Anais da Academia Brasileira de Ciências (2017) 89(3 Suppl.): 2155-2165
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201720160067
www.scielo.br/aabc | www.fb.com/aabcjournal

Aqueous extract of *Psidium guajava* leaves: phenolic compounds and inhibitory potential on digestive enzymes

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Manuscript received on March 7, 2016; accepted for publication on July 22, 2016.

ABSTRACT

Leaves of *Psidium guajava* L. (guava) have been widely used in the popular way for prevention and treatment of various diseases. Thus, the objective of this study was to evaluate the inhibitory potential of leaves aqueous extract from three cultivars of *P. guajava* (Pedro Sato, Paluma and Século XXI) on α-amylase, α-glycosidase, lipase, and trypsin enzymes, in the presence or not of simulated gastric fluid and to determine the content of phenolic compounds by high performance liquid chromatography. All cultivars presented the same composition in phenolic compounds, but in different proportions. The compounds identified are gallic acid, epigallocatechin gallate, syringic acid, o-coumaric acid, resveratrol, quercetin, and catechin (which was the major compound in all the cultivars evaluated). In the absence of simulated gastric fluid, it was observed different inhibitions exercised by the leaves aqueous extracts from three cultivars of *P. guajava* on each enzyme. In presence of simulated gastric fluid, all cultivars showed increase in the inhibition of lipase and α-glycosidase, and decrease in inhibition of α-amylase and trypsin enzymes. These results indicate that *P. guajava* leaves aqueous extracts from all cultivars evaluated possess potential of use as an adjuvant in the treatment of obesity and other dyslipidemias.

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

INTRODUCTION

Obesity is a disease resulting from the excessive accumulation of body fat, and due to the consequences caused by it and its rapid increase throughout the world, it has been considered a global epidemic, with over 1.9 billion overweight adults, from which 600 million are clinically obese. Between 1980 and 2014, the world's obesity prevalence doubled (World Health Organization - WHO 2015). Its incidence independent of socioeconomic factors and age, and its consequences range from the development of debilitating diseases (cardiovascular diseases, some types of cancer, muscle disturbs, hypertension, and type 2 diabetes mellitus) to death, directly affecting the quality of life of individuals (Wanderley and Ferreira 2010, WHO 2015).

Among the options available for the treatment of obesity, the most used ones are balanced diets,

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regular physical exercises, and drug treatments, ranging from lipase inhibitors to anorectics. However, due to side effects and the high cost of drugs traditionally used in the treatment of this disease, the potential of natural products for treatment of obesity have been widely explored, and they may be a viable alternative for future development of more effective and safe anti-obesity drugs (Park et al. 2005, Mayer et al. 2009). Mixtures of phytochemicals or isolated molecules identified from plants represent an excellent opportunity for the development of such therapeutics (Bhutani et al. 2007).

The phenolic compounds stands out among the bioactive substances in medicinal plants capable of generating new phytotherapic drugs, and that attends pharmaceutical industry interest. These compounds present several medicinal properties like antioxidants, anti-histamines, anti-inflammatory, antibacterial, anti-thrombotic (Balasundram et al. 2006), and can also be used as adjuvants in the treatment of obesity (Klaus et al. 2005, Hen et al. 2006, Alterio et al. 2007, Santiago-Mora et al. 2011, Zhang et al. 2015, Vogel et al. 2015).

The action potential of phenolic compounds in the treatment and prevention of obesity is due to their thermogenic effects, which corresponds in ability to oxidize body fat, and decrease intestinal absorption of fats and carbohydrates. These effects are result of digestive enzymes inhibition, with consequent weight loss (Klaus et al. 2005, Alterio et al. 2007).

In this context, enzymes like α-amylase and α-glycosidase, responsible for processing dietary carbohydrates, acts 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. 2006, Balasubramanian 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 (Friedman and Brandon 2001).

Studies have shown the effectiveness and therapeutic potential of enzymes inhibitors in the treatment of obesity and associated comorbidities, reinforcing the need to search for new sources of natural inhibitors (Pereira et al. 2011a, Souza et al. 2011, Simão et al. 2012). Therefore, digestive inhibitors, which assist in reducing fat and carbohydrate absorption in the small intestine, may be useful helpers in the treatment of obesity.

