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Original Article

Operculina macrocarpa: chemical and intestinal motility effect in mice

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ABSTRACT

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.

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 6×10^{-6} mbar to improve trapping efficiency, and was provided as the collision gas during the CID experiment. For MS/MS spectra,

the fragmentation amplitude varied between 0.6 and 0.9 V; the MS operated in the negative ion mode with a scan rate of 13,000 u/s. Samples were infused into the ESI source by use of a syringe pump at a flow-rate of 5 μl/min.

Animals

There were used Swiss mice (*Mus musculus)*, male, 30 days of age, between 20 and 30 g of weight, adapted to the experimental animal facility for 5 days before the begin of the biological trials. The animals had free access to food and water, and were kept in room temperature of 20 ± 1 °C, controlled humidity and a photoperiod of 12 h light/darkness. The day of the trial the animals were in fasting for 3 h, receiving water *ad libitum*.

The animals used are from the Bioterism Centre of the São Paulo State University, Botucatu. The procedures were previously approved by the Ethics Research Committee of the São Paulo State University, Araraquara, approval number 25/2006.

Preparation of solid dispersion from the resin

Considering the insolubility of the resin in water, a solid dispersion was prepared for oral administration in mice in the test of intestinal motility.

The solid dispersion was prepared dissolving the resin in an ethanolic solution of polivinilpirrolidone (10%). The ethanol was evaporated in rotary evaporator at 50 °C. The final concentration of the solid dispersion was 12.5 mg/ml of resin.

Intestinal transit with active charcoal

The animals were randomly separated in groups of ten animals each, and received the treatment by oral gavage administration. A control group received saline solution. After 45 min, the animals received a suspension of active charcoal 10% in a solution of 5% arabic gum and 0.5 ml/animal, by gavage administration. After 45 min, the mice were sacrificed in a CO_2 chamber, and it was performed an immediate extirpation of the intestine from the pylorus to the beginning of the caecum. Thereby, it was made the measurement of the total length of the small intestine and the distance travelled by the active charcoal suspension. The results were expressed in percentage of the total length of small intestine. The intestines were weighted individually in an analytical balance.

The activity in the intestinal transit was determined according to Janssen and Jageneau (1957) and Wong and Way (1981).

Intestinal transit with modified active charcoal (Marona and Lucchesi, 2004)

The animals were randomly separated in groups of ten each one, and received the oral treatment by gavage administration. A control group received saline solution. After 90 min, the animals received a suspension of active charcoal 10% in an arabic gum solution 5% and 0.3 ml/animal, by gavage administration as well. After 60 min, the animals had access again to food and water.

The mice were observed for 4 h each 5 min, and it was timed how long did it take them to eliminate of the first faeces with active charcoal.

Intestinal transit in metabolic cage

The animals were randomly separated in groups of ten and received the oral treatment by gavage administration. A control group received saline solution. The animals were placed in a metabolic cage, and after 4 h the faeces were weighted and compared to the control group.

Statistical analysis

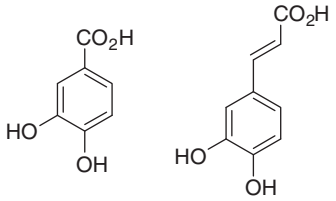
The experimental results were expressed in mean ± standard deviation. The statistical analysis was calculated by Student *t*-test ($p < 0.05$) (De Muth, 1999).

Results and discussion

The phytochemical study of the EEtOH extract from the *O. macrocarpa* resulted in the isolation of protocatechuic acid (**1**), caffeic acid (**2**) and the new compound 3,4′-dehydrodicaffeic acid (**3**).

The ^{1}H NMR analysis of the white crystal revealed the presence of a mixture of two compounds. The major compound was identified by the presence of the two doublets at 7.02 ppm (J = 2.5 Hz, 1H) and at 6.76 ppm (J = 8.5 Hz, 1H) and a double doublet at 6.95 ppm, (J = 8.5 and 2.5 Hz, 1H), This coupling pattern of three aromatic hydrogens suggested the existence of 1,3,4-trisubstituted benzene. The two large doublet at 6.17 ppm (J = 16.0 Hz, 1H) and at 7.38 ppm (J = 16.0 Hz, 1H) indicated *trans* olefinic hydrogens. These data characterized the presence of the caffeic acid (**2**). The ^{13}C NMR data corroborate this compound as the caffeic acid (Dürüst et al., 2001).

