSO decreased SBP by

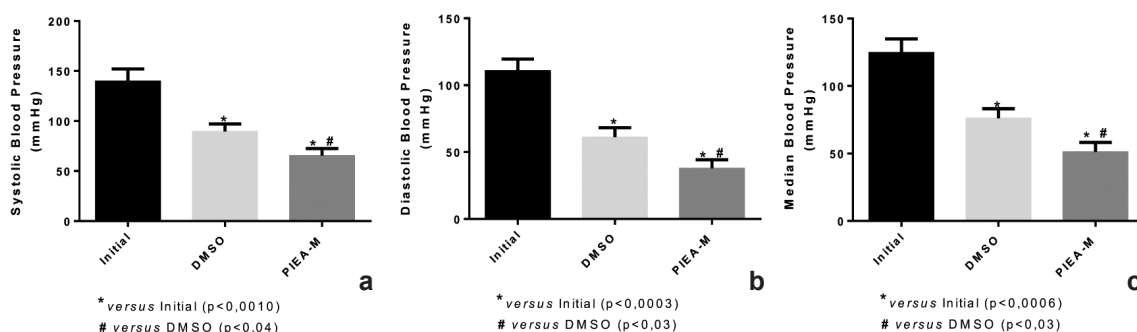


Figure 4 – a-c. Effects of the methanolic fraction (30 mg.kg⁻¹) on blood pressure of normotensive Wistar rats – a. systolic blood pressure (SBP); b. diastolic blood pressure (DBP); c. mean arterial pressure (MBP). Values are expressed as mean of five experiments.

50.1 mmHg, DBP decreased by 49.6 mmHg and MBP by 48.6 mmHg. The statistical analysis ($p < 0.05$) confirmed the hypotensive activity of the methanolic fraction.

Fraction 10 administration also significantly decreased SBP by 87.6 mmHg, DBP by 82.8 mmHg and MBP by 88.2 mmHg. DMSO caused a lower reduction in comparison to the sample: SBP

was reduced by 50.6 mmHg, DBP decreased by 60.2 mmHg and MBP decreased by 58.4 mmHg. The statistical analysis ($p < 0.05$) of the maximum effect caused by DMSO and by the sample showed the hypotensive effect of the compound present in fraction 10 (Fig. 5a-c). This is the first report of hypotensive activity of Quercetin 6''-O-acetyl-3-O-glucoside.

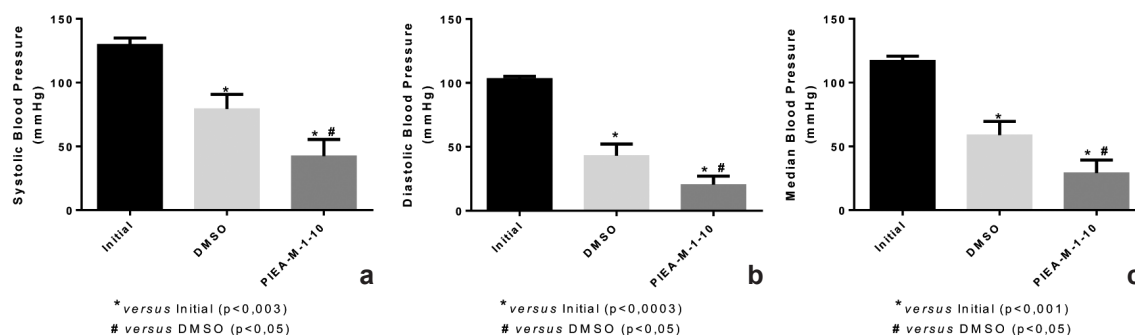


Figure 5 – a-c. Effects of the fraction 10 (30 mg.kg^{-1}) on blood pressure of normotensive Wistar rats – a. systolic blood pressure (SBP); b. diastolic blood pressure (DBP); c. mean arterial pressure (MBP). Values are expressed as mean of three experiments.

Comparing the reduction of pressure caused by administration of the control and fraction 18, we observed the sample produce a greater drop at initial blood pressure. The application of fraction 18 reduced SBP by 68.8 mmHg, DBP by 70.6 mmHg and MBP by 71.6 mmHg. While, DMSO promoted a decrease of 48.5 mmHg in SBP, 57 mmHg in DBP and 53.7 mmHg in MBP (Fig. 6a-c). Although there was not statistically significance between DMSO and sample values, a reduction of 10 mmHg in

rat pressure is quite significant and, in this case, discounting the effect of DMSO, the sample generated a reduction of 20.3 mmHg in SBP, 13.6 mmHg in DBP and 17.9 mmHg in MBP. The flavonoids quercetin 3-O-glucoside and quercetin 3-O-galactoside, which are present in fraction 18, have already had their hypotensive activity reported in the literature. In an *in vitro* study, flavonoids exhibited inhibitory activity against the angiotensin convorsor enzyme (ACE) (Balasuriya & Rupasinghe 2012).

