

Full Length Research Paper

Stem bark extracts of *Endopleura uchi* (Huber) Cuatrec: Inhibition of pancreatic lipase and antioxidant activity

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Pancreatic lipase is considered an important target for the control of hyperlipidemia. Several plants, especially those rich in phenolic metabolites, have been shown to have anti-hyperlipidemic activity and are considered a good alternative for obesity prevention. Extracts of the stem bark of *Endopleura uchi* (Huber) Cuatrec (Humiriaceae) were evaluated for the inhibitory activity on the pancreatic lipase enzyme, as well as their antioxidant potential were verified in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. In addition, the total phenolics (TPC) and flavonoids contents (TFC) were estimated. In general way, the acetonetic and ethanolic extracts showed better results than aqueous extract. At the concentration of 1 mg/mL, both acetonetic and ethanolic extracts inhibited the activity of pancreatic lipase by 49.33 and 36.88%, representing 135.26 and 102.75 of inhibited lipase activity per gram of extract (ILA/g). On the other hand, the aqueous extract inhibited lipase by 47.54% at the concentration of 2 mg/mL, which means 213.84 ILA/g. The highest antioxidant activity was observed for the acetonetic extract with a 50% effective concentration (EC₅₀) of 7.9 µg/mL, followed by the ethanolic extract with EC₅₀ of 9.7 µg/mL and the aqueous extract with EC₅₀ of 12.4 µg/mL. TPC in gallic acid equivalent per gram of sample (GAE/g) were 0.52, 0.51 and 0.35 respectively for the ethanolic, acetonetic and aqueous extracts. In turn, TFC in quercetin equivalent per gram of sample (QE/g) were 2.13, 1.89 and 1.35 for the acetonetic, ethanolic and aqueous extracts, respectively. Positive and strong correlations (r Pearson > 9.0) between TPC and antioxidant activity were found for all 3 extracts. These results suggest that both pancreatic lipase inhibition and antioxidant activity were distinguished by organic solvents and water extraction. Furthermore, organic extracts (acetone and ethanol) showed to be richer in phenolic metabolites. These metabolites may be related to the biological activities that were found, indicating the stem bark extracts of *E. uchi* as possible candidates for the development of strategies in the prevention of obesity and hyperlipidemia.

Key words: Antioxidant activity, pancreatic lipase activity, *Endopleura uchi* extracts.

INTRODUCTION

A possible imbalance between consumption of high calorie diet and lipids, body weight and lipoproteins in the body (Sharma et al., 2005; Nguyen et al., 2012) can influence the susceptibility of the organism to disorders

and changes in lipid metabolism, increasing reserves fat (Montoya et al., 2002; Coelho et al., 2011), with subsequent accumulation of adipose tissue and abdominal fat, causing weight gain (Daniels, 2006;

Barbieri, 2012).

These disorders can be accompanied by changes in the lipid profile - increased serum triglycerides, total cholesterol, high density lipoproteins (LDL) cholesterol and free fatty acids and decreased high density lipoproteins (HDL) (Bertolami et al., 2004; Wilborn et al., 2005), causing rising prevalence of dyslipidemias (Pozzan et al., 2004; Grillo et al., 2005), pro-inflammatory conditions, oxidative stress - lipid peroxidation of low density lipoproteins (LDL) (Bevilacqua et al., 2007; Singh et al., 2011) and atherosclerosis (Sorace et al., 2006; Peluso et al., 2012).

Inhibition of the pancreatic lipase, an enzyme essential for the metabolism of fats (Yun, 2010; Sukhdev and Singh, 2013) and formation of the lipoproteins: LDL, intermediate-density lipoprotein (IDL), LDL and very-low-density lipoprotein (VLDL) in the body (Sharma et al., 2005; Klop et al., 2013), has become a valuable therapeutic alternative for the treatment of dyslipidemia resulting from excess weight and obesity induced by a hyperlipidemic diet (Zhang et al., 2008; Lee et al., 2010; Lewis et al., 2012).

Plants in northern and northeastern Brazil have been used for decades as folk remedies for treatment of various diseases (Menezes and Homma, 2012). *E. uchi* (Humiriaceae) is a species native to the Brazilian Amazon and is distributed mainly in the Pará estuary (Marx et al., 2002). It is popularly known as 'uxi amarelo' (Gaia and Shanley, 2004).

The tea of the *E. uchi* bark has been traditionally used to treat diabetes, arthritis, rheumatism and other diseases, as well as to control serum cholesterol levels and eliminate or reduce fibroids (Politi, 2009; Muniz, 2013).