The same aromatic substitution pattern was observed for the minor compound **1**. The signals at 7.34 ppm (*d*, J = 2.5 Hz, 1H), 7.29 ppm (*dd*, J = 8.5 and 2.5 Hz, 1H) and 6.78 ppm (*d*, J = 8.5 Hz, 1H). The ^{13}C NMR spectrum revealed the presence of 7 signals being one of them corresponding to a carbonyl carbon at 168.7 ppm. The spectra analyze of the two-dimensional HMQC and HMBC experiments allow the identification of the minority compound as protocatechuic acid (**1**) (Yu et al., 2006).



1 **2**

Analyzing the ^{1}H NMR spectra of another phenolic acid, showed the presence of hydrogen signals with chemical shifts fairly constant and similar couplings to those of the caffeic acid. However, they are duplicated. Through the integration of the signals along with analysis of ^{13}C NMR spectra and two-dimensional HMBC and HMQC experiments it was possible to identify the new substance as the caffeic dimer bound by positions 3 of the unity I with 4′ of the unit II. The signals at 7.04 ppm (*d*, J = 2.5 Hz, 1H), 6.76 ppm (*d*, J = 8.5 Hz, 1H) and 6.90 ppm (m, 1H) refers to hydrogens H-2, H-5 and H-6, respectively the aromatic ring of unit I. These assignments can be made by the correlations observed in the HMBC spectrum. The hydrogens of the *trans* double bond of that unit I can be assigned by the signals at 6.24 ppm (*d*, J = 16.0 Hz, 1H) and 7.48 ppm (*d*, J = 16.0 Hz, 1H), consecutively. The confirmation that these signals belonging to Unit I were given by correlations between the signal at 7.48 ppm and 6.24 ppm (H-α and H-β) with a signal of a carbonyl at 166.1 ppm, as well as the correlation between proton β of the double bond (7.48 ppm) with C-6 at 121.0 ppm. These correlations allowed the unambiguous assignment of the unit I. The aromatic part of the unit II may be ascertained through signals at 7 ppm (*d*, J = 2.5 Hz, 1-H), 6.78 ppm (*d*, J = 8.5 Hz, 1-H) and 6.90 ppm (m- 1H), which refer to hydrogens H-2, H-5 and H-6, respectively. The signals at 7.45 ppm (*d*, J = 16.0 Hz, 1-H) and at 6.18 ppm (*d*, J = 16.0 Hz, 1-H) correlated with the signal at 165.6 ppm confirm their

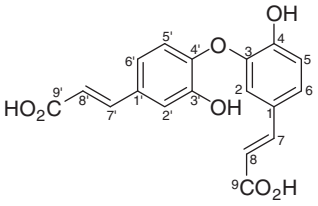
Table 1
Percentage of distance travelled by the active charcoal in the intestine of mice treated with *Operculina macrocarpa*.

Treatment	Dose (mg/kg)	Distance travelled by the active charcoal (%)	Weight of the intestin (g)
Control (saline)	10 ml/kg	72.01 ± 8.15	2.3983 ± 0.25
Infusion extract	1000	83.74 ± 12.20*	2.2830 ± 0.28
Infusion extract	500	75.34 ± 7.43	2.3139 ± 0.31
EEtOH without resin	1000	91.23 ± 13.22*	2.4160 ± 0.44
Resin fraction	400	60.62 ± 5.79	2.5684 ± 0.28

* $p < 0.05$; $n = 10$.

correlation to the double bond of the unit II of the caffeic dimer. Similarly to the unit I, it was possible to assign all signals through the analysis of the ^{1}H and ^{13}C NMR spectra as well as two-dimensional experiments (HMBC and HMQC). To establish the linkage position of the two caffeic unities was measured and a long range carbon hydrogen correlation for H-2 (unity I) → C-4 (unity II) observed. In order to corroborate this structure we realized the ESI-MS experiment. This experiment revealed the presence of a pseudomolecular ion *m*/*z* 341 $[M-H]^-$. While in the MS^2 showed abundant ions at *m*/*z* 179 (100) and 135 (90) which accords with the caffeic acid. In comparison our data with those reported by Shaheen et al. (2011) for the 3,3′-didehydrodicaffeic acid we confirmed the presence of the 3,4′-dehydrodicaffeic acid (**3**) (Plazonić et al., 2009).