Psidium guajava L., popularly known as guava, is an example of plant that stands out for its economic expression, taste, flavor, and diversity of possible uses. Gutiérrez et al. (2008) published a revision about guava highlighting pharmacological properties of bark, fruit, leaves, and roots, describing its antioxidant, hepatoprotective, anti-allergic, anti-microbial, anti-plasmodial, anti-diabetic, and anti-inflammatory functions.

Although the fruit is the most significant part from *P. guajava*, teas, infusions, and decoctions prepared from its leaves have been used by people for medicinal purposes, in the treatment of gastroenteritis, fever, diarrhea, Chagas disease, ulcers, cholera, digestive problems, and others (Vendruscolo et al. 2005). However, many of these applications have no scientific evidence of a therapeutic effect, highlighting the importance of studies that bring information on the efficacy and safety of its use.

In the chemical composition of *P. guajava* leaves are found several bioactive compounds such as essential oils, saponins and phenolic

compounds, highlighting thus, the pharmacological potential of these leaves and different perspectives to their therapeutic application (Haida et al. 2011). In addition, the leaves from *P. guajava*, shows rich composition in phenolic compounds, reinforcing the importance of investigations about their possible inhibitory effect on digestive enzymes.

Considering the increasing search for effective therapeutic alternatives, less expensive and of proven security, as well as the lack of scientific information for a wide use of the leaves of *P. guajava*, the objective of the present study was to evaluate the potential of leaves infusions from different cultivars of *P. guajava* (Paluma, Pedro Sato and Século XXI) as a source of α-amylase, α-glycosidase, lipase, and trypsin inhibitors, as well as determine the phenolic compounds by high performance liquid chromatography, contributing with information for its future use as an adjuvant in the treatment of obesity and associated diseases.

MATERIALS AND METHODS

OBTENTION AND PREPARATION OF PLANT SAMPLES

Fresh leaves, without lesions induced mechanically or by pathogens, of *Psidium guajava* L. (Paluma - PL, Pedro Sato - PS and Século XXI – SEC, cultivars) were collected in an orchard located in Lavras city, Minas Gerais, Brazil, 845m altitude, latitude 21:15 ° S and longitude 45.22 ° W, in March 2015.

The leaves were identified by the College of Agriculture at Lavras Herbarium where a voucher specimen was deposited which received the voucher number: PL n° 26276, PS n° 26277 and SEC n° 26278.

The leaves were washed in running water, kept in a 0.1% sodium hypochlorite solution for 1 hour, washed in distilled water and dried in an oven for 48 hours, at a temperature of 35 °C. The dried leaves were ground in a Willey mill and the obtained powder was subjected to infusion in boiling water at a 1:25 (w v^{-1}) ratio for 30 minutes. The extract was then centrifuged at 10,000 x g for 10 minutes (206 BL Fanem Baby®I) and the supernatant was collected. The supernatants were then lyophilized (FreeZone LABCONCO 4.5 L benchtop lyophilizer) and weighed. Posteriorly, this lyophilized supernatant was dissolved in water, for use in the assays and named aqueous extract.

IDENTIFICATION AND QUANTIFICATION OF PHENOLIC COMPOUNDS

The high performance liquid chromatography (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). Analysis 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.

The phenolic standards used as identification parameters were gallic acid, catechin, epigallocatechin gallate, epicatechin, syringic acid, *o*-coumaric acid, *p*-coumaric acid, ferulic acid, vanillin, 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 extracts 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 extracts were identified by comparison with retention times of standards and by co-elution performing the elution of samples together with 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

In these assays the following enzymes were used: porcine pancreatic lipase (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 Simão et al. (2012).

α-AMYLASE ACTIVITY

The α-amylase activity was determined using the methodology proposed by Noelting and Bernfeld (1948). Thus, aqueous extracts and α-amylase enzyme 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 that, 100 µl of substrate were added and the mixture was incubated for four periods of time. The reaction was interrupted adding 200 µl of 3.5 dinitrosalicylic acid and the product measured in spectrophotometer at 540 nm.

