Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

ASSESSMENT OF THE CHEMICAL PROFILE, POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY IN EXTRACTS OF *PSIDIUM GUAJAVA* L. FRUITS

BRUNA GALDORFINI CHIARI[1], JULIANA APARECIDA SEVERI[1], PRISCILA ABACKERLI DE PAULI-CREDENDIO[1], CÉLIA MARIA DE SYLOS[1], WAGNER VILEGAS[2], MARCOS ANTONIO CORRÊA[1], VERA LUCIA BORGES ISAAC[1]*

[1]Faculdade de Ciências Farmacêuticas, UNESP – Univ Estadual Paulista, Departamento de Fármacos e Medicamentos, c.p. 355; zip code: 14801-902, Araraquara, Brazil; [2]Instituto de Química, UNESP - Univ Estadual Paulista, c.p. 355; zip code: 14800-900, Araraquara, Brazil. Email: lacos.unesp@gmail.com, veraisaac@fcfar.unesp.br

Received: 27 Aug, 2012, Revised and Accepted: 03 Oct, 2012

ABSTRACT

Fruits and vegetables that are rich in polyphenolic compounds, especially flavonoids, may be used to benefit human health by reducing the incidence of cancers and cardiovascular diseases. Previous studies have demonstrated the antioxidant activity of guava, a fruit widely available in Brazil, possibly due to the presence of these polyphenolic compounds. The aim of this study was to analyze the total phenolic and flavonoid contents of various guava extracts, assay their antioxidant activity and record the chromatographic profiles of these extracts, to determine a simple and low way of extracting these compounds efficiently from guava. The results confirmed the presence of polyphenols in guava, including flavonoids, and its antioxidant activity. Furthermore, it was demonstrated that the 70% ethanol (by volume) was the most effective solvent to extract these compounds from the fruit, among those tested.

Keywords: Antioxidants, Flavonoids, Guava, Polyphenols, *Psidium guajava* L.

INTRODUCTION

Despite the scarcity of studies in this regard, it has long been known that plants are a rich source of organic compounds that can be extracted economically and in sufficient quantities to be used as raw materials for scientific studies and commercial and technological applications[1,2].

The interest in using these organic compounds derived from plants arises from the fact that the consumption of fruits and vegetables is associated with reduced incidence of cancer and cardiovascular diseases, while many bioactive compounds are beneficial to human health. Recently, scientists have found evidences that specific combinations of phytochemicals are more effective in protecting against diseases than the isolated compounds[3], pointing to a need to study the synergy among active compounds in plants, for example, by experimenting with plant extracts.

Psidium guajava L. is an evergreen tree of the Myrtaceae family found in several tropical and subtropical regions in the world, which produces the guava, one of the most nutritious fruits of these regions[4,5], which has been gaining visibility in the agro-food industry due to its taste and high nutritional value[6]. The products that result from its industrial processing are juice, squash, jam, jelly and the guava itself. The fruit contains sugars, iron, calcium, phosphorus and vitamins A, B and C in higher concentrations than most other fruits. It is rich in fiber and carotenoids, particularly lycopene, a substance important in the prevention of some types of cancer[4,7-8].

Many pharmacological activities of this plant have been described: antioxidant, hepatoprotective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, antitussive, antidiabetic, anti-inflammatory and antinociceptive activities, stimulating many studies with this plant[9].

In guava there are phenolic compounds, especially the flavonoids, and vitamin C, both of which are powerful antioxidants, of great importance in preventing the action of free radicals in the body and, therefore, able to prevent cancer and help to prevent premature skin aging[10-12]. The health-related properties possessed by phenolic compounds are based mainly on their antioxidant activity, particularly as free-radical scavengers[13] and as metal chelators capable of catalyzing the peroxidation of lipids[14,15].