The anti-inflammatory (Nunomura et al., 2009; Borges, 2010), antimicrobial (Politi et al., 2010) and anti-steatotic (Castelo et al., 2013) activities of the species are well documented and have been mainly attributed to the action of phenolic compounds and derivatives, as derived gallic acid, found in various parts of the plant, but especially in the stem bark (Bilieri et al., 2011; Muniz, 2013).

Based on the traditional uses in the treatment of dyslipidemias and diseases associated with oxidative damages, this study aimed to quantify the total phenolic and flavonoid contents and to evaluate the antioxidant and anti-lipase activities of extracts obtained from *E. uchi* barks using *in vitro* assays.

MATERIALS AND METHODS

Plant material

The finely crushed dried bark of *E. uchi* (Huber) Cuatrec

(Humiriaceae) was obtained from Cha e Cia Ervas Mediciniais Ltda., São Paulo, SP, Brazil (IBAMA Registration 187956; Product no.001010125361/98; Lot 003; harvest 90/2012, validity 09/2017).

Aqueous extract (AE)

Dried and ground plant material (200 g) was extracted by static maceration in 1 L of purified water, supplemented by decoction for 1 h, every day for 2 weeks. At the end of each week the extractive mixture was filtered through filter paper. The filtrates had their volumes reduced by evaporation under reduced pressure and then lyophilized. The yielding dried extract was weighed and packed in a hermetically sealed amber bottle, identified and stored in a freezer at -20°C.

Extracts in ethanol (EE) and acetone (AcE)

For organic extracts, 80% ethanol and 80% acetone, dried and ground plant material (200 g) was extracted by static maceration in 1 L of solvent for two weeks. At the end of each week, the extractive mixtures were filtered through filter paper. The filtrates had their volumes reduced by evaporation under reduced pressure and then lyophilized. Each of the dried extracts was weighed and packed in a hermetically sealed amber bottle, identified and stored in a freezer at -20°C.

Total flavonoids content (TFC)

The total flavonoid contents were determined by measuring the absorbance of the complex formed with aluminum chloride, in medium containing glacial acetic acid and pyridine, at 415 nm (Petry et al., 2001; Petacci et al., 2012). Quercetin was used as standard and the calibration curve was constructed with solutions ranging from 1, 2, 4, 6, 8 to 10 µg/mL. The results were expressed as mg equivalent of quercetin per gram of sample (QE/g).

Total phenolic content (TPC)

The Folin-Ciocalteu reaction was used to determine the concentration of phenolic compounds in the extracts. The samples in concentrations ranging from 2.5 to 20 µg/mL were prepared in methanol and then reacted with RFC in an alkaline medium. Then, the absorbance was read at $\lambda = 760$ nm. Gallic acid was used as a standard and the calibration curve was constructed with solutions ranging 0.5, 1.25, 2.5, 3.75, 5 and 6.75 µg/mL. The results were expressed as gram equivalent of gallic acid per gram of sample (GAE/g) (Trevisan et al., 2009; Rocha et al., 2011; Petacci et al., 2012).

Reduction assay of radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The reaction with DPPH was used to estimate the samples antioxidant potential. For this purpose, 1 mL of 0.3 mM DPPH ethanol solution was added to 2.5 mL of samples diluted in ethanol, with final concentration ranging from 2.5 to 20 µg/mL. After 30 min

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of reaction in the dark, the absorbances were read at $\lambda = 516$ nm (Sharma and Bhat, 2009; Martins et al., 2015). For comparison, a standard extract of *Ginkgo biloba* (Fagron) was tested under the same conditions as the samples. The percentage of antioxidant activity for each concentration tested was calculated according to

$$\% \text{ Antioxidant Activity} = \frac{100 - [\text{sample absorption} - \text{solvent absorption}] \times 100}{\text{blank absorption}}$$

The antioxidant activity results of the extracts were expressed as 50% effective concentration (EC_{50}).

Inhibition of pancreatic lipase activity

To measure the inhibition of the enzymatic activity of pancreatic lipase, 50 μ L of each EE, AcE and AE extract solution, 100 μ L of the pancreatic lipase enzyme solubilized in Tris-HCl 0.05 mol/L pH 8.0 buffer and 50 μ L of 8 mmol/L *p*-nitrophenyl palmitate substrate containing 0.5% Triton-X100, were incubated for four time periods (10, 20, 30 and 40 min).