α-GLYCOSIDASE ACTIVITY

The α-glycosidase activity was determined according to Kwon et al. (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, aqueous extracts and α- glycosidase enzyme were incubated in a water bath, at 37 °C, for four periods of time, and after that, the substrate was added . The reaction was interrupted adding 1.000 µl of 0.05 mol L^{-1} NaOH and the product was measured in a spectrophotometer at 410 nm.

LIPASE ACTIVITY

The lipase activity was determined according to Simão et al. (2012), using 4 mmol L^{-1} *p*-nitrophenyllaurate in Tris-HCl 0.05 mmol L^{-1}, pH 8.0 buffer containing 0.5% Triton-X100 as substrate. In this assay, aqueous extracts and lipase enzyme were incubated in a water bath, at 37 °C, for four periods of time, and after that, the substrate was added. The reaction was stopped, transferring the tubes to an ice bath and adding 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*-nitrophenyllaurate, was measured in a spectrophotometer at 410 nm.

TRYPSIN ACTIVITY

The trypsin activity was determined according to the methodology proposed by Erlanger et al. (1961). Thus, aqueous extracts and trypsin were incubated in a water bath, at 37 °C, for four periods of time, and after that the *p*-benzoyl-DL-arginine-p-nitroanilide substrate (BAPNA), prepared in Tris 0.05 mol L^{-1}, pH 8.2, was added. The reaction was interrupted adding 200 µl of 30% acetic acid and the product measured in a spectrophotometer at 410 nm.

DETERMINATION OF INHIBITION

For each assay of enzymatic activity, the concentrations of aqueous extract were different and its dilution ranged so that the enzyme inhibition ranged from 40% to 80%, according to the methodology.

The inhibition of the enzymes were obtained from the determination of the slopes of the straight lines (absorbance x time) corresponding to values obtained for the control enzyme (without aqueous extract) and enzymes + inhibitor (with aqueous extract) in the activity assays. The slope of the straight line correspond to the speed of product formation per minute of reaction and the presence of the inhibitor causes a decrease of this inclination. The absorbance values were converted into micromoles of product based on data obtained from a standard curve elaborated with glucose for the amylase and with *p*-nitrophenol for glycosidase and lipase, while, for the trypsin, the molar extinction coefficient of BAPNA was determined by Erlanger et al. (1961).

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 aqueous extract was 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. Subsequently, it was neutralized with sodium bicarbonate salt to pH 7.0 and, only then, the activity assays were realized.

STATISTICAL ANALYSIS

All data were collected in three repetitions and presented as the mean ± standard deviation. The data were statistically evaluated by analysis of variance, and the means were compared using the Scott Knott test ($P < 0.05$) with the aid of the R software (R Development Core Team 2012).

RESULTS AND DISCUSSION

Mass yield percentages obtained for the Paluma (PL), Pedro Sato (PS) and Século XXI (SEC) cultivars were 3.88 ± 0.05%, 4.45 ± 0.02% and 3.52 ± 0.23%, respectively.

In the figure 1 are demonstrated the chromatograms obtained to phenolic compounds presents in the leaves aqueous extracts from *P. guajava* cultivars and the phenolic compounds used as standards. The results of chromatographic analysis to the phenolic compounds quantification in the leaves aqueous extracts from *P. guajava* cultivars are presented in Table I. All cultivars showed the same phenolic composition but with different levels of gallic acid, catechin, epigallocatechin gallate, syringic acid, *o*-coumaric acid, resveratrol, and quercetin.

The catechin was the major compound between phenolic compounds identified in the cultivars, followed by gallic acid and resveratrol, however the levels vary in the different cultivars, except for gallic acid. The cultivar PL presented the highest level of catechin; PS of epigallocatechin gallate and resveratrol; and the SEC of syringic acid, o-coumaric acid and quercetin. The PL cultivar presented the highest content of total phenolic compounds.

The compounds epicatechin, *p*-coumaric acid, ferulic acid, vanillin, and salicylic acid were not identified in the aqueous extract of the leaves of three cultivars of *P. guajava*.