It has been demonstrated that polyphenols have the ability to counteract the toxicity, mutagenicity and carcinogenicity of various chemical products[16-18]. Since these compounds can be beneficial to human health, the aim of this study was to improve the extraction procedure of secondary metabolites from guava fruits in order to increase the levels of phenolic compounds and flavonoid in the extract, assessing their contents by colorimetric assays associated with the study of its chromatographic profile and antioxidant activity. The techniques employed in this study to assess the extracts are constantly used because they are simple, inexpensive and provide meaningful data[19,20].

Other studies have assessed the phenolic and flavonoid contents of guava fruit[6,21,22], but usually it has been done in the fresh fruit and without stabilizing and concentrating the active compounds by drying the guava.

MATERIAL AND METHODS

Chemicals and reagents

Sodium hypochlorite 0.2% (LabSynth), ethanol (LabSynth and Chemis), Folin-Ciocalteau reagent (Imbralab), sodium carbonate (Na_2CO_3) (Quimex), gallic acid (Sigma), sodium nitrite ($NaNO_2$) (Merck), aluminum chloride ($AlCl_3$) (Merck), sodium hydroxide (NaOH) (Chemis), rutin (Sigma), quercetin (Sigma), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Sigma-Aldrich), methanol PA and HPLC grade (Merck, Chemis), ascorbic acid (Galena), ABTS (Sigma), potassium persulfate (Synth), solid phase extraction cartridges (SPE) of reverse phase (C18) 200 mg/3 mL (Phenomenex), Phenomenex Synergi Hydro C_{18} column (250 × 4.6 mm i.d.; 3.5 µm particle size), membrane filter 0.45 µm pore size (Millipore), acetonitrile (Mallinckrodt, Baker Inc), trifluoroacetic acid (Riedel-deHaën).

Plant material

P. guajava L. (Myrtaceae) fruits, Paluma cultivar, were picked trees in March 2009 in São Lourenço do Turvo, São Paulo (Brazil).

Preparation of fruit extracts

The whole guavas were disinfected in a sodium hypochlorite solution (0.2%) for 2 hours and then rinsed in distilled water. Next, fruits were cut into small pieces, dried at 60 ^{0}C to constant weight (around 72 hours) in an air circulation chamber, and finely powdered in a knife mill (590 µm mesh size). Samples of 50 g of the milled plant were percolated with 1.5 L of ethanol (70 vol%, 90 vol% or absolute) at room temperature, or macerated with the same volume of deionized water (Gehaka, São Paulo, Brazil) at 5±2 ^{0}C for 96 hours. Before the percolation process, 300 mL of the water-ethanol mixture was left in contact with the fruit powder for 18 hours, after which 1.2 L of the mixture was added and the percolation process was carried out for 78 hours.

The extracts were filtered through filter paper, concentrated under reduced pressure (MA-120, Marconi rotavapour) at 60 °C, frozen, and lyophilized (Modulyo D Freeze Dryer, Thermo Scientific). The fruit extracts were stored in amber flasks at a controlled temperature (5 ±2 °C) and assigned according to their extractor liquid as E70 (70% aqueous-ethanol), E90 (90% aqueous-ethanol), E100 (absolute ethanol), EAq (distilled water) extracts.

