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Full Length Research Paper

Inhibition of digestive enzymes by medicinal plant aqueous extracts used to aid the treatment of obesity

Simão, Anderson Assaid*, Corrêa, Angelita Duarte and Chagas, Pricila Maria Batista

Chemistry Department, Biochemistry Laboratory, Federal University of Lavras-UFLA, P.O. Box 3037, Zip Code 37200.000, Lavras, MG, Brazil.

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The objective of this work was to perform digestive enzyme inhibition assays with aqueous extracts of the medicinal plants: *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr., *Tournefortia paniculata* Cham. (marmelinho) and with the aqueous extract of the combination of those plants in the proportions 1:1, 5:0, 5:1, 5:0, 5, used as aids in the treatment of obesity. The α-amylase, α-glycosidase, lipase and trypsin enzyme inhibition analyses were conducted in the presence and absence of a simulated gastric fluid. In the absence of the simulated gastric fluid, the enzymes underwent variable inhibition by the plant extracts, except for lipase, that did not undergo any inhibition. In the presence of the simulated gastric fluid, only the α-amylase and α-glycosidase enzymes were inhibited by the plant extracts. The combination of the plants did not cause inhibition in any of the evaluated digestive enzymes. Those results reveal that the aqueous plant extracts aloe, carqueja and marmelinho present potential as adjuvant in the treatment of obesity and of other dyslipidemias, because they inhibit α-amylase (marmelinho) and α-glycosidase (aloe, carqueja and marmelinho) after the gastric digestion simulation; the same cannot be said of the plants in combination.

Key words: α-amylase, α-glycosidase, lipase, trypsin, medicinal plants, obesity.

INTRODUCTION

Obesity is a chronic disease resulting from the excessive accumulation of body fat, which causes health damage in adults, adolescents and children, both in developed and in developing countries, with significant losses not only in the quality of life, but also in longevity.

The prevalence of obesity has been increasing at alarming rates throughout the world, and has become a major health problem in modern society. Approximately, 1.6 billion are overweight, of which 400 million are obese, leading the disease to a global epidemic status (Tucci et al., 2010).

Obesity causes psychological problems, frustration, unhappiness and it predisposes the organism to a series of diseases, in particular cardiovascular diseases, some types of cancer, diabetes and hypertension, causing an increase of economical costs for governments as well as for society (Bray, 2004).

Weight loss strategies and obesity treatments usually involve a combination of dietary changes, increase of physical activity, behavioral therapy, pharmacotherapy, and, in extreme cases, surgery (Celleno et al., 2007). Another widely used option are the natural products that have had a considerable increase in their consumption in recent years, mainly for the fact that the population believes they are ingesting medicines that do not cause harm to health and present low cost.

Research on therapeutic alternatives, mainly with medicinal plants, has been gaining space and importance in the pharmaceutical industry, being revealed as a quite promising option for the discovery of new phytomedicines and phytotherapeutics, due to the high number of still unstudied plant species, representing a vast field of substances to be discovered (Viegas et al., 2006).

For the treatment of obesity, molecular targets, such as enzymes and receptors present in natural products, have

*Corresponding author. E-mail: andersonbsbufla@yahoo.com.br.

been studied in order to search for medicines based on the enzymatic inhibition mechanism that causes beneficial alterations in the metabolism, being an excellent alternative for the safe and effective development of anti-obesity drugs (Bhutani et al., 2007).

In this context, the enzymes α-amylase and α- glycosidase are responsible for carbohydrate processing, operating in the breakdown of starch and in the absorption of monosaccharides by the enterocytes (Kandra, 2003; Ota et al., 2009). Inhibitors of those enzymes, present in plants, offer a promising strategy to aid in the treatment of obesity, hyperglycemia associated to type 2 diabetes and hypertension, through the reduction of the starch breakdown and of the glucose absorption in the intestine (Kwon et al., 2006).

Besides those, lipase, involved in the lipid metabolism, is also shown as an interesting target of inhibitors, because its inhibition promotes reduction in triglyceride absorption, causing a decrease of caloric use and weight loss. On the other hand, trypsin inhibition, involved in protein digestion, unlike the other inhibitions, is characterized as having a harmful effect because it impedes the complete absorption of amino acids, which are of fundamental importance for the organism, present in foods (Friedman and Brandon, 2001).

