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Chemical composition and inhibitory activities on dipeptidyl peptidase IV and pancreatic lipase of two underutilized species from the Brazilian Savannah: *Oxalis cordata* A.St.-Hil. and *Xylopia aromatica* (Lam.) Mart

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ABSTRACT

Brazil has the greatest vegetal biodiversity in the world, but products derived from native species are not optimally utilized. *Oxalis cordata* and *Xylopia aromatica* are two underutilized species whose leaves and fruits, respectively, have been used as food in the 19th century. In this study, we used chemical and *in vitro* assays to evaluate the potential of these species as functional foods. The inhibitory activity on pancreatic lipase and DPP-IV were evaluated using the crude extracts and fractions ethyl acetate, butanol and water of these two species. For polyphenols determination, samples were prepared with different solvents and these were analysed by chromatographic and spectroscopic methods. Finally, fatty acids profile was determinated by gas chromatography. The crude extract (IC_{50} = 0.84 mg/ml), ethyl acetate extract (IC_{50} = 0.88 mg/ml) an aqueous fraction (IC_{50} = 0.63 mg/ml) of *C. cordata* were inhibitory on pancreatic lipase but inactive against dipeptidyl peptidase IV (DPP-IV). Extracts from *X. aromatica* were inactive against the lipase pancreatic enzyme, but a butanolic fraction inhibited DPP-IV (IC_{50} = 0.71 ± 0.05 mg/ml). The phenolic acids orientin/isorientin, chlorogenic acid (0.32 g/100 g) and the flavonoid derivatives rutin (0.27 g/100 g), quercetin and luteolin were observed in all products. Additionally, fatty acid quantification showed that oleic (7.5 g/100 g) and linoleic acid (6.5 g/100 g) were predominant in *X. aromatica* fruit. This study confirms the potential for the use of both plants as functional foods due to their nutritional value, biological activity and important phytochemical content.

1. Introduction

Spices and condiments are commonly considered nutritious and rich in phytochemicals and are used to treat or prevent a wide range of diseases such as cancer, obesity, diabetes and cardiovascular disorders (Bogusz et al., 2018; Wootton-Beard & Ryan, 2011). The search for bioactive products from plants is increasing, and Brazil is a hotspot due to its extensive biodiversity. However, useful native Brazilian plants are not optimally utilized. This is a consequence of the intense destruction of native vegetation, as well as the miscegenation of cultures during the last centuries, which has contributed to the popularization of exotic rather than indigenous plants for cooking and medicine (Brandão, Grael, & Fagg, 2011). During the last decades, our research group has focused on investigating these underutilized Brazilian plants and promoting their better use (Brandão et al., 2008; Brandão et al., 2011). The leaves of *Oxalis cordata* A. St.-Hil. and the fruits of *Xylopia aromatica* (Lam.) Mart. are two of these interesting underutilized spices.

The Portuguese popular name of *O. cordata* is “azedinha”, which means “little sour”, which was conferred because of the taste (Correa, 1984). Historical data describe the use of the other “azedinhas” *O. hirsutissima* and *O. repens* as an antiscorbutic, antipyretic and for angina treatment in the past (Brandão, Pignal, Romaniuc, Grael, & Fagg, 2012). The genus *Oxalis* has approximately 900 species commonly found in tropical and subtropical regions of Africa and Asia (Bhattacharjee, 1998), and *O. corniculata* is the most studied species. The leaves of *O. corniculata* contain carbohydrates, proteins, amino acids, fibres and phytosterols and a high content of oxalate that gives the sour taste to *Oxalis* species (Vishwakarma & Dubey, 2011). Its leaves have many biological activities including antioxidant, inhibition of pancreatic lipase and anti-diabetic by *in vitro* studies.

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X. aromatica is still in use in very remote regions in Brazil as a substitute for black pepper, due to its similar but milder taste (Oliveira, Yamada, Fagg, & Brandão, 2012). Other species of *Xylopia* are native to Brazil, including *X. frutescens* and *X. sericea*, and have a less spicy taste (Lorenzi & Matos, 2002). They are also used in traditional medicine as an aphrodisiac and carminative (Correa, 1984). The genus *Xylopia* has 160 species distributed in the savannah and tropical areas (Maas, Lobão, & Rainer, 2014) and the fruits of *X. aethiopica* have positive effects on the metabolism of glucose and lipids by preclinical studies (Ezekwesili, Nwodo, Eneh, & Ogbunugafor, 2010; Nwozo, Orojobi, & Adaramoye, 2011). The observed activities were attributed to the presence of phenolic compounds and phytosterols in the extracts. In a previous study, we have shown that a crude extract of *X. aromatica* fruit improves the glucose intolerance induced by a high-carbohydrate diet in mice, which was attributed to the presence of organic acids and alkaloids (Oliveira, Ferreira, Oliveira, Teixeira, & Brandão, 2014).

In our continuous efforts to contribute to better knowledge and the use of Brazilian food species, in this study, we evaluated the chemical composition and *in vitro* inhibitory activity on dipeptidyl peptidase IV and pancreatic lipase of *O. cordata* leaves and X. *aromatica* fruits. The final objective of this work was to verify the potential use of these plants as functional foods.