Phytochemical analysis of fruit extracts

The phytochemical screening of guava fruit extracts was performed according to standard procedures in order to identify the major secondary metabolites present[23,24]. A combination of tests was applied to hydroethanolic solutions of extracts, which were subjected to thin-layer chromatography (silica gel 60 F_{254} aluminum sheets, 20 × 20 cm, 0.2 mm thickness, Sorbent Technologies). Alkaloids were investigated by spraying the TLC plates with Dragendorff's and iodoplatinate reagents, associated with precipitation reactions with Bertrand, Mayer, Bouchadart, and Hager reagents. Flavonoids were detected by their intense fluorescence under UV light on TLC after spraying with natural product/polyethylene glycol (NP/PEG) and 5% aluminum chloride, in addition to the results from Shinoda, Pew, Pacheco and Taubock reactions. Tannins were characterized by precipitation reactions with 5% ferric chloride in methanol and with 1% gelatin solution. Coumarins were investigated by their fluorescence under UV light after spraying TLC plates with 10% potassium hydroxide in methanol. Triterpenes and steroids were analyzed by Liebermann-Buchard, Keller-Kiliani, Pesez and Kedde reactions, and by spraying anisaldehyde-sulphuric acid reagent on TLC (observed after heating the plate for 5 min at 100 °C). Anthraquinones were investigated by the Borntrager reaction. For saponins was used the foaming test. The Guignard (picrate) reaction was used for cyanogenic glycosides and Fehling's reagent for reducing sugars.

The fruit extracts were additionally analyzed by High Performance Liquid Chromatography coupled to Photodiode Array Detection (HPLC-UV/PAD). A Jasco 2000 HPLC system was used, fitted with a PU-2089 Plus pump, a MD-2010 Plus photodiode array detector, and an AS-2055 Plus autosampler. Prior to HPLC analysis, fruit extracts (40 mg) were subjected to solid-phase extraction cartridges (Sep-Pak C_{18}, 200 mg, Phenomenex), eluted with methanol:water (8:2, 2.0 mL, v/v). The HPLC eluents were filtered through a 0.45 µm polytetrafluoroethylene (Millex, Millipore) membrane filter and subjected to HPLC-PAD analysis. Separation was achieved with a Phenomenex Synergi Hydro C_{18} column (250 × 4.6 mm i.d.; 3.5 µm particle size) equipped with a Phenomenex security guard column (4.0 × 2.0 mm i.d.). The mobile phase consisted of water (eluent A) and acetonitrile (eluent B), both containing 0.05% of trifluoroacetic acid. The gradient program was as follows: 5-10% B for 10 min, 10-40% B for 40 min, and 40-95% B for 60 min, at a flow of 1.0 mL/min. Peaks were monitored by PAD, scanning from 200 to 600 nm. The program EZCrom Elite 3.1.7 (Agilent Technologies) was used for data acquisition and processing. The room temperature was kept at 25 °C by an air-conditioning system.

Total Phenolic Content

Total phenolic content was determined by reaction with Folin-Ciocalteu reagent (Imbralab), as described by Singleton and Rossi (1965)[25] and Kähkönen *et al.* (1999)[26]. Briefly, solutions of the four extracts in deionized water were prepared to give a final concentration of 6 mg/mL. Each solution (400 µL) was mixed with 2 mL of Folin-Ciocalteu reagent (which had previously been diluted 10-fold with distilled water) and allowed to stand at room temperature for 8 min; 1.6 mL of 7.5% Na_2CO_3 solution was added to the mixture. Contents of the tubes were mixed and allowed to stand in the dark for 30 min, after which the absorbance was measured at 765 nm (Hitachi U-2001). The results were expressed as gallic acid equivalents per 100 mg of crude extract (mg GAE/100 mg extract).

Flavonoid content

Flavonoid content was determined by the procedure described by Zhishen *et al*. (1999)[27]. Solutions of the four extracts in deionized water were prepared to give a final concentration of 6 mg/mL. Each solution (5 mL) was mixed with 0.3 mL of 5% aqueous $NaNO_2$ (w/v) and allowed to stand at room temperature for 5 min; 0.6 mL of 10% $AlCl_3$ solution (w/v) was added to the mixture. After 6 min, 2 mL of 1M NaOH and 2.1 mL of water were added to the mixture. The absorbance was measured at 510 nm (Hitachi U-2001). The results were expressed as quercetin (Q) and rutin (R) equivalents per 100 mg of crude extract (mg Q or R/100 mg extract).