Phenolic compounds, such as caffeic and chlorogenic acid, catechin, epigallocatechin gallate and quercetin have thermogenic effect, ability to oxidize fats, control appetite, regulate levels of hormones related to obesity and inhibit digestive enzymes involved in the absorption of carbohydrates and lipids (Lin and Lin-Shiau 2006, Alterio et al.

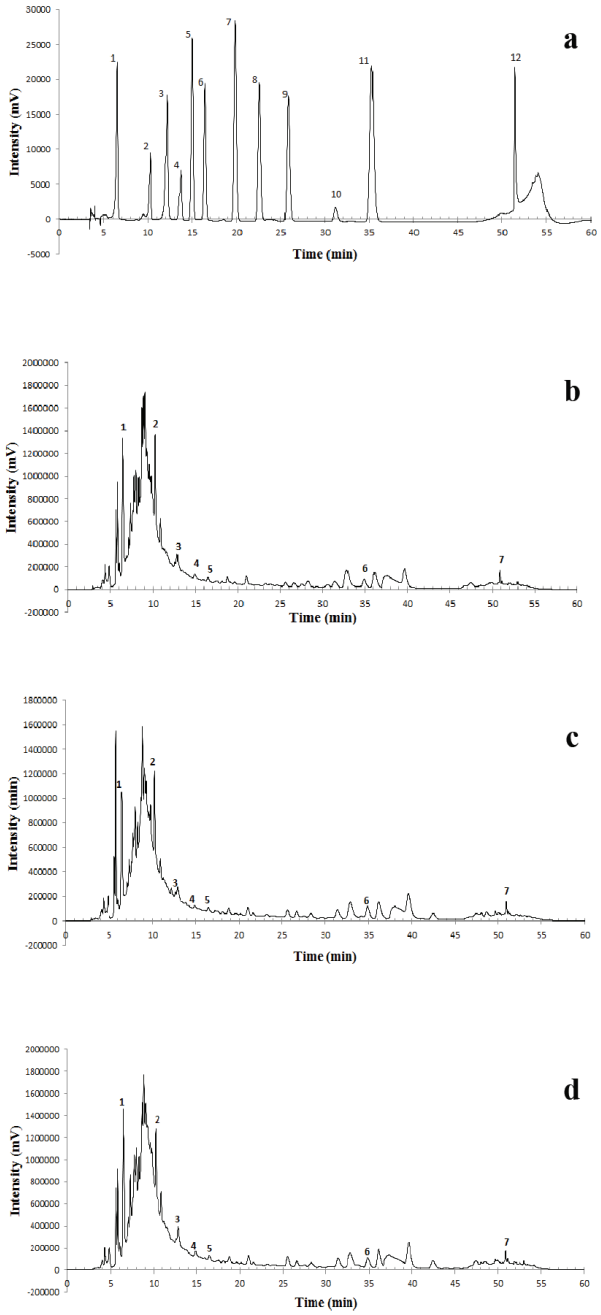

Figure 1 - Chromatogram of phenolic compounds in the aqueous extract of the leaves from three cultivars of *Psidium guajava*. (**a**) Identification standards: 1 = Gallic acid; 2 = Catechin; 3 = Epigallocatechin gallate; 4 = Epicatechin; 5 = Syringic acid; 6 = *o*-coumaric acid; 7 = *p*-coumaric acid; 8 = Ferulic acid; 9 = Vanillin; 10 = Salicylic acid; 11 = Resveratrol; 12 = Quercetin. (**b**) Paluma. (**c**) Pedro Sato. (**d**) Século XXI.

TABLE I
Phenolic compounds, in mg 100g^{-1}, present in the aqueous extract of the leaves from three cultivars of *Psidium guajava*.