Much research has demonstrated the effectiveness, importance and the potential use of those enzyme inhibitors in the treatment of obesity and associated comorbidities and they reinforce the need for the search of new sources of those inhibitors: amylases (Obiro et al., 2008; Udani et al., 2009), glycosidases (Kwon et al., 2006) and lipases (Sharma et al., 2005; Souza et al., 2010). As such, digestive enzyme inhibitors that help limit the intestinal absorption of carbohydrates and fats in the initial phase may prove to be useful as aids in the treatment of obesity.

Various medicinal plants such as *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr., and *Tournefortia paniculata* Cham. (marmelinho) are used as aids in the treatment of obesity. However, studies related to the presence of enzymatic inhibitors in the extracts of those plants that can participate or even be responsible for their anti-obesity properties, are scarce in the literature.

Based on the foregoing, the objective in this work was to conduct assays of digestive enzyme inhibition with aqueous extracts of aloe, calunga, carqueja, *G. cambogia* and marmelinho and with the aqueous extract of the combination of those plants, so that these plants can be used as aids in the treatment of obesity.

MATERIALS AND METHODS

Sample collection and preparation

The plants *S. ferruginea* St. Hil. (calunga), *B trimera* (Less.) DC (carqueja) and *T. paniculata* Cham. (marmelinho) were acquired in the municipal market of Belo Horizonte, Minas Gerais, Brazil, and transported to the Biochemistry Laboratory in the Chemistry Department of Federal University of Lavras (UFLA). The marmelinho and carqueja leaves were washed under running water and distilled water and soon afterwards placed together with the bark obtained from the calunga trunk in forced-air ovens for drying for 48 h, at temperatures ranging from 30 to 35°C. After drying, the leaves and the bark were ground in a Willy type mill and the flours stored in hermetically sealed flasks until the analyses. The commercial powder of *A. vera* (L.) Burm. (aloe) (mucilage) and that of *G. cambogia* Desr. (fruit) were acquired from FLORIEN, a pharmaceutical supply distributor.

The flour of the plants were mixed for the elaboration of a simulated phytotherapeutic, from the combination of aloe, calunga, carqueja, G. *cambogia* and marmelinho in the proportion 1:1,5:0,5:1,5:0,5, respectively; the same combination is used in the elaboration of the phytotherapeutic known by the trade name ‘Moder Diet’.

Moisture determination

The moisture determination was carried out in triplicate in the medicinal plant flours (MPF) according to the Association of Official Analytical Chemists - AOAC (2005) method that consists of the water loss by dehydration, at temperatures ranging from 100 to 105°C.

Extract preparation

The MPF were mixed with distilled water in the proportion 1:25 (p/v), and placed in a horizontal agitator at room temperature for 1 h. Soon afterwards, the mixture was filtered in filter paper and used as inhibitors in the enzymatic analyses.

Enzyme obtention

For the assays, the pancreatic porcine α-amilase type VI (SIGMA) was used, as well as the pancreatic porcine trypsin and the porcine lipase type II (MERCK). The α-glycosidase was obtained from fresh porcine duodenum provided by the Amimal Science Department of UFLA, that was triturated in blender with Tris-HCl 0.5 mol L^{-1}, pH 8.0 buffer at 4°C, for extraction of the enzymes from the enterocyte membranes and processed in mixer until complete homogenization. The homogenate was filtered in nylon mesh and centrifuged for 10 min, at 2500 x g, at 4°C. The supernatant was collected and used as an enzymatic extract (Souza et al., 2010).

α-amylase activity

The α-amylase activity was determined according to the methodology proposed by Noelting and Bernfeld (1948). Thus, 50 µl of the plant extracts and 50 µl of α-amylase were pre-incubated for 20 min, in a water bath at 37°C. The substrate was the 1% starch prepared in Tris 0.05 mol L^{-1}, pH 7.0 buffer with 38 mmol L^{-1} NaCl and 0.1 mmol L^{-1} $CaCl_2$. After addition of 100 µl of the substrate, the mixture was incubated for four periods of time. The reaction was interrupted adding 200 µl of 3.5 dinitrosalicylic acid and the product read in spectrophotometer at 540 nm.

α-glycosidase activity

The α-glycosidase activity was determined according to Kwon et al.

Table 1. Inhibition of digestive enzymes by aqueous extracts of medicinal plants given in enzyme inhibition units (EIU[1]).