DPPH Method

The radical scavenging activity was determined by using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Sigma-Aldrich), as in Mensor *et al.* (2001)[28] and Falcão *et al.* (2006)[29], with modifications. Solutions of the four extracts in deionized water were prepared to give final concentrations in the range of 0 to 30 mg/mL. An aliquot of each solution (1 mL) was mixed with 2.5 mL of 0.004% DPPH solution in methanol. After 30 minutes of incubation in the dark, the absorbance was measured at 515 nm. The antioxidant activity was expressed as IC_{50}, defined as the final concentration of the tested sample required to scavenger 50% of the DPPH radical. Linear regression analysis was used to calculate the IC_{50} values[30]. The same procedure was followed with ascorbic acid, a standard antioxidant substance.

Statistical Analysis

All determinations were performed at least in triplicate; average values and standard deviations (SD) were calculated. Differences were analyzed by ANOVA and Tukey's test if necessary. Significance was accepted if $p<0.05$.

RESULTS AND DISCUSSION

Preparation of fruit extracts

When the plant material was dried to constant weight, it lost 84.48 ± 0.33% of its original weight, showing that this is the percentage of water in guava. This result corroborates those of Medina and Pagano (84.3 ± 0.1%)[31] and Franco (86.07%)[32]. This step allowed better preservation of the plant material, since high humidity enables the proliferation of microorganisms and occurrence of enzymatic reactions which could impair its quality[33].

During the powdering process, the hard seeds remained unbroken and were separated from the powder, despite the presence of phenolic compounds, because the presence of cytotoxic compounds in the seeds, such as phenylethanol glycosides, could have undesired effects when using these extracts in high concentrations in food, pharmaceutical or cosmetic products[34,35].

The size of the fruit particles is an important parameter that should be taken into consideration to optimize the phenol content in the extracts. For this reason 590 µm mesh was selected for the extract preparation. The reduction of the particle size led to increase of the contact surface area between plant material and extraction solvent, promoting a more efficient extraction process and increasing the yield of extract, while reducing the volume of solvent used and decreasing the extraction time[36].

The extraction procedure was carried out by using water and ethanol as extractor liquids. This choice was based on two concepts: the molecular affinity between the antioxidant compounds and these solvents and, also, the inherent safety in using these liquids and their low impact on the environment[37-39]. Another advantage is easy availability and low cost[39].

Four different solvent systems were used: water, absolute ethanol, and aqueous ethanol at 90 vol% and 70 vol%. According to a review of techniques[37], the steps employed in the present study to choose the best extraction method for bioactive compounds from guava were appropriate, since, for the identification and isolation of bioactive compounds from natural sources is suggested the extraction with solvents of varying polarities. This procedure allows results to be compared, so as to optimize extraction efficiency.

The use of percolation as the method of extraction was based on the simplicity of this process, which does not waste energy and needs no

filtration step, and can be done at room temperature and without incidence of radiation. The two last characteristics are of great importance when dealing with compounds sensitive to light and to temperature, such as phenolic compounds. Moreover, according to Kassing *et al*. (2010)[40], in the percolation process there is a constantly high concentration gradient which results in an almost complete leaching of extractable components.

However, in the case of extraction with water as solvent, the fine particle size hindered the flow of the solvent through the plant material, excluding the use of percolation as the extraction method in this case. For this reason, the aqueous extract was obtained by maceration.

The extraction was performed at room temperature, thus avoiding high temperatures, as the plant extract is rich in polyphenols whose structures are readily modified.

The yield of each extract is shown in Table 1. Statistically, the yield of the extraction in 70 % ethanol (E70), 90% ethanol (E90) and water (EAq) were equal. Only the extract obtained in absolute ethanol (E100) had a lower yield. Hence, on the industrial scale, E100 would probably be of less interest. However, this parameter cannot be used alone in choosing the best extraction process, since the amounts of compounds of interest present in the extracts should also be analyzed. For this reason, phytochemical analyses were performed, to examine the contents of such compounds in each extract.