2. Materials and methods

2.1. Plant material and chemicals

Fresh *O. cordata* leaves and dried *X. aromatica* fruits were obtained in Curvielo, Minas Gerais, Brazil, in March 2013. The botanical identification was performed by K. M. Gomes-Bezerra. Authorization for the collection and the studies was obtained from SISBIO (33104-1) and CNPq (010183/2013-2). Vouchers of plants are deposited in DATAPLAMT, Federal University of Minas Gerais (DAT-110/2013 and DAT-125/2015). After drying at 35 °C in an oven with circulating air until dryness, the plant materials were pulverized.

All reagents used were analytical grade. The standards caffeic acid (Sigma-Aldrich SLBB5479V), chlorogenic acid (Sigma-Aldrich SLBB6914V), gallic acid (Sigma-Aldrich 021M0035V), rutin (Sigma-Aldrich BCBH63231) and quercetin (Sigma-Aldrich SLBD8415V) were purchased from Sigma-Aldrich®, São Paulo, SP, Brazil. DPP-IV, diprotin-A (Sigma, St. Louis, MO), emodin (Sigma, St. Louis, MO), gly-pro-4-na (G0513, Sigma, St. Louis, MO), lipase activity assay kit II (Sigma-Aldrich, St-Louis, MO, EUA) and porcine pancreatic lipase were purchased (Sigma-Aldrich®, St-Louis, MO, USA). Organic solvents (ACS or HPLC grade) were also obtained from Sigma Aldrich.

2.2. Total phenols and flavonoids by spectrophotometry

We used the Folin-Ciocalteu (Folin & Ciocalteu, 1927) and aluminium chloride assay described by Amorim et al. (2008), respectively, with adaptations (Oliveira et al., 2014).

2.2.1. Quantification of total phenol content

Exactly 1 g of dried powdered *O. cordata* leaves and *X. aromatica* fruits was refluxed with 50 ml of 60 ml/100 ml methanol containing 0.1 ml/100 ml HCl for 30 min and placed in a water bath for 2 h at 85 °C. After cooling, the phenol solution was filtered and made to a final volume of 100 ml. An aliquot of 5 ml of the solution was transferred to a 25 ml volumetric flask and completed with distilled water. In a 25 ml volumetric flask, 2 ml of the phenol/water solution, 1 ml of Folin–Ciocalteu reagent and 10 ml of distilled water were combined and a 10.6 ml/10 ml solution of anhydrous sodium carbonate was added to the volume to 25 ml. After 30 min in the dark, the absorbance at 760 nm was measured in a glass cuvette. Aqueous solutions of gallic acid (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 μg/ml) were used to obtain a standard curve. The mean of three readings was used, and the results are expressed as mg of gallic acid equivalents (GAE) per 100 mg of dry weight.

2.2.2. Quantification of total flavonoids content

5 g sample of dried pulverized *O. cordata* leaves and *X. aromatica* fruits was refluxed with 50 ml of methanol for 45 min. After cooling, the solution was filtered, and methanol was added to a final volume of 100 ml. A 5 ml aliquot of this solution, 0.6 ml of glacial acetic acid and 2.5 ml of 8 ml/100 ml aluminium chloride in methanol were added to a 25 ml volumetric flask, and methanol was added to bring the total volume to 25 ml. After 30 min in the dark, the absorbance at 420 nm was measured in a glass cuvette. Methanol solutions of rutin (5, 10, 20, 30 and 40 μg/ml) were used to construct a standard curve. The mean of three readings was used, and the results were expressed as mg of the flavonoid rutin equivalents (RE) per 100 mg of dry weight.

2.3. Determining the lipid profile of X. aromatica

A 100 g sample of *X. aromatica* was extracted with hexane by percolation and hydrolysed and methylated as described elsewhere (Jham, Teles, & Campos, 1982). Briefly, 1 ml of HEXA was added to 2.0 ml of KOH/MeOH (0.5 mol/l). The solution was heated in a water bath for 5 min with addition of 1 ml of HCl (4:1 *v*/v), and the mixture was heated again for 15 min. The fatty acid fraction was obtained by extraction with ether. Fatty acid methyl esters were determined using a Shimadzu QP-5000 equipped with an ion detector and a HP-5MS column (30 m × 0.25 mm × 0.25 mm). The parameters used were injector temperature: 280 °C; detector temperature: 200 °C; column temperature: 180–210 °C at 5 °C/min; carrier gas: ultrapure helium; flow rate: 1 ml/min; injection volume: 1.0 μl in MeOH; run time: 25 min. The conversion of the proportions of the lipid profile was done according to Holland, Unwid, Buss, Paul, and Southgate (1991).

$$Cl = \%A \times \%L \times fc/100 \quad \text{Eq. 1}$$

where: Cl = concentration of fatty acid in g/100 g, %A.

2.4. Preparation of extracts and fractions

Dried and pulverized plant materials were extracted by percolation with 70% hydroalcoholic solution. The solution was evaporated in a vacuum until dryness to yield a crude extract (CEOC and CEXA, respectively). A portion of the CEOC and CEXA was diluted in water and extracted successively with hexane, ethyl acetate and butanol saturated with water. Ethyl acetate and butanol were evaporated to dryness at max 50 °C to obtain an ethyl acetate (EAF), butanol (BF) and aqueous (AF) fraction. The objective of this process was to obtain products enriched in bioactive compounds with different polarities.