^{13}C NMR (DMSO-d_6, 125 MHz) for the 3,4′-dehydrodicaffeic acid was: δ for unity I: 114.7 (C-α), 114.8 (C-2), 115.8 (C-5), 121.0 (C-6), 125.5 (C-1), 145.5 (C-3), 144.6 (C-β), 148.2 (C-4), 166.1 (COOH). For the unity II: 114.2 (C-α), 114.6 (C-2), 115.7 (C-5), 121.3 (C-6), 125.6 (C-1), 145.5 (C-3), 144.9 (C-β and C-4), 148.2 (C-4), 165.6 (COOH).



3

Evaluation of the laxative activity

Since the population uses *O. macrocarpa* as a laxative, this activity was evaluated using three experimental models.

The first model evaluated was the one that uses active charcoal as marker in order to observe the intestinal transit. The activity is determined by the distance travelled by the marker in the small intestine, compared to the control group. This method is widely used to evaluate the activity of vegetal extracts (Michelin and Salgado, 2004; Figueiredo et al., 2005; Salgado et al., 2006).

The second model, proposed by Marona and Lucchesi (2004), uses the same methodology as the first one; however, in this model the animals are not sacrificed, and can be used later on in another experiment, fact that helps with the wellbeing of the animal.

In the third model, animals are placed in a metabolic cage and the activity is determined analyzing the faeces, differently of the other two models. In this model, the animals can also be used again in other experiments since there is no need to sacrifice them.

According to literature, until now it is the first study evaluating the activity of *O. macrocarpa* in the intestinal transit, except by the preliminary study, previous to this one, carried out by our research group (Michelin and Salgado, 2004).

Intestinal transit with active charcoal

This test was carried out in 1957 by Janssen and Jageneau, and it was evaluated the inhibition of the gastrointestinal propulsion with a charcoal suspension in mice. Then, Wong and Way (1981) evaluated the effect of aspirin and acetaminophen in the inhibition of the gastrointestinal propulsion induced by morphine in mice. The trial was performed according to the second experimental model developed by Janssen and Jageneau (1957) and Wong and Way (1981).

The infusion extract and the standards of the caffeic, chlorogenic and ferulic acid were evaluated using this experimental model.

The results shown in Table 1, demonstrate the significant increase of the intestinal motility, when compared to the control group, caused by the infusion extract using a dosage of 1000 mg/kg. Using a dosage of 500 mg/kg the result appeared to be not significant.

The EEtOH extract was previously tested using a dosage of 1000 mg/kg, and the results showed a significant increase of the intestinal motility of mice (69.99%) when compared to the control group (47.87%) (Michelin and Salgado, 2004). The low solubility presented by the resin in water implied the preparation of a solid dispersion to test in mice, however it did not show significant activity in the concentration used.

The evaluation of the EEtOH extract after the precipitation of the resin at the dose of 1000 mg/kg has showed a significant increase of the intestinal motility. Despite the higher quantity of 1–3 compounds, were submitted to the same test. The three acids showed a significant increase of the intestinal motility in mice when compared to the control group (Table 2).

The acids were tested in dosages of 10 and 20 mg/kg. The caffeic acid presented activity in the dosage of 20 mg/kg, and travelled a distance of 86.80% of the small intestine, while in the control group it travelled a distance of 81.53%. The ferulic acid showed significant activity in the tested dosages, travelling 86.43 and 91.45% of the small intestines using the dosages of 10 and 20 mg/kg, respectively. Finally, the results obtained for the chlorogenic acid, were

Table 2
Percentage of the distance travelled by the active charcoal in the small intestine of mice treated with caffeic, ferulic and chlorogenic acids.

Treatment	Dose (mg/kg)	Distance travelled by the active charcoal (%)	Weight of the small intestin (g)
Control (saline)	10 ml/kg	81.53 ± 9.75	1.5632 ± 0.19
Caffeic acid	10	79.70 ± 13.05	1.6160 ± 0.15
Caffeic acid	20	86.80 ± 10.99[a]	1.6400 ± 0.37
Ferulic acid	10	86.43 ± 16.63[a]	1.4864 ± 0.09
Ferulic acid	20	91.65 ± 13.60[a]	1.5282 ± 0.17
Chlorogenic acid	10	78.62 ± 9.34	1.3862 ± 0.07
Chlorogenic acid	20	86.08 ± 7.16[a]	1.4385 ± 0.20

[a] $p < 0.05$; $n = 10$.