After the incubation periods, the reaction was stopped with 1.0 mL of Tris-HCl buffer at 0.05 mol/L, and the reaction product was analyzed in a spectrophotometer at $\lambda = 410$ nm (Pereira et al., 2011; Souza et al., 2011; Marques et al., 2012; Simão et al., 2012; Souza et al., 2012). Orlistat (Sigma Aldrich) was used as a positive standard in the same assay conditions. For each extract, controls were tested without enzyme (substrate control) without substrate (enzyme control).

The results obtained were expressed as percentage inhibition (%) and lipase activity inhibited per gram of plant (LA/g), corresponding to 1 μ mol of *p*-nitrophenol not produced per minute due to the presence of the inhibitor under the test conditions.

The inhibition percentages of each extract were obtained from the slope of the graph (absorbance \times time) and calculated from the difference between the slopes in the absence and presence of the inhibitor. The absorbance values obtained from the slope differences were converted into micromol of product by a standard *p*-nitrophenol curve.

Statistical analysis

The results were expressed as the average of three replicates \pm standard deviation. Analysis of variance followed by the Tukey test was used to compare means. The correlation between the antioxidant activity of the extracts and the content of phenolic metabolites was evaluated by Pearson coefficient. The statistical tests were performed using GraphPad Prism, version 5 (2007) with a confidence interval of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

According to Table 1, the EE and AcE extracts showed the highest yields, 17.12 and 13.32%, respectively, compared to AE with 11.73%. Hydro-organic mixtures induce better swelling (Koffi et al., 2010) and increases porosity (Hismath et al., 2011) of the crushed material, influencing the extractability (Cheng et al., 2013) and dissolution of phenolic compounds (Crişan et al., 2013), as well as sugar, colorants and reserve and unsaponifiable substances (Andreo and George, 2006),

the equation:

resulting in higher extractive contents. On the other hand, water promotes better extraction of sugars, polysaccharides (Jensen et al., 2007), few inorganic salts and some polyphenols (Andrade et al., 2015).

Usually, aqueous solutions containing organic solvent ranging from 50 to 80%, tend to modulate the polarity of the solvent mixture (Do et al., 2014) and the variety of polyphenolic constituents, unlike a pure aqueous solvent (Mazandarani et al., 2012). This may explain the higher values for both total flavonoids and total phenolics ($p < 0.05$) found for EE (1.89 mg QE/g and 0.52 g GAE/g) and AcE (2.13 mg QE/g and 0.51 g GAE/g) compared to AE (1.35 mg QE/g and 0.35 g GAE/g) (Table 1).

Other factors such as dielectric constant, chemical and physicochemical properties of the extraction process, even as the stereochemistry of polyphenolic compounds influence their solubility in solvents with intermediate polarity (Jouki and Khazaei, 2010; Wissam et al., 2012).

In such cases, the extraction is facilitated by the presence of hydroxyl, carboxyl (Anokwuru et al., 2011) and polar and non-polar fragments of polyphenol molecules (Diciaula et al., 2014), which allow hydrogen bonding between them and the electronegative oxygen atoms of ethanol and acetone (Galanakis et al., 2013).

The lower TFC and TPC in the AE could also be related with the extraction temperature that AE decoction underwent (above 80 °C for 60 minutes), and the fact that heating at around 40 to 70°C safely removes a larger amount of substances from the sample (Dent et al., 2013), by increasing its solubility (Tan et al., 2013): using temperatures above 80°C for extended time periods causes the polyphenol content to decrease (Chew et al., 2011). This is caused by the reduction of the number of hydrogen bonds between phenolic compounds and water molecules (Galanakis et al., 2013), as well as the appearance of artifacts (Yalavarthi, 2016), influencing the separation and recovery of polyphenols (Garcia-Marquez et al., 2012) and the degradation of the biologically active fraction (Druzynska et al., 2007; Chan et al., 2009).

The highest antioxidant activity was observed in AcE, with EC_{50} of 7.9 μ g/mL, followed by EE, with EC_{50} of 9.7 μ g/mL and AE with EC_{50} of 12.4 μ g/mL ($p < 0.05$) (Table 2).