2.4.1. Chromatographic analysis of the extracts and fractions

The plant products were prepared at 0.25 mg/ml in methanol, while chemical standards of gallic, chlorogenic and caffeic acids, rutin and quercetin were prepared at 0.07 mg/ml. For the quantification, calibration solutions were prepared at the following concentrations (mg/ml): gallic acid (0.02 to 0.4), chlorogenic acid (0.024 to 0.48), caffeic acid (0.012 to 0.24), rutin (0.014 to 0.27) and quercetin (0.001 to 0.02). The concentration of each substance was measured by the area of their respective peaks.

Aqueous solutions of gallic acid were used to obtain a standard curve de (0.5, 1.0, 1.5, 2.0, 2.5 e 3.0 μg/ml). The mean of three readings was used, and results are expressed as mg of gallic acid equivalent (GAE) per 100 mg dry weight. Absorbance response (y) of gallic acid, ($y = 133.2 \times -0.0365$, $r^2 = 0.998$) were with linear concentrations. Methanol solutions of rutin (Sigma-Aldrich®, São Paulo, SP, Brazil) were used to obtain a standard curve (0.5, 1.0, 1.5, 2.0, 2.5 e 3.0 μg/ml). The mean of three readings was used, and the results are expressed as mg of rutin equivalent (RE) per 100 mg of dry weight. Absorbance