Table 3
Elimination time of the faeces with marker in mice treated with *Operculina macrocarpa*.

Treatment	Dose (mg/kg)	Time to eliminate the faeces with marker (min)
Control (saline)	10 ml/kg	194.6 ± 30.37
EEtOH extract	1000	156.4 ± 23.29[a]
Infusion extract	1000	65.1 ± 4.70[a]

[a] $p < 0.05$; $n = 10$.

Table 4
Weight of the faeces eliminated by mice treated with *Operculina macrocarpa*.

Treatment	Dose (mg/kg)	Weight of the faeces (g)
Control (saline)	10 ml/kg	1.80 ± 0.23
EEtOH extract	1000	2.01 ± 0.60[a]
Infusion extract	1000	3.98 ± 0.71[a]

[a] $p < 0.05$; $n = 10$.

significant in the dose of 20 mg/kg, having travelled a distance of 86.08%.

Based on these results, it can be suggested that phenolic acids have stimulant activity of the intestinal motility, since they are present in the EEtOH and infusion extracts of *O. macrocarpa*, where this activity was also found.

Intestinal transit with modified active charcoal (Marona and Lucchesi, 2004)

In this model were evaluated the EEtOH and the infusion extracts of *O. macrocarpa*. This model of intestinal transit also uses active charcoal as marker, however it was modified in 2004 by Marona and Lucchesi to improve the wellbeing of the animal, since in this model the animals are not sacrificed and can be used again in other experiments.

The results displayed in Table 3, show a significant decrease of the elimination time of the marker when it is compared to the control group.

Intestinal transit in metabolic cage

In this model were evaluated the EEtOH and the infusion extracts of *O. macrocarpa*. It is important to highlight that in this model, animals are placed in a metabolic cage and the activity is determined by the weight of the faeces eliminated during the observation period, thus animals are not sacrificed and can be used again in other experiments.

Table 4 shows the results. It can be observed a significant difference of the weight of the faeces between the treated groups and the control group, indicating that the EEtOH and infusion extracts of *O. macrocarpa* also have stimulant activity of the intestine transit in this model.

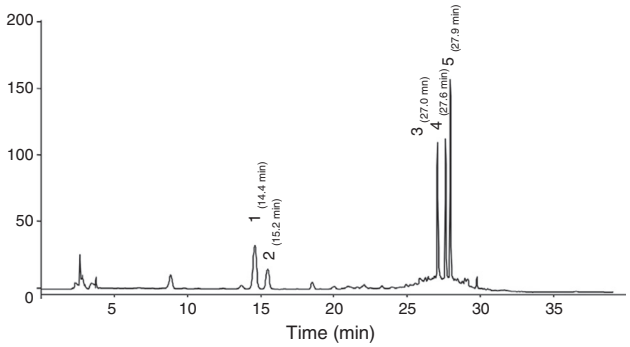


Fig. 1. HPLC separation chromatogram, in analytic mode (210 nm) of the EEtOH extract of *Operculina macrocarpa*. **1**. clorogenic acid; **2**. caffeic acid; **3**. 3,4′-dehydrodicaffeic acid; **4** and **5**. Not identified.

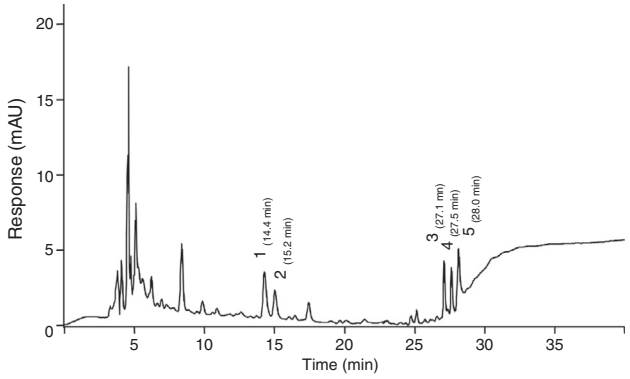


Fig. 2. HPLC separation chromatogram, in analytic mode (210 nm) of the infusion extract of *Operculina macrocarpa*. **1**. clorogenic acid; **2**. caffeic acid; **3**. 3,4′-dehydrodicaffeic acid; **4** and **5**. Not identified.