Acetone and ethanol extracts usually have higher antioxidant activities due to the possible formation of complexes of some phenolic compounds (Rockenbach et al., 2008; Wong et al., 2014) with a high number of antioxidant groups present (Tatiya et al., 2011). The

Table 1. Yield (mass in grams and % g/g) in relation to dry plant material, content of flavonoids and total phenols* of bark extracts of *Endopleura uchi* (Huber) Cuatrec.

| Sample | Yield | | Total flavonoids (mg) QE/g sample | Total phenolic compounds (g) GAE/g sample |
|--------|-------|-------|--------------------------------------|--|
| | g | % | | |
| AE | 23.46 | 11.73 | 1.35 ± 0.067 ^b | 0.35 ± 0.031 ^b |
| EE | 34.24 | 17.12 | 1.89 ± 0.071 ^a | 0.52 ± 0.009 ^a |
| AcE | 26.65 | 13.32 | 2.13 ± 0.046 ^a | 0.51 ± 0.052 ^a |

QE, Quercetin equivalent; GAE, gallic acid equivalent; AE, aqueous extract; EE, 80% ethanol extract; AcE, 80% acetone extract.

* Values expressed as three mean ± standard deviation; different letters in the same column differ statistically (Tukey, $p < 0.05$).

Table 2. 50% effective concentration (EC_{50}) antioxidant activity in the DPPH test * and correlation between concentration of phenolic derivatives and percentage of antioxidant activity of bark extracts of *E. uchi* (Huber) Cuatrec.

| Sample | EC_{50} ($\mu\text{g/mL}$) | Gallic acid concentration × %AOA <i>r</i> Pearson ($p > 0.05$) |
|--|--------------------------------|---|
| AE | 12.4 ± 0.083 ^b | 0.9992 |
| EE | 9.7 ± 0.381 ^c | 0.9904 |
| AcE | 7.9 ± 0.315 ^d | 0.9880 |
| Dry extract of <i>Ginkgo biloba</i> (standard) | 37.8 ± 2.496 ^a | - |

EC_{50} , 50% effective concentration; %AOA, average percentage of antioxidant activity; AE, aqueous extract; EE, 80% ethanol extract; AcE, 80% acetone extract. * Values expressed as mean ± standard deviation; different letters in the same column differ statistically (Tukey, $p < 0.05$).

extraction of one or more phenolic compounds (Sineiro et al., 2008) with low molecular weight, or phenolic compounds with a smaller number of active hydroxyl groups (Dai and Mumper, 2010; Anwar and Przybyski, 2012), essential for high antioxidant activity, is characteristic of pure extracts in water.

The three extracts varied significantly in antioxidant activity compared to the dry standardized extract of *Ginkgo biloba* (37.8 $\mu\text{g/mL}$) ($p < 0.05$), which has proven antioxidant activity due to its high content of flavonoids (Aboshora et al., 2014; Sardsaengjun and Jutiviboonsuk, 2010).

In the comparative evaluation of the data, the relationship between the concentration of total phenolic derivatives and the ability to reduce free radicals of the extracts obtained from *E. uchi* bark is significant. The three extracts showed strong positive correlation, with Pearson $r > 0.9$ ($p > 0.05$).

However, the better antioxidant activity of AcE and EE, as well as AE, can also probably be related to the total flavonoid content. These metabolites, even at low concentrations, can contribute to the high antioxidant potential of plant species (Savini et al., 2013), mainly due to the presence of conjugated double bonds, hydroxyl groups at C4 and C3 and the catechol group in ring B (Degáspari and Waszczyński, 2004) in their structures, making them targets of attack by free radicals, with displacement of the electron cloud around the aromatic ring (França et al., 2013) and electron donation, thereby

blocking the phase propagation of chain reactions (Halliwell, 2001) and giving greater stability to the radical form.

Accordingly, phenolic derivatives, specially flavonoids may have an important roles in obesity and the ability to prevent oxidation of serum lipoproteins (Niki et al., 2005; Sen et al., 2010), as well as cytotoxic effects of oxidized LDL, since they can break the propagation of the peroxidation chain of lipids and lipoproteins (Dornas et al., 2007; Sikder et al., 2014). Besides that, flavonoids can regenerate active alpha-tocopherol by donating hydrogen atoms to lipid peroxidation radicals (Amirkhizi et al., 2008; Annuzzi et al., 2014), favoring the body's endogenous antioxidant defense system (Jellinger et al., 2012; Olusi, 2002).

According to Table 3, the three extracts showed very similar results to Orlistat 1 mg/mL (65.01% and 200.03 ILA/g), which act by inhibiting pancreatic lipase in the intestinal lumen effective in 30% hydrolysis of triglycerides (Yesilbursa et al., 2005; Coutinho, 2009). It is observed that AcE was able to inhibit pancreatic lipase by 49.33% (135.26 ILA/g), while the AE and EE inhibited it by 47.54% (213.84 ILA/g) and 36.88% (102.75 ILA/g), respectively ($p < 0.05$). However, AcE and EE inhibited the enzyme at a concentration of 1 mg/mL, so they were more effective than AE, which required twice the concentration (2 mg/mL) to act on the pancreatic lipase.