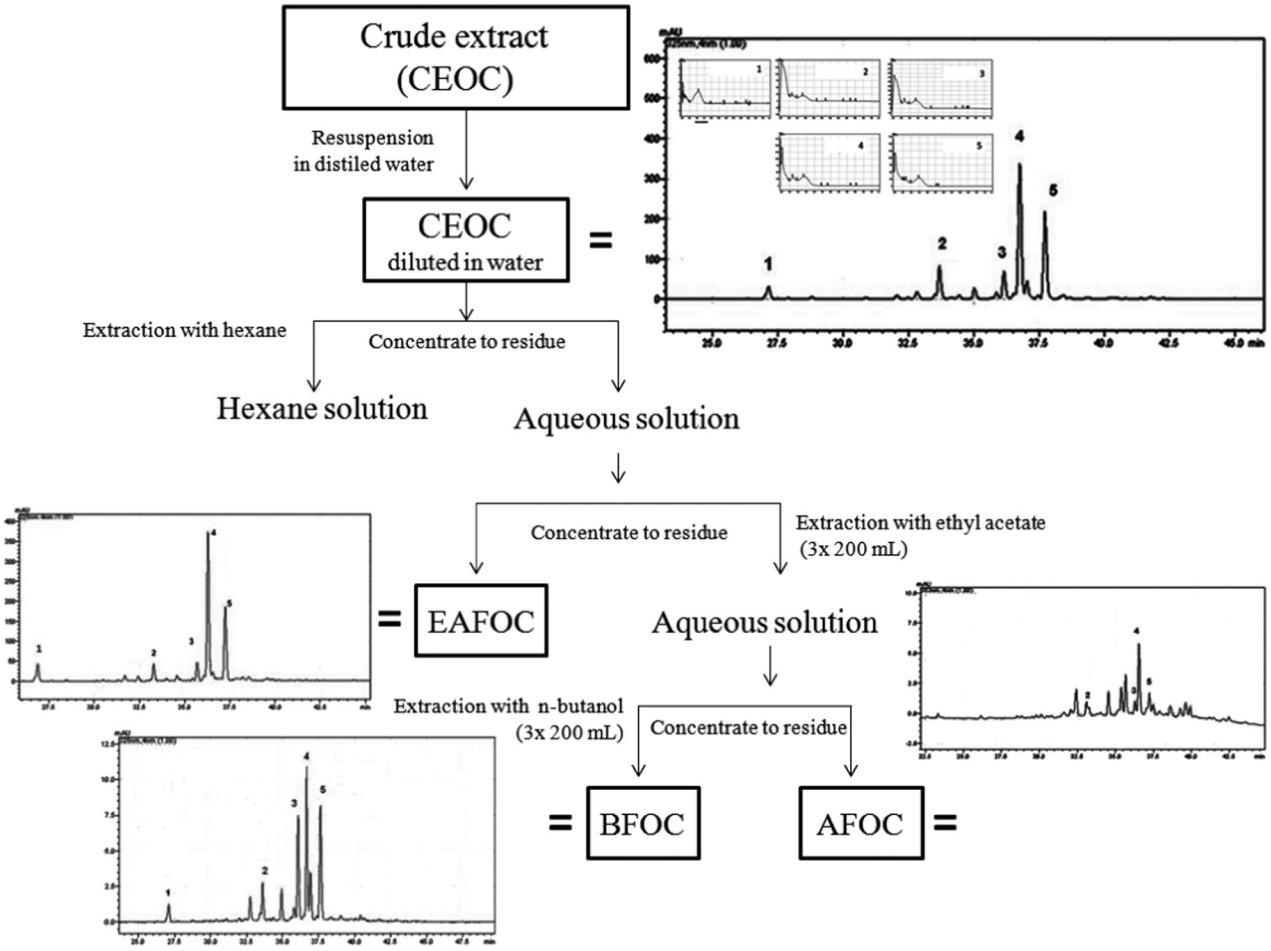

Fig. 1. Route of obtention of crude extract (CEOC) and fractions (EAFOC, BFOC and AFOC) from *O. cordata* leaves, and corresponding chromatograms in HPLC/DAD. Retention times and identification of the substances by HPLC/MS in CEOC: (1) R_T 24.5 min, p-Coumaroyl quinic acid, ($[M-H]^-$ 337.0730), error 70.3 ppm; (2) R_T 31.9 min, orientin/isoorientin, ($[M-H]^-$ 447.0681), error 55 ppm; (3) R_T 34.8 min, vitexin ramnosídeo, ($[M-H]^-$ 577.1248), error 54,6 ppm; (4) R_T = 35.4 min vitexin/isovitexin ($[M-H]^-$ 431.0738), error 57 ppm and (5) R_T = 36.7 min, orientin metil éter, ($[M-H]^-$ 461.0832).

response (y) of rutin (y = 33.75 × − 0.0128, r^2 = 0.999), were with linear concentrations.

Analyses were performed in a Shimadzu HPLC with a DAD/UV detector. The column used was a VP-ODS (250 mm × 4.6 mm, 5 μm) at 30 °C. The mobile phases were 0.3 ml/ml formic acid (A) and acetonitrile, water and formic acid (1:1:0.3) (B). The elution gradients were as follows: 98:2 (A:B) for 0–5 min, 98:2 to 90:10 for 5–10 min, 90:10 to 20:80 for 10–50 min, and 20:80 to 98:2 for 50–70 min, with a flow rate of 1 ml/min. The injection volume was 5 μl, and the detection was performed at 325 nm. To confirm the presences of organic acids and flavonoids, the extracts were analysed in a MAXIS 3G - Bruker Daltoni liquid chromatograph. The ionization was performed in the negative mode using an electrospray source (ESI (−) − MS), with an ion trap accumulator (IT) and a Time of Flight (TOF) mass analyser. The temperature of the heating block was 200 °C, with a capillary voltage of 4.5 kV. The mass/charge peaks (*m/z*) of interest were fragmented at collision energy of 20 eV. The isotopic masses and the peak fragments obtained were compared with the phenolic substances described in the literature for other species with botanical similarity to the extract studied. The mass fragments of the predominant peaks were compared with those present in the database massbank.jp/.

2.5. DPP-IV in vitro assay

The assay is based on spectrophotometric determination of the cleavage of Gly-Pro-4-NA a synthetic chromogenic substrate of DPP-IV (Al-masri, Mohammad, & Tahaa, 2009). Samples of 10 mg of CEOC, CEXA, EAFOC, EAFXA, BFOC, BFXA and AFOC were dissolved in Tris-HCL buffer (50 mM, pH 7.5) in microcentrifuge tubes. The tubes were centrifuged at 5000*g* for 5 min, and the supernatant was collected to make a stock solution with a concentration of 10 mg/ml. The following concentrations (10, 5, 2.5, 1.0, 0.5, and 0.25 mg/ml) were prepared from the stock and tested. *Assay*: After adding 50 μl of Tris-HCL buffer, 20 μl of rat plasma and 50 μl of sample, standard or buffer, the mixture was pre-incubated for 10 min at 37 °C. This was followed by addition of 50 μl of substrate. The final incubation was at 37 °C for 30 min. The experiments were done in triplicate. The results were compared with a negative control that contained no inhibitor. Diprotin A was used as a positive control. The inhibition percentage was calculated using the following formula: % inhibition = (Absorbance of control − Absorbance of inhibitor) × 100 absorbance of control. The IC_{50} was also calculated.

2.6. Pancreatic lipase in vitro assay

A lipase inhibition assay was performed according to the method of Choi, Hwang, and Kim (2003) with some adaptations. The reaction mixture contained 10 ml/100 ml DTNB Porcine lipase was prepared as a stock at 1 mg/ml in potassium phosphate buffer (0.1 mM, pH 7.0, 0.1 ml/100 ml Tween 80) and stored at − 80 °C. Samples of 2 mg of CEOC, CEXA, EAFOC, EAFXA, BFOC, BFXA and AFOC were dissolved in potassium phosphate buffer (0.1 mM, pH 7.0, 0.1 ml/100 ml Tween 80)