In order to relate the pharmacological activity observed with the metabolites found in this plant was performed the chromatographic profile by HPLC-UV-PAD from the EEtOH and infusion extracts of *O. macrocarpa*. The UV data showed intense bands in the spectral range between 190–220 and 260–280 nm confirming the presence of five phenolic acids in these extracts (Saldanha et al., 2013) (Figs. 1 and 2). For each compound assignment, we used the comparison of retention time of the sample chromatogram with those of standards, additionally we comparing the absorption spectra for peaks obtained with those of standards analyzed under the same chromatographic conditions. It was performed by co-injection of the authentic patterns or the metabolites isolated from this plant (Figs. 1 and 2). The compounds **4** and **5** observed in the chromatographic profile of EEtOH and infusion extracts were not identified, but could be quantified through the same profile displayed on the UV absorption spectra. According to the quantification of phenolic acids in the EEtOH extract there was a greater caffeic acid concentration (4.7 mg/g), followed by the

Table 5
Quantity of phenolic acids determined in the extracts of *Operculina macrocarpa*.[a]

Substances	Concentration (mg/g) ± standard deviation		
	EEtOH extract	EEtOH without resin	Infusion extract
Caffeic acid, **2**	4.7 ± 2.9	5.1 ± 4.1	2.4 ± 3.1
Chlorogenic acid, **1**	1.1 ± 5.1	1.1 ± 9.8	0.4 ± 5.5
3,4′-Dehydrodicaffeic acid, **3**	1.9 ± 2.9	1.5 ± 1.7	0.4 ± 2.9
NI 1	1.2 ± 2.4	1.0 ± 4.2	0.3 ± 1.5
NI 2	2.4 ± 1.2	1.9 ± 4.7	0.3 ± 3.5

[a] Each value represents de mean ± standard deviation (%) for 3 independent experiments ($n = 3$); NI = not identified.

3,4′-dehydrodicaffeic acid (1 mg/g) and chlorogenic acid (1.1 mg/g) (Table 5). The EEtOH extract without the resin showed a higher concentration of caffeic acid (5.1 mg/g) and a lower concentration of 3,4′-dehydrodicaffeic acid (1.5 mg/g). It was possible to observe the same level of chlorogenic acid in both EEtOH extract as in EETOH extract without resin. The infusion has a significant reduction in the content of both quantified acids.

Conclusions

The phytochemical analysis of the EEtOH extract of the roots of *O. macrocarpa* guided to the isolation and identification of the caffeic and protocatechuic acids and the new 3,4′-dehydrodicaffeic acid.

The EEtOH and infusion extracts were subjected to HPLC-UV-PDA chemical profiling and 5 principal peaks (1–5) were detected (Figs. 1 and 2). The caffeic and chlorogenic acids and 3,4′-dehydrodicaffeic acid were quantified in *O. macrocarpa* extracts. The caffeic acid was found to be the characteristic component of theses extracts.

The results obtained with the three experimental models tested showed the laxative activity with increasing the intestinal motility of all extracts of *O. macrocarpa*. The caffeic, ferulic, chlorogenic acids and the 3,4′-dehydrodicaffeic acid evaluated showed the laxative action. This effect is directly related to the content of caffeic, chlorogenic acids and the 3,4′-dehydrodicaffeic acid in the extract. The correlation between the phenolic acids structures and the intestinal motility we can suggest that activity involve the skeleton with the conjugated acid portion and/or the phenolic without necessarily with the *orto*-dihydroxi system. Our results confirm the traditional use of this specie a laxative.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

DMP contribution included the execution of intestinal motility effect in mice, the isolation of the compounds and elaboration the paper. MS contribution included the identification of the compounds, analyzing the results and preparing the paper. DR contribution included the analysis chromatographic by HPLC-UV-PDA. WV and HRNS contribution included to the provision of all laboratory equipment and infrastructure for the development of all experimental part of the phytochemical and pharmacological tests of this work.

Conflicts of interest

The authors declare no conflicts of interest.

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