Phenolic compound	Paluma	Pedro Sato	Século XXI
Gallic acid	681.12 ± 35.76^{Ba}	650.08 ± 3.25^{Ba}	630.38 ± 21.95^{Ba}
Catechin	846.19 ± 9.84^{Aa}	756.31 ± 30.73^{Ab}	771.97 ± 16.64^{Ab}
Epigallocatechin gallate	10.30 ± 0.15^{Fb}	61.04 ± 6.40^{Da}	6.29 ± 0.25^{Fc}
Syringic acid	22.70 ± 1.52^{Db}	14.17 ± 0.46^{Ec}	27.91 ± 0.34^{Ea}
o-Coumaric acid	11.17 ± 0.09^{Eb}	11.86 ± 1.02^{Fb}	36.66 ± 2.04^{Da}
Resveratrol	71.03 ± 5.78^{Cb}	93.77 ± 4.78^{Ca}	61.74 ± 0.81^{Cc}
Quercetin	0.03 ± 0.00^{Gb}	0.03 ± 0.00^{Gb}	0.07 ± 0.01^{Ga}
∑ Phenolic compounds	1,642.54	1,587.26	1,535.02

Data from three repetitions, with mean ± standard deviation. Uppercase letters in columns compare among phenolic compounds and lowercase on the lines compare among cultivars. Same letters do not differ among themselves by the Scott-Knott test at 5% probability.

2007, Cho et al. 2010, Rains et al. 2011). Thus, this study shows that the aqueous extract of the leaves from *P. guajava* presents potential to be explored by the pharmaceutical industry in search of drugs to control obesity and related diseases.

The results for enzymatic inhibition of aqueous extract of the leaves of three cultivars of *P. guajava* are shown in Table II. All enzymes studied were inhibited by *P. guajava* (PS, P and SEC) leaves extracts, before and after exposure to simulated gastric fluid.

For α-amylase enzyme, SEC cultivar induced an inhibition significantly greater than the other cultivars, before the exposure to gastric fluid (Table II), but, after exposure to gastric fluid, there was no significant difference among the three cultivars.

However, a decrease in enzyme inhibition of 19.20% (PS) to 25.42% (SEC), was observed.

The inhibitory potential presented by the *P. guajava* aqueous extract, from different cultivars, exceeds the one found by Pereira et al. (2011b), who analyzed the white bean crude extract and detected an inhibition of 54.1 μmol min^{-1} g^{-1} sample; as well as those from Simão et al. (2012), that studying aqueous extracts of medicinal plants, observed an inhibition of 2,512.55 μmol min^{-1} g^{-1} sample for *Tournefortia paniculata* Cham (marmelinho).

Infusions, decoctions, and teas rich in α-amylase inhibitors appears to be an interesting strategy in the prevention and treatment of hyperglycemia, by slowing postprandial glucose levels in blood after the ingestion of carbohydrates (Vadivel et al. 2011).

The PL cultivar induced the greatest inhibitory activity of α-glycosidase, before and after exposure to gastric fluid (Table II). The inhibition of α-glycosidase by the aqueous extract of the leaves from three cultivars found in this paper surpasses the ones verified by Simão et al. (2012), who, studying the aqueous extracts of medicinal plants, found inhibitions of 1.23 μmol min^{-1} g^{-1} dry matter for *Aloe vera* (L.) Burm., and 0.58 umol min^{-1} g^{-1} dry matter for *Baccharis trimera* (Less.) DC, which are lower than values found for *Tournefortia paniculata* Cham. (5.46 μmol min^{-1} g^{-1} dry matter), as well as those described by Pereira et al. (2011a), who analyzed commercial samples of *Hoodia gordonni*, used as an auxiliary in the treatment of obesity and found inhibitions of 10.40 e 16.70 μmol min^{-1} g^{-1} dry matter.

The inhibition of α-glycosidase extends gastric emptying, leading to satiety and weight loss, effects that can be useful in the treatment of obesity (Chen et al. 2008). Therefore, the inhibition of α-amylase and α-glycosidase by natural products can provide an alternative for the treatment of obesity in substitution to synthetic drugs, besides controlling glucose levels in blood in type 2 diabetes patients (McDougall et al. 2005a).

TABLE II
Inhibition of digestive enzymes by aqueous extract of the leaves from three cultivars of *Psidium guajava*, before and after exposure to simulated gastric fluid.