Medicinal plant	α-amylase	α-glycosidase	Lipase	Trypsin
Aloe	nd[2]	1.23±0.05	nd	nd
Calunga	nd	nd	nd	nd
Carqueja	nd	0.58±0.03	nd	10.38±0.81
Garcinia cambogia	nd	nd	nd	nd
Marmelinho	2,907.13±7.64	35.46±0.58	nd	176.68±7.05
Phytotherapeutic[3]	nd	nd	nd	nd

Data are average of triplicates ± standard deviation. Moisture content of medicinal plant flour in g 100 g^{-1}: aloe = 8.53; calunga = 8.42; carqueja = 8.56; *Garcinia cambogia* = 3.94; marmelinho = 9.90. [1]One EIU is equal to 1 μmol min^{-1} g^{-1} of dry matter. [2]nd: inhibition not detected. [3]Phytotherapeutic: elaborated from the combination of aloe, calunga, carqueja, *Garcinia cambogia* and marmelinho in the proportion 1:1, 5:0, 5:1, 5:0, 5, respectively.

(2006), using 5 mmol L^{-1} p-nitrophenyl-α - D-glucopyranoside in a 0.1 mol L^{-1} pH 7.0 citrate-phosphate buffer as substrate. In the assay, 50 μl of the plant extracts and 100 μl of enzyme were incubated in a water bath, at 37°C, for four periods of time, after addition of 50 μl of the substrate. The reaction was interrupted adding 1.000 μl of 0.05 mol L^{-1} NaOH and the product was read in a spectrophotometer, at 410 nm.

Lipase activity

In each analysis, the mixture of 100 μl of lipase, 50 μl of the plant extracts and 50 μl of 4 mmol L^{-1} *p*-nitrophenyl laurate substrate in Tris-HCl 0.05 mmol L^{-1} pH 8.0 buffer containing 0.5% Triton-X100 was incubated for four periods of time. The reaction was stopped, transferring the tubes to an ice bath and adding 1.000 μl of Tris-HCl 0.05 mmol L^{-1} pH 8.0 buffer. The *p*-nitrophenol, of yellow coloration, a product of the lipase action on *p*-nitrophenyl palmitate, was read in a spectrophotometer at 410 nm (Souza et al., 2010).

Trypsin activity

The trypsin activity was determined according to the methodology proposed by Erlanger et al. (1961); in which 200 μl of the plant extracts and 200 μl of enzyme were incubated in a water bath, at 37°C, for four periods of time, after addition of 800 μl of *p*-benzoyl-DL-arginine-p-nitroanilide substrate (BApNA) prepared in 0.05 mol L^{-1}, pH 8.2 TRIS (tris(hydroxymethyl)aminomethane) buffer with 20 mmol L^{-1} $CaCl_2$. The reaction was interrupted adding 200 μl of 30% acetic acid and the product read in a spectrophotometer at 410 nm.

Determination of inhibition

The enzyme inhibition was obtained from the determination of the slopes of the straight lines (absorbance x time) of the control enzyme (without plant extract) and enzymes + inhibitor (with plant extracts) activity assays. The slope of the straight line is due to the speed of product formation per minute of reaction and the presence of the inhibitor causes a decrease in that inclination. From that inclination, the absorbance values were converted into micromoles of product through a standard glucose curve for the amylase and of *p*-nitrophenol for glycosidase and lipase, while, for the trypsin, the of BApNA molar extinction coefficient determined by Erlanger et al. (1961) was used.

Preparation of simulated gastric fluid

With the objective of simulating the digestion process in the stomach *in vitro*, enzymatic activity assays in the presence of a simulated gastric fluid were also carried out. For such, the plant extracts were incubated with the simulated gastric fluid prepared according to The United States Pharmacopeia - USP (2005), for 1 h in a water bath at 37°C. After that period, they were neutralized with sodium bicarbonate salt to physiological pH and only then were the inhibition activity determination assays conducted.

RESULTS AND DISCUSSION

The digestive enzyme inhibition is a promising alternative for obesity treatment, mainly by the fact that they act on the small intestine, without acting on the central nervous system, where the usual anorexigens act. The results of the enzymatic inhibition by the medicinal plant aqueous extracts are presented in Table 1.

α-amylase was only inhibited by the marmelinho extract that presented an inhibition potential of 2,907.13 μmol min^{-1} g^{-1} dry matter (DM). This potential is considered high when compared to Pereira et al. (2010), who analyzed different extraction conditions (solvents, times and temperatures) of α-amylase inhibitors in white bean, found inhibition between 36.88 and 66.89 μmol min^{-1} g^{-1} DM. Souza et al. (2010), analyzing the inhibitory potential of different carqueja extracts, also did not detect inhibition of α-amylase.

