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Original Article

Metanolic extract of *Malpighia emarginata* bagasse: phenolic compounds and inhibitory potential on digestive enzymes

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ABSTRACT

Adding value to fruit residues is of great interest, since they can be presented as a viable solution in search of new drugs for the treatment of obesity and related diseases, due to bioactive substances, especially phenolic compounds. Thus, the objective of this study was to prepare the methanol extract of acerola bagasse flour, in order to evaluate its potential as a source of inhibitors of the enzymes α-amylase, α-glucosidase, lipase and trypsin, and determine the content of phenolic compounds by high performance liquid chromatography. Enzymatic inhibition assays were conducted in the presence or absence of simulated gastric fluid. In the methanol extract of acerola bagasse flour, the following phenolic compounds were identified: gallic acid, syringic and *p*-coumaric acid, catechin, epigallocatechin gallate, epicatechin and quercetin; epicatechin was the major compound. In the absence of gastric fluid, simulated enzymes had a variable inhibition of the acerola bagasse flour extract, except for lipase, which was not inhibited. In the presence of simulated gastric fluid, there was an inhibition of 170.08 IEU (Inhibited Enzyme Unit in $\mu mol\ min^{-1}\ g^{-1}$) for α-amylase and 69.29 IEU for α-glucosidase, indicating that this extract shows potential as an adjuvant in the treatment of obesity and other dyslipidemia.

Introduction

Obesity is a disease resulting from the excessive accumulation of body fat, and brings multiple outcomes for health, such as the prevalence and progression of cardiovascular diseases (especially heart diseases and stroke), which were the major causes of death in 2012; Some types of cancer (endometrium, breast and colon); skeletal muscle disturbs (specially osteoarthritis – a highly incapacitating degenerative disease); hypertension and type 2 diabetes mellitus (Wanderley and Ferreira, 2010; WHO, 2015).

Between 1980 and 2014, the world's obesity prevalency doubled. Data from the World Health Organization report that, in 2014, more than 1.9 billion adults were overweight and, among them, more than 600 million were obese (WHO, 2015).

One way to fight this epidemic disease is drug treatment. Medicine to fight weight gain, which has the objective to restrict energy absorption and cause weight loss, is widely available (Boniglia et al., 2008). However, these drugs cause side effects and are prohibited by Anvisa since 2011 (Abeso, 2014). Another alternative broadly employed is the use of plant extracts. Over the last years, there was a substantial increase in its use, by the fact that the population believes its intake is harmless, with a low cost, and may inhibit digestive enzymes, leading to beneficial changes in metabolism (Simão et al., 2012). However not all natural products are beneficial and further studies are necessary to evaluate their effects on the organism.

Enzymes like α-amylase and α-glycosidase, responsible for processing dietary carbohydrates, act on starch breakdown, resulting in monosaccharide absorption by enterocytes. Therefore, their inhibition offers a promising strategy for the prevention of obesity, as well as type 2 diabetes associated to hyperglycemia, by inhibiting starch breakdown and glucose absorption in the small intestine (Kwon et al., 2008; Balasubramaniam et al., 2013).

Lipase, involved in fat metabolism, is also an important target for inhibitors, since its inhibition limits triacylglycerol absorption, leading to a decrease in caloric yield and weight loss. On the other hand, trypsin inhibition, involved in protein digestion, has a malefic effect, once it impairs the complete amino acid absorption in food, essential for the organism.

Research has been carried out for evaluating the effects of natural products on the treatment of obesity and associated comorbidities, reinforcing the need for the search of new sources of

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amylase, glycosidase and lipase inhibitors (Souza et al., 2011; Pereira et al., 2011a; Simão et al., 2012). Therefore, digestive inhibitors who assist in reducing fat and carbohydrate absorption in the small intestine may be useful helpers in the treatment of obesity.

Natural products have been gaining space and importance in the pharmaceutical industry, since they have bioactive substances capable of inspiring new phytomedicines and phytotherapic products. Phenolic compounds are among those substances. These compounds present chemical structures with hydroxyls and aromatic rings, which can be simple structures or polymers, originated from plant secondary metabolism and largely found in fruits (Angelo and Jorge, 2007). Many studies report the benefits of phenolic compounds as an adjunct in the treatment of obesity (Klaus et al., 2005; Hen et al., 2006; Alterio et al., 2007; Santiago-Mora et al., 2011; Vogel et al., 2015; Zhang et al., 2015).

Alterio et al. (2007) and Klaus et al. (2005) report that phenolic compounds act in the prevention of obesity due to their thermogenic effects, ability to oxidize body fat and by decreasing intestinal absorption of fats and carbohydrates caused by the inhibition of digestive enzymes, resulting in weight loss. Phenolic compounds, such as tannins, have the ability of combining with digestive enzymes, proteins and other polymers (such as carbohydrates), forming stable complexes, impairing absorption and, therefore, making them possible inhibitors of some of these digestive enzymes (Won et al., 2007; Gholamhoseinian et al., 2010).

In this context, the use of agro industrial residues of fruits is promising for the extraction of active principles that may be employed as an alternative to the treatment of obesity and correlated diseases. By discarding these residues, secondary metabolites of great aggregated value with possible applications in pharmaceutical and food industries, are also eliminated. For example, the acerola bagasse originated in juice processing is, according to Marques et al. (2013), rich in phenolic compounds, with record contents of 10.82 g 100 g^{-1} dry matter; however, these phenolic compounds were not yet identified.