Enzyme	Cultivar	Inhibition (IEU[1])*	
		Before exposure	After exposure
α-amylase	Paluma	13,776.93 ± 79.18^{Ba}	10,633.73 ± 58.05^{Ab}
	Pedro Sato	13,130.47 ± 21.94^{Ca}	10,608.40 ± 100.54^{Ab}
	Século XXI	14,410.60 ± 38.00^{Aa}	10,747.73 ± 58.05^{Ab}
α-glycosidase	Paluma	2.28 ± 0.02^{Ab}	2.59 ± 0.06^{Aa}
	Pedro Sato	2.20 ± 0.02^{Bb}	2.33 ± 0.04^{Ba}
	Século XXI	1.99 ± 0.04^{Cb}	2.38 ± 0.04^{Ba}
Lipase	Paluma	28.82 ± 0.79^{Cb}	33.15 ± 1.64^{Ca}
	Pedro Sato	36.45 ± 0.68^{Ab}	43.33 ± 1.80^{Aa}
	Século XXI	31.89 ± 0.45^{Bb}	38.20 ± 1.17^{Ba}
Trypsin	Paluma	142,075.70 ± 918.22^{Ba}	21,470.40 ± 795.20^{Ab}
	Pedro Sato	124,051.20 ± 4,207.80^{Ca}	11,132.8 ± 791.31^{Cb}
	Século XXI	147,377.10 ± 918.21^{Aa}	15,108.8 ± 1,590.40^{Bb}

Data from three repetitions, with mean ± standard deviation. [1]IEU = Inhibited Enzyme Unit in μmol min^{-1} g^{-1} sample. *The aqueous extract of the leaves from three cultivars of the *Psidium guajava* measured for each of the enzymes was diluted to provide an inhibition between 40% and 80%, in order to ensure result reliability. Uppercase letters in columns compare among cultivars and lowercase on the lines compare before and after the exposure to simulated gastric fluid. Same letters do not differ among themselves by the Scott-Knott test at 5% probability.

A greater lipase inhibition was observed to PS cultivar and the lower inhibition to PL, before and after exposure to gastric fluid (Table II).

Studies show the presence of lipase inhibitors in alcoholic vegetal extracts mainly methanolic

extracts (Sharma et al. 2005, Sugimoto et al. 2009, Souza et al. 2011). These studies suggest that organic compounds soluble in methanol, exhibit some structural feature that results in binding and inhibition of pancreatic lipase. The three aqueous extracts from *P. guajava* varieties, analyzed in this study, demonstrated an inhibitory potential of pancreatic lipase, and the phenolic compounds may be responsible for this inhibition, since these compounds are also present in medicinal plant alcoholic extracts.

The three varieties of *P. guajava* induced high percentages of inhibition of the trypsin activity before exposure to gastric fluid, having a significant reduction in inhibitory activity after exposure to gastric fluid, ranging from 84.88% (PL) at 91.02% (PS).

When trypsin inhibitors are present in the diet, these may lead to a reduction in growth rate in animals, followed by a decrease in protein digestibility, leading to weight loss and endogenous protein catabolism (McDougall et al. 2005a). Therefore, the trypsin inhibitors are considered as anti-nutritional factors. Thus, this reduction in trypsin inhibition, after exposure to gastric fluid, is considered positive, since protein digestibility is little affected.

In this study, the inhibition of digestive enzymes can probably be explained by the presence of phenolic compounds in the aqueous extract of the leaves from three *P. guajava* cultivars, whose levels were different for each cultivar assessed (Table I). PL cultivar, that showed the highest content of catechin, exerted greater inhibition on α-glycosidase enzyme, the PS, with the major content of epigallocatechin gallate and resveratrol, showed the greatest inhibition on lipase, while SEC cultivar, rich in syringic acid, *o*-coumaric acid and quercetin, showed greater potential inhibition on the α-amylase and trypsin enzymes. The synergy between the phenolic compounds must be taken in account for a better understanding of the inhibitory action of the extracts on digestive enzymes.