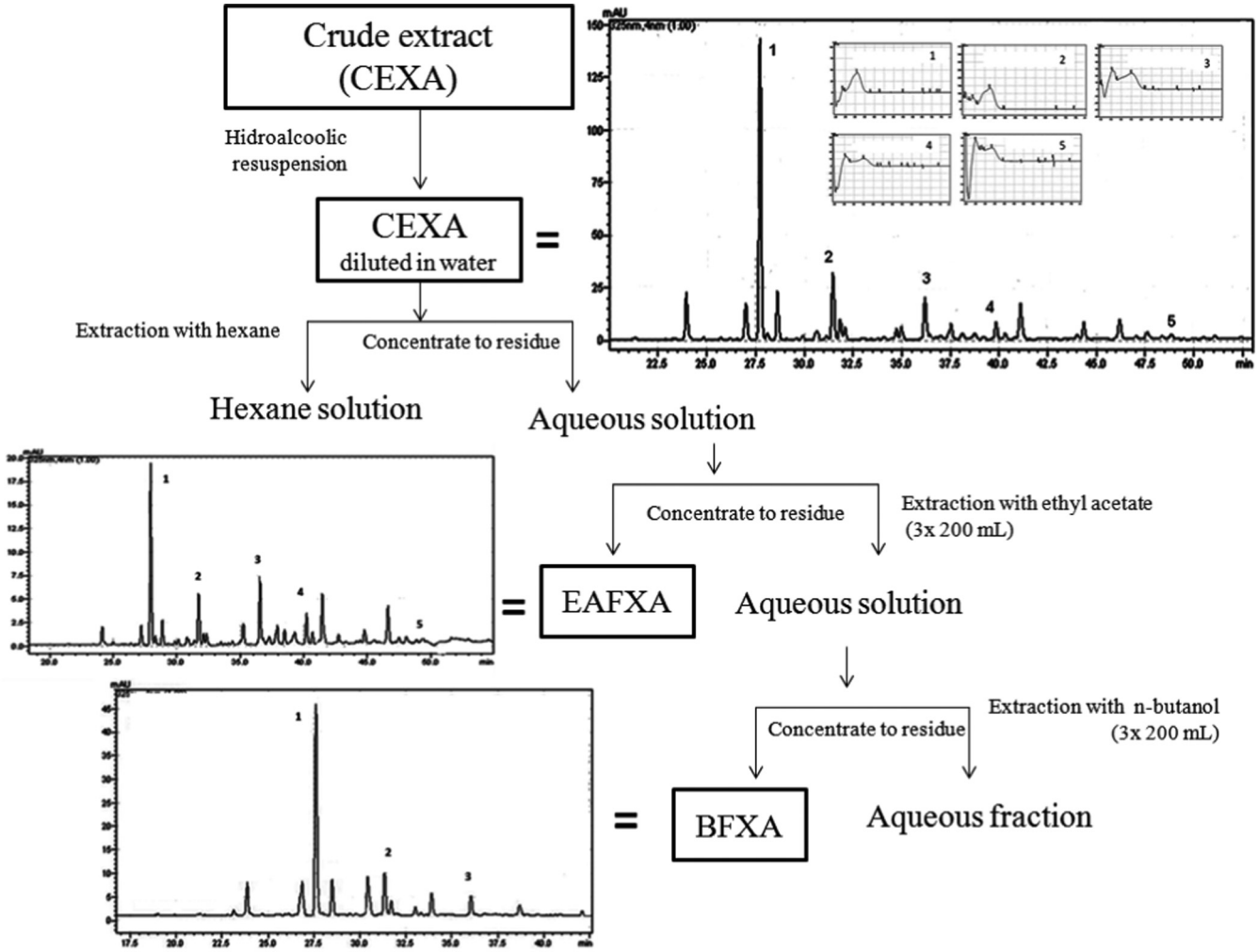

Fig. 2. Route of obtention of crude extract (CEXA) and fractions (EAFXA and BFXA) from *X. aromatica* fruits, and corresponding chromatograms in HPLC/DAD. Retention times and identification of the substances by HPLC/MS in CEXA: (1) R_T 27.6 min, chlorogenic acid, ($[M-H]^-$ 353.0639) error 67.7 ppm; (2) R_T 31.9 min, not identified, ($[M-H]^-$ 337.0695), (3) R_T 36.3 min, rutin, ($[M-H]^-$ 609.1074) error 63.5 ppm; (4) R_T = 39.8 min quercetin-3-arabinoside ($[M-H]^-$ 433.0493), error 61.6 ppm and (5) R_T = 48.9 min, quercetin and luteolin, ($[M-H]^-$ 301.0145, error 69.4 ppm and 285.0192, error 74.7 ppm).

Table 1
Polyphenols of *O. cordata and X. aromatica* on dry matter.

	Total polyphenols gGAE/100 g	Total flavonoids gRUT/100 g
O. cordata	6,2 ± 1,0[a]	3,2 ± 0,5[a]
X. aromatica	2,09 ± 1,4[b]	0,12 ± 0,05[b]

* Data are expressed in dry matter as means ± standard deviation (n = 3). GAE = gallic acid equivalents, RUT = rutin. Different letter in the same row are significantly different (P < 0,05).

or DMSO in microcentrifuge tubes. The tubes were centrifuged at 5000*g* for 5 min, and the supernatant was collected to make a stock solution with a concentration 2 mg/ml. The following working concentrations (1.0, 0.75, and 0.5 mg/ml) were prepared from the stock and tested. *Assay*: After adding 40 µl of Tris-HCL buffer and 100 µl of sample, standard or buffer, the mixture was pre-incubated for 60 min at 37 °C. This was followed by addition of 20 µl of substrate. The final incubation was at 37 °C for 30 min. The experiments were done in triplicate. The results were compared with the negative control that had no inhibitor. The inhibition percentage was calculated using the following formula: % inhibition = (Absorbance of control − Absorbance of inhibitor) × 100 absorbance of control. The IC_{50} was also calculated.