Given the above, the objective of the present study was to prepare the methanol extract of acerola bagasse flour (ABF), evaluate its potential as a source of α-amylase, α-glycosidase, lipase and trypsin inhibitors, and determine the phenolic compounds by high performance liquid chromatography (HPLC), aiming to use it as an auxiliary in the treatment of obesity and correlated diseases, aggregating value to this residue.

Material and methods

Preparation of acerola bagasse flour

Acerola *Malpighia emarginata* DC., Malpighiaceae (BRS 238 Frutacor) bagasse was obtained from plants grown in the municipality of Perdões, MG, Brazil (21°05′27″ S; 45°05′27″ W, 848 m altitude); the local climate according to the Köppen system is classified as Cwa: mild and rainy summers with moderate temperatures, annual average temperature below 21 °C, average annual precipitation of 1529.7 mm, and relative humidity of 76% (Emater, 2002). Acerola fruits were used for pulp extraction, and the residual bagasse was provided in three batches by a fruit pulp plant firm located in Perdões, MG, Brazil.

Acerola bagasse (4 kg) was frozen at −18 °C and lyophilized in glass containers protected from light for 7 days to obtain 450 g dry bagasse. After lyophilization, acerola bagasse was homogenized using mortar and pestle, was passed in sieves and most flour particles were retained on sieves sized 40 mesh (0.425 mm) to 80 mesh (0.180 mm), thus, classified as fine and then placed in a hermetically sealed flask, protected from light in a refrigerator at 4 °C.

Obtention of the extract

To obtain the methanol extract of acerola bagasse flour (ABF), 1 g of acerola bagasse lyophilized powder was transferred to a 250 ml erlenmeyer and then added 50 ml of 50% methanol solution in three repetitions. Afterwards, it covered with a ground glass joint and put on a hot plate at 80 °C. After boiling for 15 min, the extract was filtered in filter paper and collected to a 250 ml becker. The residue was once again put on an erlenmeyer and this process repeated for two more times. After the third filtration, the becker was taken to the hot plate to evaporate the methanol until the volume reaches 16 ml (AOAC, 2012), and then submitted to enzymatic inhibition analysis.

For the chromatography process, the becker was taken to the hot plate to evaporate the methanol, posteriorly frozen and lyophilized (Free Zone® 2.5 liter Freeze Dry Sustems). Lyophilyzed extract (1 g) was solubilized in 16 ml ultrapure water obtained from a Milli-Q system (EMD Millipore, Billerica, MA, USA).

Identification and quantification of phenolic compounds

HPLC was performed using a Shimadzu UHPLC chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with two LC-20AT high-pressure pumps, an SPD-M20A UV–vis detector, a CTO-20AC oven, a CBM-20A interface, and an automatic injector with an SIL-20A auto sampler. Separations were performed using a Shim-pack VP-ODS-C18 (250 mm × 4.6 mm) column, connected to a Shim-pack Column Holder (10 mm × 4.6 mm) pre-column (Shimadzu, Japan).

The mobile phase consisted of the following solutions: 2% acetic acid in water (A) and methanol:water:acetic acid (70:28:2, v/v/v) (B). Analyses were performed for a total time of 65 min at 40 °C, flux of 1 ml min^{-1}, wavelength of 280 nm, and injection volume of 20 μl in a gradient-type system (100% solvent A from 0.01 to 5 min; 70% solvent A from 5 to 25 min; 60% solvent A from 25 to 43 min; 55% solvent A from 43 to 50 min; and 0% solvent A for 10 min) until the end of the run. Solvent A was increased to 100%, seeking to maintain a balanced column. Acetic acid and methanol (HPLC grade; Sigma–Aldrich, USA) were used in the preparation of the mobile phase.

Addition of standards to the extracts was also used as an identification parameter. The phenolic standards used were gallic acid, catechin, epigallocatechin gallate, epicatechin, syringic acid, *p*-coumaric acid, ferulic acid, salicylic acid, resveratrol and quercetin all obtained from Sigma–Aldrich (St. Louis, MO, USA). The stock standard solutions were prepared in methanol (HPLC grade; Sigma–Aldrich, USA).

The ABF extract and the standards were filtered through a 0.45-μm nylon membrane (EMD Millipore, USA) and directly injected into the chromatographic system, in three replicates. The phenolic compounds in the extract were identified by comparison with retention times of standards. Quantification was performed by the construction of analytical curves obtained by linear regression using Origin 6.1 computer software (OriginLab, Northampton, MA, USA) and considering the coefficient of determination (R^2) equal to 0.99.

Enzyme obtention

Were used in the assays the enzymes: porcine pancreatic lypase (EC 3.1.1.3) type II, Sigma; porcine pancreatic α-amylase (EC 3.2.1.1) type VI B, Sigma and porcine pancreatic trypsin (EC 3.4.21.4), Merck. The α-glycosidase (EC 3.2.1.20) was obtained from fresh porcine duodenum according to Souza et al. (2011). The supernatant was collected and used as an enzymatic extract.