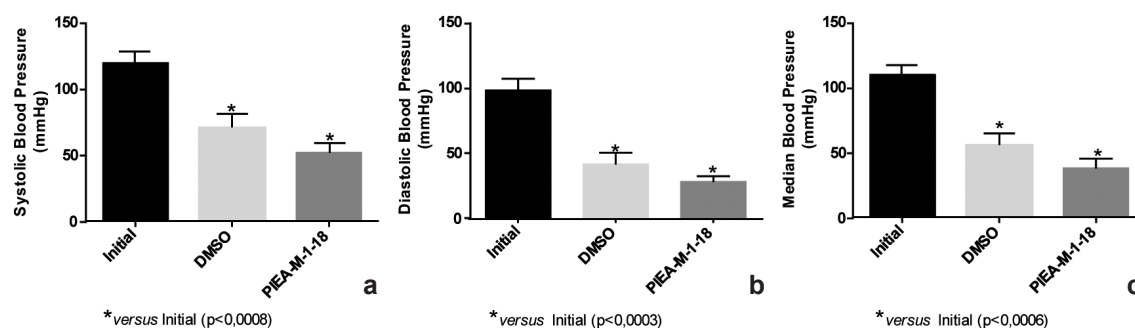


Figure 6 – a-c. Effects of the fraction 18 (30 mg.kg^{-1}) on blood pressure of normotensive Wistar rats – a. systolic blood pressure (SBP); b. diastolic blood pressure (DBP); c. mean arterial pressure (MBP). The values are expressed as mean of three experiments.

The Rota-Rod test revealed the extract PIEA and PIEA-M impair locomotor activity of the mice at 15 and 30 min after administration (Fig. 7). Although the number of falls decreased in-between each evaluation, the results were statistically significant when compared to the control group. On the other hand, at 60 and 120 min, the extracts were unable to cause significant effects on motor coordination, while diazepam maintained its effects. It is worth noting that PIEA and PIEA-M had not shown statistical difference.

Phenolic compounds, such as flavonoids and hydroxycinnamic derivatives, are known to possess effect on the central nervous system, displaying neuroprotective and sedative properties (Johnston 2015; Silva *et al.* 2015; German-Ponciano *et al.* 2018). Thus, mice motor incoordination could be related to beta adrenergic inhibition, which promotes decrease in psychomotor function. For example, propranolol, a beta blocker used in hypertension, causes motor deficiency (Broadhurst 1980). Flavonoids have some antihypertensive mechanisms already confirmed, such as endothelin-1 inhibition, a strong vasoconstrictor agent, and stimulation of nitric oxide production, a potent vasodilator through induction of nitric oxide synthase, an endothelial enzyme. This class of compounds also reduces angiotensin II levels through inhibitory activity against angiotensin converting enzyme

(ACE), and increases hypotensive action of bradykinin (B2 receptor agonist), which releases nitric oxide and prostacyclin and contributes to blood pressure control (Loizzo *et al.* 2008; Loke *et al.* 2008; Balasuriya & Rupasinghe 2012). Besides, flavonoids also show inhibitory effect on phosphodiesterase, increasing cyclic adenosine monophosphate (cAMP), thus contributing to vasodilatation (Duarte *et al.* 1993; Röhrig *et al.* 2017). Phenolic compounds and flavonoids present in these samples are classes with high antioxidant capacity, which is fundamental for controlling physiological balance and inhibiting oxidative stress. Previous studies indicate increased oxidative stress can contribute to hypertension (Touyz & Schiffrin 2004; Silva *et al.* 2015; German-Ponciano *et al.* 2018).

With the results obtained in this study, it was possible to verify that phenolic compounds are the substances responsible for the anti-hypertensive activity empirically attributed to *Ipomoea pes-caprae*.

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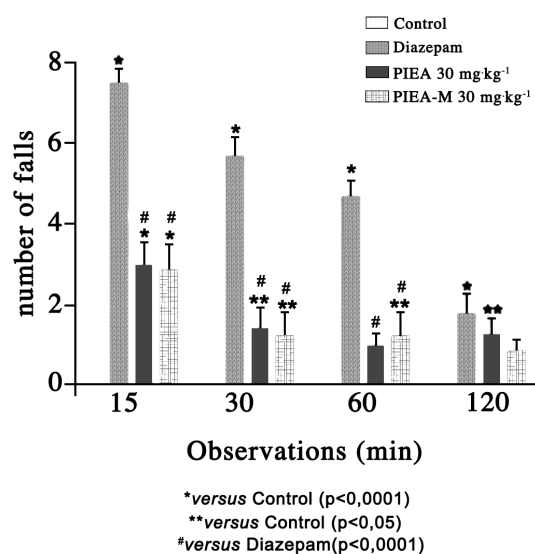


Figure 7 – Effects of extract and methanolic fraction of *Ipomoea pes-caprae* on Swiss mice in Rota-Rod test. Values are expressed as mean of ten experiments.

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