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Original Article

# *Operculina macrocarpa*: chemical and intestinal motility effect in mice

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A B S T R A C T

*Operculina macrocarpa* (L.) Urb., Convolvulaceae, is used by the population as a laxative. In this work we described the isolation of the three phenolic acids present in the hydroethanolic extract of the *O. macrocarpa* roots. The quantification of the caffeic, chlorogenic acids and of the new caffeic dimer in the hydroethanolic and infusion extracts was performed by high-performance liquid chromatography coupled photodiode array detector. These analyses showed the higher content of the chlorogenic, caffeic and the new 3,4′-dehydrodicaffeic acid in hydroethanolic and hydroethanolic extracts without resin in which infusion. The acid found in greater quantity is caffeic acid followed by the 3,4′-dehydrodicaffeic acid. The laxative activity was evaluated by different experimental models of intestinal transit with the hydroethanolic and infusion extracts, and the resin fraction, caffeic, chlorogenic and ferulic acids. The results showed all extracts and compounds tested had significant activity in the experimental model tested. These results obtained are essential for the future development of a pharmaceutical product with safety and efficacy.

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## Introduction

The therapy based on plants dates back to the origins of humanity and, has always had an important role in the community health over time. According to the World Health Organization (WHO), medicinal plants are the best sources to obtain drugs (WHO, 2002).

Medicinal plants have an important role in the Brazilian culture and tradition. Nowadays, the majority of the urban population, as well as the rural communities, use phytomedicines as medicinal treatments although a few number of species were studied by the chemical and pharmacological point of view. In this sense, the evaluation of the therapeutic potential and chemical composition of these plants can contribute to the development of the Brazilian phytotherapy. The security and efficiency in the use of phytotherapics should be based on the existence of relevant scientific literature grounded on the demonstration of its pharmacological activity, clinical efficacy and its toxicity. As for security, the knowledge that guarantees the use of many phytotherapics comes from traditional medicine and the ethnomedicinal knowledge gathered for centuries (Carvalho, 2005).

*Operculina macrocarpa* (L.) Urb., Convolvulaceae, popularly known as "batata-de-purga" or "jalapa", (syn. *Ipomoea purga* Hayne) (Xavier et al., 1994) from the northeast of Brazil, and widely used by population due to its laxative, purgative and depurative activity against skin diseases and in the leucorrhea treatment (Matos, 1982; Martins et al., 2000).

According to Kohli et al. (2010) in Ayurveda, *O. turpethum* has been included in the group of 'ten purgative herbs'. The presence of resin glycosides in Convolvulaceae family has been established associated with the laxative properties of the herbal drugs. Resin glycosides were classified into two groups: jalapin and convolvulin. The jalapin group presents the common structure of a macrolactone with one acylated glycosidic acid while the convolvulin groups are oligomers of glycosidic acids (Pereda-Miranda et al., 2006). Previously we have reported the intestinal motility from the dichloromethane, ethyl acetate and residual fractions, and the powder preparation of *O. macrocarpa* (Michelin and Salgado, 2004). Xavier et al. (1994) isolated from the leaves of this species two *C*-flavonoids. Despite the popular use of this specie it is fundamental a meticulous study about the chemical composition and its

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laxative effect. The present study is therefore, aimed to the isolation the phenolic compounds presents in the hydroethanolic extract of roots of the *O. macrocarpa* and the evaluation of the laxative effect of the extracts, resin fraction and the compounds isolated of this plant.

## Material and methods

### *Plant material*

*Operculina macrocarpa* (L.) Urb., Convolvulaceae, was collected in the garden of Medicinal Plants Uniararas, and the exsiccate was identified and included in the collection of the ESA-Agriculture School Luiz de Queiroz Herbarium, Department of Biological Sciences ESALQ/USP, registration number ESA114652.

The roots were previously shaved and dried in an air heater, at a temperature of 45 °C until it was reached a constant weight, and then they were crushed in a ball grinder to reduce the size of the particles.

### *Chemicals*

Caffeic acid, chlorogenic acid and ferulic acid, both solid state, were purchased from Sigma (St. Louis, MO, USA). Analytical grade acetic acid was purchased from Riedel-de Haën (Seelze, Germany). Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). HPLC-grade water (18 MΩ cm) was obtained using a Direct Q5 Milli-Q purification system (Millipore Co., Bedford, MA, USA).

### *Extraction*

The powder of the roots (200 g) of *O. macrocarpa* was previously moistened with ethanol 70°GL and maintained during 2 h. Then, this mixture was transferred in to the percolator and completed with 2 l of the solvent. The reduction of the solvent volume was obtained by the utilization of a rotatory evaporator. During this process was observed the precipitation of a yellowish brown solid, the resin fraction (5 g; 2.5% of yield). This material was separated by decantation. After all the process were obtained 36 g of dry hydroethanolic extract (EEtOH; 18% of yield).