In the present study, among the phenolic compounds identified in the leaves from *P. guajava*, gallic acid is considered a hydrolysable tannin, when found in the form of gallic acid esters, while catechin and epicatechin gallate, when found in the form of flavonoids, are considered condensed tannins. These compounds have strong interactions with metal ions and macromolecules such as polysaccharides, besides the ability to form soluble complexes with several proteins, as digestives enzymes (Won et al. 2007, Gholamhoseinian et al. 2010).

Several studies have shown that phenolic compounds present in medicinal plants and fruits have anti-obesity properties by exerting different mechanisms of action, especially by inhibition of digestive enzymes.

McDougall et al. (2005b) reported that red fruit extracts rich in phenolic compounds inhibit α-amylase and α-glycosidase, *in vitro.* In a similar way, recent studies with red fruits reported inhibition of α-amylase and α-glycosidase, and mentioned that tannins were the most effective compounds in inhibiting these enzymes (Boath et al. 2012). Kam et al. (2013) described that the methanol extract from the pomegranate flower, where the phenolic compounds gallic acid and ellagic acid are found, exhibits a potent inhibitory effect on α-amylase and α-glycosidase enzymes.

Studies conducted *in vivo* by Klaus et al. (2005) demonstrated that rats fed with diet supplemented by epigallocatechin gallate, purified from green tea, had an obesity decrease, due to a reduction in energy absorption and an increase in lipid oxidation. In other study, Bryans et al. (2007) reported that black tea is efficient in reducing postprandial blood glucose levels and related this fact to the presence of phenolic compounds such as epigallocatechin, epigallocatechin gallate, epicatechin, and epicatechin gallate.

Wenzel (2013) reported that quercetin limits carbohydrate digestion and controls postprandial glucose levels in blood, thus confirming the result obtained by Tadera et al. (2006), who reported the inhibitory activity of quercetin on α-amylase.

Other anti-obesity action mechanism attributed to flavonoids is by their ability to affect the sympathetic nervous system through the modulation of noradrenaline, thus increasing thermogenesis and fat oxidation. It also prevents the increase in the size and number of adipocytes, therefore preventing the deposition of fat in the body and regulating body weight (Lin and Lin-Shiau 2006).

Phenolics, like *p*-hydroxybenzoic acid, syringic acid, trans-*p*-coumaric acid, epicatechin gallate, quercetin and kaempferol presents in lentil extracts, showed to be effective inhibitors of lipase and α-glycosidase, contributing to control glucose levels in blood, as well as obesity (Zhang et al. 2015).

In addition, the aqueous extract of leaves from *Tournefortia paniculata* Cham., rich in phenolic compounds (Simão et al. 2014) presented *in vitro* inhibition of the α-amylase and α-glycosidase enzymes before and after exposure to gastric fluid simulation (Simão et al. 2012), and later, when administered to *Wistar* rats submitted to high calorie diet resulted in weight, food intake, liver fat, glucose and serum triglycerides reduction (Simão et al. 2015). The results described by these authors highlights the resistance of inhibitors present in *P. guajava* leaves to go through simulated gastric fluid, there is maintenance of inhibitory action *in vivo*.

Most phenols previously mentioned were found in the aqueous extract of the leaves from three cultivars of *P. guajava*, which could have led to a complexation with digestive enzymes, contributing to its inhibition. The inhibition of digestive enzymes by these compounds is a promising alternative for the treatment of obesity and type 2 diabetes, especially because they act in the small intestine, without acting in the central nervous system, where anorexigenic drugs usually act.

CONCLUSIONS

The aqueous extracts of leaves from *Psidium guajava* (Paluma, Pedro Sato and Século XXI) that contains the phenolic compounds gallic acid, catechin, epicatechin gallate, syringic acid, *o*-cumaric acid, resveratrol, and quercetin, were able to inhibit *in vitro* the digestive enzymes α-amylase, α-glycosidase, and lipase, with less inhibitory effect on trypsin, after exposure to simulated gastric fluid. The data shows that the aqueous extract of the leaves from *Psidium guajava* cultivars may represent a good source of inhibitors and can be used as an auxiliary in the treatment of obesity, associated comorbidities and in the control of type 2 diabetes.

ACKNOWLEDGMENTS

The authors would like to thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the grants provided.

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