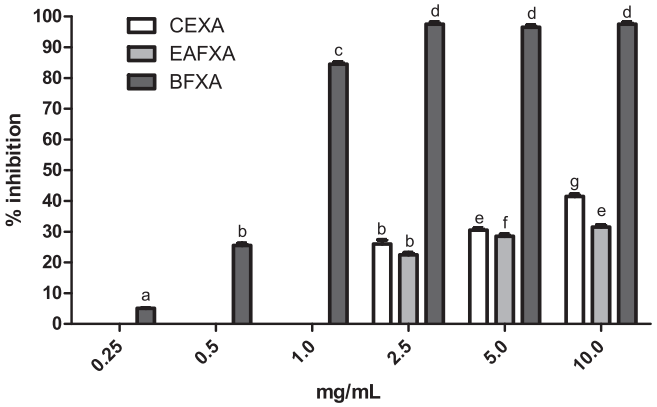

Fig. 3. Inhibitory activity on the LP enzyme of *O. cordata* EBOC, FAEOC, FBOC and FAOC, in concentrations 0.5, 0.75 and 1.0 mg/ml (< 0.05).

2.7. Statistical analysis

All values are expressed as the mean ± standard deviation. The statistical data were evaluated using GraphPad Prism5 software. The data were statistically analysed by a one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were considered

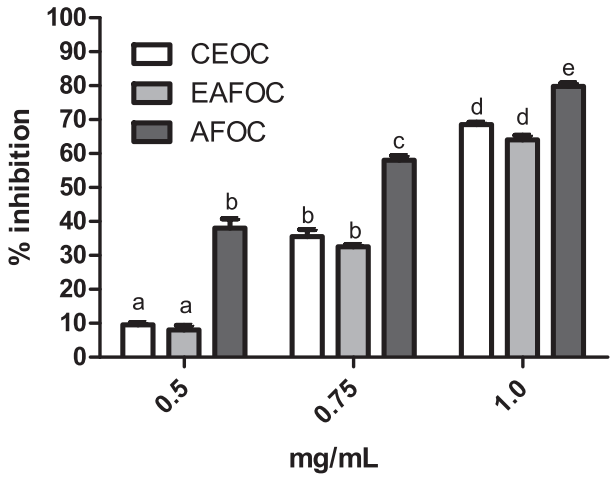

Fig. 4. Inhibitory activity on the DPP-IV enzyme of *X. aromatica* EBXA, FAEXA e FBXA, in different concentrations (< 0.05).

statistically significant at $P < 0.05$.

3. Results and discussion

The increasing prevalence of metabolic disorders such as obesity and type 2 diabetes mellitus highlights the need to identify new and effective natural therapies that do not cause undesirable side effects as commonly used synthetic drugs do (Lazarini et al., 2016; Liu et al., 2016). In the present study, we aimed to characterize two Brazilian native plant species that are traditionally used in specific regions of the country and demonstrate their potential use as functional foods.

Figs. 1 and 2 respectively show the experimental procedures performed to obtain the *O. cordata* and *X. aromatica* products for study, as well as the results of the chromatographic analysis for each. Organic acids and flavonoids were present in both plants as indicated by the UV spectra in DAD. However, these substances were present in different proportions. Fig. 1 shows that a crude extract of *O. cordata* (CEOC) has more peaks correlated with flavonoids, and the peaks with the highest concentrations were identified by their mass spectra as orientin and vitexin derivatives (Fig. 1, numbers 4 and 5). A weak peak was identified as coumaroylquinic acid (1). On the other side, the *X. aromatica* fruits showed many peaks for organic acids, and chlorogenic acid was the most abundant (Fig. 2, number 1). Only a few flavonoid peaks (*i.e.*, rutin, quercetin and luteolin) were observed.

The presence of flavonoid and phenolic acids in the plants are consistent with the results obtained by quantitative analysis (Table 1). The concentration of flavonoids in the *O. cordata* leaves was 3.2 g/100 g, while for total phenolics was 6.2 g/100 g (Table 1). This high concentration of flavonoids can explain the historic traditional use of this species as an antiscorbutic (Saint-Hilaire, 2014). In *X. aromatica* fruits, only 0.12 g/100 g was observed in a total of polyphenols. This low concentration of flavonoids is *X. aromatica* fruits was already detected by Oliveira et al. (2014) (0.07 ± 0.01 mg RE/100 mg) while the concentration of phenolic acids was much higher (2.01 ± 0.06 mg GAE/100 mg). It is interesting to note that the value for polyphenols in *X. aromatica* is higher than for black pepper (1.7 g/100 g) (Liu, Qiu, Ding, & Yao, 2008) and the total flavonoids content is similar to known spices, such as oregano (0.16 g/100 g), basil (0.13 g/100 g) and cumin (0.10 g/100 g) (Kim, Yang, Lee, & Kang, 2011). The concentration of phenolic substances in the both species is also similar to other plant extracts that showed positive results in *in vivo* metabolic dysfunction models (Kim et al., 2011; Williams et al., 2013). Total phenolics in green and black tea normally ranged from 3.2 g/100 g to 14.7 g/100 g (Prior & Cao, 1999). Polyphenols, especially flavonoids (Talhouk, Karam, Fostok, El-Jouni, & Barbour, 2007) have anti-inflammatory and antioxidant activities and have been considered as potential anti-diabetic agents. They have hypoglycaemic and anti-hyperglycaemic action, mimicking and stimulating insulin secretion, with an action similar to drugs such as metformin, which increases insulin sensitivity but not its release (Chuang et al., 2010; Wu, Wu, Huang, Jao, & Yen, 2009).