This inhibition ability of *E. uchi* extracts on the activity of pancreatic lipase may be directly related to the

Table 3. Pancreatic lipase activity in the presence of bark extracts of *E. uchi* (Huber) Cuatrec.

| Sample | Inhibition percentage (I%)* | ILA/g* |
|----------|-----------------------------|-----------------------------|
| AE | 47.54 ± 2.576 ^b | 213.84 ± 9.432 ^a |
| EE | 36.88 ± 1.075 ^c | 102.75 ± 7.523 ^c |
| AcE | 49.33 ± 3.494 ^a | 135.26 ± 7.156 ^b |
| Orlistat | 65.01 ± 2.026 ^d | 200.03 ± 8.466 ^d |

AE, Aqueous extract (2 mg/mL); EE (1 mg/mL), 80% ethanol extract; AcE (1 mg/mL), 80% acetone extract. Orlistat, 1 mg/mL. ILA/g, lipase activity inhibited per gram of sample. *Values expressed as mean ± standard deviation; Different letters in the same column differ statistically (Tukey, p<0.05).

polyphenol content found.

As shown in Table 1, EE and AcE have the highest flavonoid and total phenolic concentrations, and the better inhibition of pancreatic lipase activity when compared to the AE.

According to the literature, phenolic compounds interact with certain amino acids at the catalytic active site of lipase (Bhutani et al., 2014). They form stable complexes and decrease structural flexibility of the enzyme (Pereira et al., 2011; Souza et al., 2011), making it insoluble to the reaction medium, inactivating it (Bhutani et al., 2007; Shikov et al., 2012).

Several studies have verified the relationship between the *in vitro* inhibition of pancreatic lipase, and the levels of phenolic and flavonoid derivatives.

Moreno et al. (2006) showed that the polyphenol content of the stem bark ethanolic extracts (10-1000 µg/mL) of *Mangifera indica* L. had strong correlation with lipase inhibition, reducing the enzyme activity by 75%. Jian Zhang et al. (2008) confirmed that ethanolic extract (250 µg/ml) of *Taraxacum officinale* with high flavonoid content inhibited the pancreatic lipase by 86.3%. Meza and Valdés (2015) showed that ethanolic fractions of *Smilax sonchifolius* rich in phenolic compounds and flavonoids inhibited the activity of the pancreatic lipase enzyme around 60 to 61%. The authors noted a correlation between the flavonoid and phenolic content with the inhibition of pancreatic lipase, suggesting these compounds may be responsible for inhibition of this enzyme.

Oliveira et al. (2015) concluded that inhibition of pancreatic lipase by ethanolic extract of *Araucaria angustifolia* rich in condensed tannin, more specifically catechin chains, epicatechin and esters of gallic acid were responsible for inhibiting approximately 50% of the enzymatic activity.

Other study, such as those of Nakai et al. (2005) have shown inhibition of pancreatic lipase by aqueous and hydro-acetone extracts of green tea, white tea and oolong due to the presence of polyphenolic metabolites with unique structural features, such as the presence of specific substituent groups.

Chemical substituents, such as carbonyl, hydroxyl or amino groups in the B ring of some flavonoids (Nguyen et

al., 2012); a C-glycosylated group in position C6 or C8 of the A-ring of flavonoid derivatives (Lee et al., 2010); tannin constituents (Okuda and Ito, 2011); proanthocyanidins and procyanidins (Oliveira et al., 2015); and flavan-3-ols and gallate and galoil derivatives like catechins and epicatechins (Nakai et al., 2005; Gondoin et al., 2010) can all inhibit pancreatic lipase activity and the antilipidemic effect (Meza and Valdés, 2015).

With the inhibition of pancreatic lipase, hydrolysis products will not be ready for transport through the intestinal microvilli; chylomicron formation and transportation of lymph vessels into the venous system to the liver; formation of lipoprotein (HDL, IDL, LDL and VLDL); and mainly transport to adipose tissue for metabolism and storage (Bhutani et al., 2007; Lu et al., 2009).

Conclusion

The results indicate that the extracts from *E. uchi* barks constitute a natural promising source of phenolic compounds and present antioxidant and anti-lipase activities, which may be important inputs for the development of herbal medicines to treat overweight, obesity, dyslipidemia and associated complications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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