α-glycosidase was inhibited by the aloe, carqueja and marmelinho extracts, and this latter plant presented an inhibition potential of 35.46 μmol min^{-1} g^{-1} DM, a potential quite superior to that of the other plants that inhibited α-glycosidase. The inhibitory potential of marmelinho in the present work outperforms that verified by Pereira et al. (2011), who, analyzing commercial samples of *Hoodia gordonni*, a plant used as an aid in the treatment of obesity, found inhibition between 10.40 and 16.70 μmol min^{-1} g^{-1} DM. However, the potentials of aloe and carqueja are lower than those of *H. gordonni*. The inhibition of those enzymes induces carbohydrate

Table 2. Inhibition of digestive enzymes by aqueous extracts of medicinal plants after exposure to simulated gastric fluid given in enzyme inhibition units (EIU[1]).

Medicinal plant	α-amylase	α-glycosidase	Lipase	Trypsin
Aloe	nd[2]	0.49±0.02	nd	nd
Calunga	nd	nd	nd	nd
Carqueja	nd	0.39±0.04	nd	nd
Garcinia cambogia	nd	nd	nd	nd
Marmelinho	2,512.55±8.54	25.90±1.12	nd	nd
Phytotherapeutic[3]	nd	nd	nd	nd

Data are average of triplicates ± standard deviation. Moisture content of medicinal plant flour in g 100 g^{-1}: aloe = 8.53; calunga = 8.42; carqueja = 8.56; *Garcinia cambogia* = 3.94; marmelinho = 9.90. [1]One EIU is equal to 1 µmol min^{-1} g^{-1} of dry matter. [2]nd: inhibition not detected. [3]Phytotherapeutic: elaborated from the combination of aloe, calunga, carqueja, *Garcinia cambogia* and marmelinho plants in the proportion 1:1, 5:0, 5:1, 5:0, 5, respectively.

tolerance, extends gastric emptying, causes satiation and weight loss; all of which are effects that can be useful in the treatment of obesity (Chen et al., 2008).

Inhibition of lipase involved in the lipidic metabolism, was not detected for any of the aqueous extracts of the medicinal plants analyzed. Studies described in the literature show that, in the alcoholic extracts of plants, mainly methanol, lipase inhibitors have been isolated (Sharma et al., 2005; Sugimoto et al., 2009). Those studies suggest that organic compounds soluble in methanol present some structural characteristics that have the capacity to bond and inhibit the pancreatic lipase.

For trypsin, a high inhibition was observed: 176.68, µmol min^{-1} g^{-1} DM, (marmelinho) and 10.38 (carqueja). Trypsin inhibitors present in the diet can cause a growth rate reduction in animals accompanied by a protein digestibility decrease which may lead to weight loss (McDougall et al., 2005). The combination of the plants did not cause inhibition under any of the digestive enzymes analyzed.

For the ingestion of the medicinal plant extract, the passage through the gastrointestinal tract can lead to structural modifications in the inhibitors due to the acid pH of the stomach, causing their deactivation. Considering the expressive inhibition of α-amylase, α-glycosidase and trypsin in the presence of the plant extracts, they were submitted to a test of possible gastric fluid action on the extract inhibitory activity (Table 2).

It was observed that there was a 14% decrease of the marmelinho extract inhibitory potential on the α-amylase in the presence of the simulated gastric fluid. For the α-glycosidase, the simulated fluid provoked a reduction in the inhibitory potential of the plant extracts of 60 (aloe), 32.8 (carqueja) and 27% (marmelinho). However, even with the decrease of enzyme inhibition potential by the plant extracts, the results did not cease to be significant.

The carqueja and marmelinho extracts lost their activity on the trypsin enzyme, which indicates that the trypsin inhibitors present in these extracts are not resistant to the passage through the gastric fluid that can lead to modifications in the inhibitor molecule due to the acidic pH of the stomach or the presence of proteinases, inactivating it.

The inhibition results suggest that aloe, carqueja and mainly marmelinho might be a good source of α-amylase and α-glycosidase inhibitors, which could be used as aids in the treatment of obesity that can be caused by an elevated level of carbohydrates in the diet. The resistance of the inhibitors, when passing through the simulated gastric fluid, is a strong indication that those results will repeat in *in vivo* assays.

Conclusions

The aloe, carqueja and marmelinho aqueous plant extracts present potential as adjuvant in the treatment of obesity and of other dyslipidemias, because they inhibit the α-amylase (marmelinho) and the α-glycosidase (aloe, carqueja and marmelinho) after the gastric digestion simulation; the same cannot be said of the plants in combination (phytotherapeutic).

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