A strategy used in the discovery of natural anti-obesity agents is to search for potent lipase inhibitors from plant extracts. Pancreatic lipase is a key enzyme for the absorption of dietary triglycerides, and it

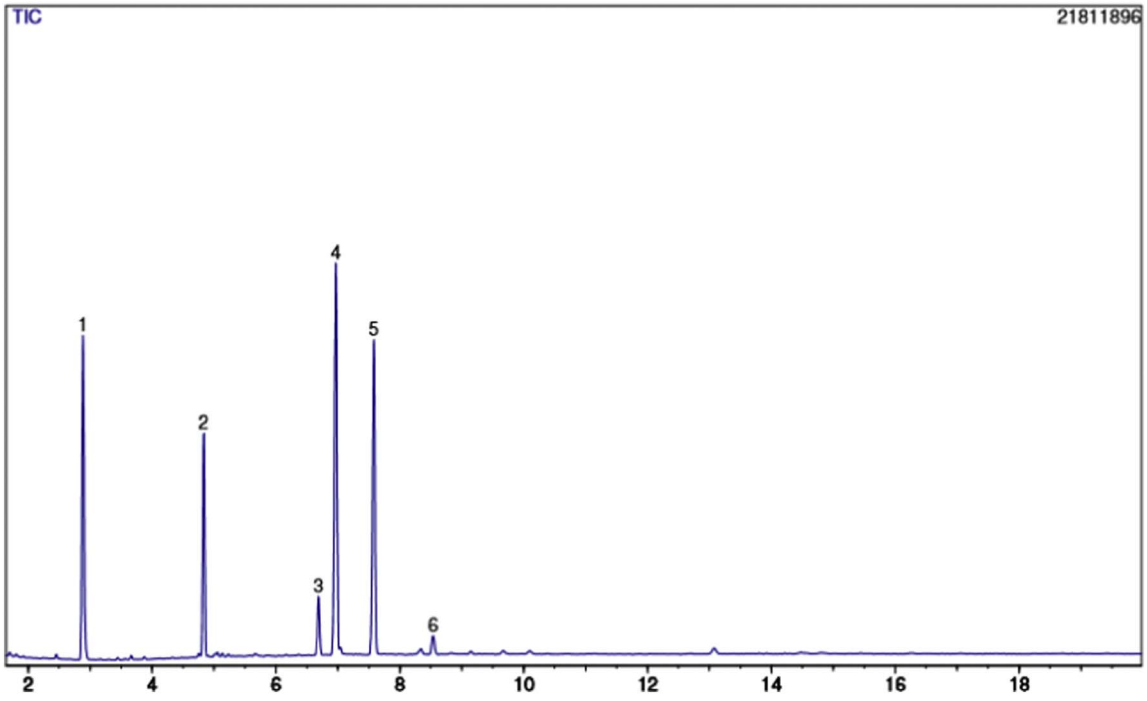

Fig. 5. Lipid profile of *X. aromatica*. Peaks: (1) T_R 2.88 min (22.06 g/100 g) BHT; (2) 4.83 (13.79 g/100 g) palmitic acid; (3) T_R 6.69 (4.29 g/100 g) stearic acid; (4) T_R 6.97 (31.40 g/100 g) oleic acid; (5) T_R 7.58 (27.40 g/100 g) linoleic acid (isomer 1); (6) T_R 8.54 (1.43 g/100 g) linoleic acid (isomer 2).

converts triglycerides to 2-monoglycerol and two free fatty acids. In this study, the inhibitory activity on pancreatic lipase (PL) by extracts and fractions of the plants was determined with emodin as a positive control (Zheng, Duan, Gao, & Ruan, 2010). A liner dose-response for emodin was observed between the concentrations tested ($y = 36.25 \times + 39.194$ $_R^2 = 0.99490.5$ mg/ml of emodin inhibit 57.3% ± 1.5 of porcine pancreatic lipase).

Fig. 3 shows the percentage of pancreatic lipase inhibition by CEOC, EAFOC and AFOC from 0.5, 0.75 and 1.0 mg/ml. BFOC was not tested. The three tested concentrations of CEOC and EAFOC had similar inhibitory activities, which were lower than those observed for AFOC. The IC50 of CEOC, EAFOC and AFOC were 0.84 ± 0.01 mg/ml, 0.88 ± 0.01 mg/ml and 0.63 ± 0.03 mg/ml, respectively. However, the inhibition observed for all three extracts were higher than that of emodine that was 0.49 mg/ml. These results suggest that these products, mainly AFOC, may have the potential to reduce fat absorption and be beneficial in obesity treatment.