### *Preparation of the infusion*

The dry and pulverized roots (200 g) were heated and boiled for 10 min in 2 l of water to extract effective components, and removed. After filtration, the infusion extract was concentrated in a rotary evaporator and it was frozen and lyophilized, furnishing 46.2 g of the infusion extract (23% of yield).

### *Chromatographic methods*

#### *Extract chromatographic profile by HPLC-UV-PDA*

The analysis of the chemical profile of the *O. macrocarpa* extracts were performed in a High Performance Liquid Chromatograph with a Photodiode Array Detector (HPLC-UV-PDA), brand Varian™ model *ProStar* 210/330. The separation was performed on a reverse phase Luna $C_{18}$ column (250 mm × 4.6 mm, 5 μm) (Phenomenex™), equipped with a Phenomenex Security Guard (4 mm × 3 mm, 5 μm). Samples and patterns were injected through a Rheodyne™ 7125 injector with a 20 μl loop.

Approximately 20 mg of each EEtOH and infusion were dissolved in 1 ml of methanol/water (1:1, v/v). For the removal of possible lipophilic compounds, each solution was purified by solid phase extraction (SPE), using Phenomenex Strata $C_{18}$ cartridges (500 mg of stationary phase), previously activated with 5 ml of methanol and equilibrated with 5 ml of HPLC-grade water. The samples were eluted from cartridges using 5 ml of a mixture of methanol/water (1:1, v/v). The samples were then filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter and aliquots of 20 μl were directly injected into HPLC.

The mobile phase compositions used were water (eluent A) and acetonitrile (eluent B), both containing 0.05% trifluoroacetic acid. The gradient programme was as follows: 28–40% B (20 min), 40–70% B (1 min), 70–100% B (10 min) and 100% (9 min). Total run time was 40 min. The flow-rate of the mobile phase was 1.0 ml/min. Star LC Workstation software was used both for the operation of the detector and for data processing.

#### *Isolation of phenolic acids*

The EEtOH extract (4 g) was fractionated by gel permeation column chromatography. The column was packed with Sephadex LH-20 (57 cm × 3.0 cm i.d.) and soaked with methanol. The column was then eluted with the same solvent mixture yielded 156 fractions (5 ml each one). After TLC analysis, similar fractions were combined to yield 28 subfractions.

Part of subfraction 17 (25 mg) was refrationated on a silica gel column using the mixture of chloroform/methanol/water (43:37:20, v/v, organic phase) as mobile phase. This procedure furnished a white precipitated (13 mg, caffeic and protocatechuic acids). The other compound was obtained from the subfraction 25 (50 mg) by column chromatography (CC) using as mobile phase the mixture of ethyl acetate/acetic acid/water (15:0.5:0.1, v/v). This procedure furnished a white precipitated (14 mg, the new compound 3,4′-dehydrodicaffeic acid).

#### *Quantitative analysis of phenolic acids*

The quantitative analysis of phenolic compounds in the *O. macrocarpa* extract and infusion was performed by HPLC-UV-PDA using the conditions describe in *Extract chromatographic profile by HPLC-UV-PDA* section.

These experiments permitted the determination of the chlorogenic and caffeic acids, and caffeic dimer. The identification was performed by comparison retention time, by spiking with known standards, and by comparison with previously isolated compounds under the same conditions. Methods using external standards were used to quantify each compound.

These curves were obtained from seven stock solutions (1000 μg/ml) in the range of concentrations between 1 and 100 μg/ml. The dilutions were made in water/methanol (1:1, v/v), and were injected in the HPLC-UV-PDA. All experiments were made in triplicate. The analytical curves had good linearity in the concentration range studied and presented correlation coefficients ($r$) with values above 0.999, which suggests as a good correlation between the areas and the concentrations studied.

#### *NMR analysis*

The $^{1}$H NMR and $^{13}$C NMR 1D and $^{1}$H NMR 2D-NMR $^{13}$C g-HMBC experiments were obtained using a Bruker® spectrometer at 300 MHz frequency (7.0 T) and on a Varian Inova® spectrometer at 500 MHz frequency (11.7 T). The samples were dissolved in dimethyl sulfoxide (DMSO-$d_6$) containing TMS for chemical reference.

#### *Direct-injection ESI/MS and ESI/MS/MS analysis*

The ESI analysis was carried out on a LCQ FLEET Thermo Scientific® equipped with an ion trap analyzer system: data were acquired using Xcalibur 2.1.0.2.40 software. The source temperature was set at 250 °C, and the source voltage was constant at 3.5 kV. Nitrogen was used as sheath and nebulizer gas at 5 l/min and 10 psi. Helium was introduced into the system at an estimated pressure of $6 \times 10^{-6}$ mbar to improve trapping efficiency, and was provided as the collision gas during the CID experiment. For MS/MS spectra,

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