Various plant species rich in polyphenols such as *O. corniculata*, *Litchi chinensis*, *Vitis rotundifolia*, *Rosmarinus officinalis* and *Camellia sinensis*, have been shown to inhibit pancreatic lipase (You, Chen, Wang, Jiang, & Lin, 2012; Wu et al., 2013). *Camellia sinensis* induces the highest PL inhibition (IC50 = 0,091 mg/ml), which has been related to the types of polyphenols present in this plant. Certain flavonoids and phenolic acids, such as luteolin, quercetin, orientin, isorientin, gallic acid, caffeic acid and chlorogenic acid, have been shown to inhibit PL (Buchholz & Melzig, 2015). We can speculate that the PL inhibitory property of extracts and fractions of *O. cordata* is a result of the presence of orientin and luteolin glycosides, which were the flavonoids found in the highest concentrations in the fractions. An explanation for the greater inhibitory activity of AFOC could be the presence of a high concentration of condensed tannins in this fraction, as these substances have already been shown to inhibit PL (Eidenberger, Manuel, Fuerst, & Krennhuber, 2014).

Recent therapies developed for the treatment of Type 2 diabetes are dipeptidyl peptidase (DPP-IV) inhibitors, such as vildagliptin, sitagliptin, and saxagliptin, which are used as antihyperglycemic agents (Tomkin, 2014). Administration of DPP-IV inhibitors blocks dipeptidyl peptidase and thereby lengthens the half-life and increases the biological activity of glucagon-like peptide-1 (GLP-I). GLP-I improves β-cell growth, insulin secretion and glycogenesis in muscles and liver (Fehmann & Habener, 1992). Bioactive substances such as chlorogenic acid, resveratrol and berberine have been shown to improve GLP-1 levels in *in vivo* and *in vitro* models, as well as in patients (Dao et al., 2011; Unnikrishnan, Suthindhiran, & Jayasri, 2014).

We evaluated the *X. aromatica* extracts CEXA, EAFXA and BFXA for the inhibition of DPP-IV with diprotin A as a positive control (Fig. 4). BFXA had the highest inhibitory activity (IC50 of 0.71 mg/ml). Previous studies have identified an isoquinoline alkaloid in the fruit of *Xylopia* species (Silva, Tavares, Queiroga, Agra, & Filho-Barbosa, 2009), which could be responsible for the observed inhibitory activity. Indeed, berberine, an isoquinoline alkaloid, has been shown to inhibit DPP-IV (Al-masri et al., 2009; Unnikrishnan et al., 2014). EAFXA is rich in compounds of intermediate polarity, including flavonoids. Bower, Real Hernandez, Berhow, and de Mejia (2014) showed that dried herbs used as condiments, including rosemary, Mexican oregano, and marjoram, contain several flavonoids that inhibit DPP-IV, such as cirsimaritin, hispidulin and naringenin. An ethanolic extract of *Psidium guajava* leaves also induced the inhibition of DPP-IV activity, which was related to flavonol glycosides including hyperoside and isoquercitrin (Eidenberger, Selg, & Krennhuber, 2013). Chlorogenic acid is a very abundant phenolic compound in coffee and was shown to increase GLP-1 production and secretion by L-cells and to improve glycaemia in mice (Johnston et al., 2003). A previous report published by our group showed that the major compound present in CEXA was chlorogenic acid (Oliveira et al., 2014). The inhibition of DPP-IV by CEXA and EAFXA could be associated with this substance.

The *X. aromatica* fruit also showed a high relative percentage of monounsaturated fatty acids (40.2 g/100 g), followed by polyunsaturated (36.93 g/100 g) and saturated fatty acids (23.1 g/100 g) (Fig. 5). The most abundant fatty acids were oleic (40.2 g/100 g) and linoleic acid (35.1 g/100 g). Linoleic acid is the major fatty acid found in spices commonly present in the diet, including black cumin, fenugreek, black pepper and clove oil (Al-Jasass & Al-Jasser, 2012), and oleic acid is thought to protect against inflammatory disease. Previous studies have found an inverse relationship between dietary oleic acid, inflammatory parameters and insulin resistance (Sala-Vila et al., 2011).

It is important to note that the results of this study are from a single lot of sample. Variations in the nutrient and phytochemical levels and the antioxidant activities due to environmental factors and genetic diversity were not considered. Toxicity is a concern associated with the introduction and promotion of the consumption of lesser known foods. The consumption of Brazilian pepper has been reported in traditional medicine, in a previous study, we show that crude extract of *X. aromatica*, at the maximum dose 250 mg/kg of BW, showed no liver alteration in BLAB/c mice (Oliveira et al., 2014). No studies on toxicology were performed with the leaves of *O. cordata*. *O. corniculata* is a very used plant in India, with important biological activity confirmed by many studies, including antioxidant, antidiabetic and anticancer. It must be also considered that both species have previous registration of consumption as food and tea on historical literature (Oliveira et al., 2012).

4. Conclusion

This study revealed that the *O. cordata* leaves and *X. aromatica* fruit are rich in phenolic substances that could be useful in the management of metabolic disorders.

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