Table 1: Total yield of extracted solids in 70 vol% and 90 vol% aqueous ethanol, absolute ethanol and water.

Extract	Extraction 1	Extraction 2	Extraction 3	Mean	SD	Yield (%)
E70	22.30	22.73	23.17	22.73[a]	0.43	45.03
E90	23.89	23.11	21.63	22.88[a]	1.15	45.76
E100	15.27	17.48	15.81	16.19[b]	1.15	32.37
EAq	18.68	22.64	20.32	20.55[a]	1.99	41.10

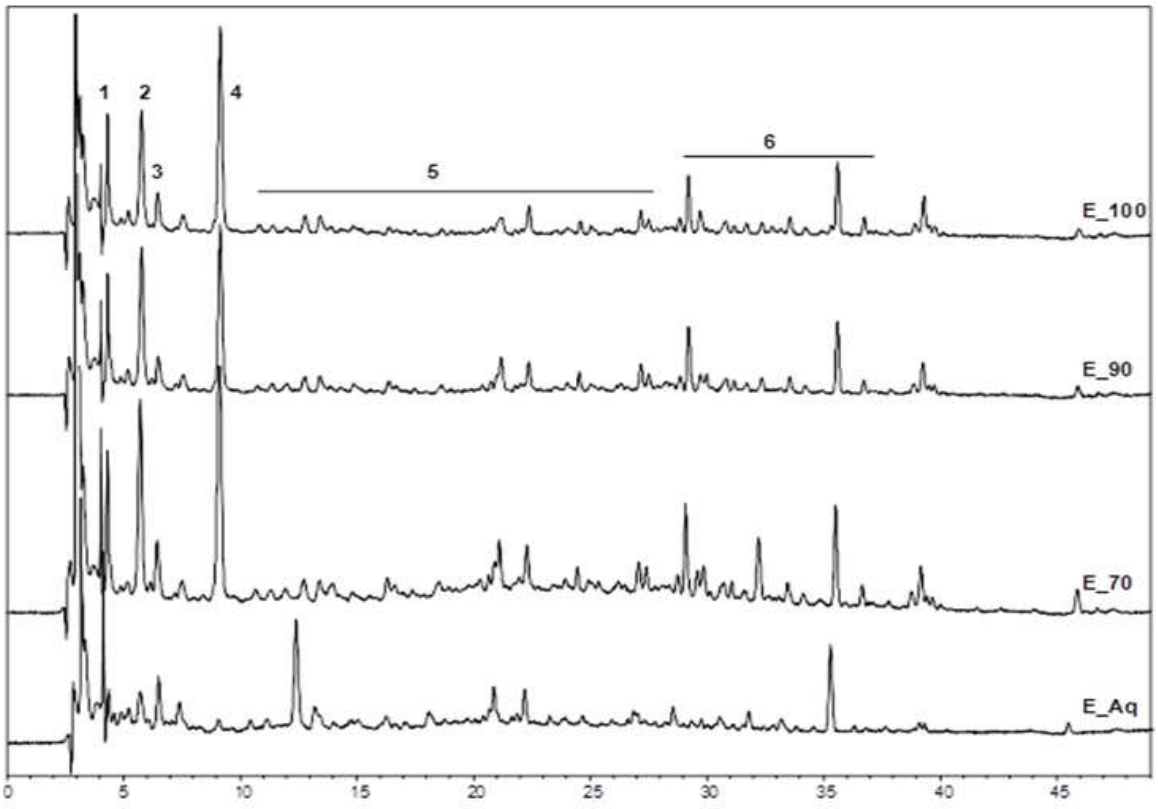
[a,b] At $p<0.05$, the yields are different for different letters.

Phytochemical analysis

Phytochemical screening by TLC indicated a qualitatively similar chemical composition in all fruit extracts. The main classes of secondary metabolites were suggested to be phenolic compounds, flavonoids, tannins, terpenes, steroids and reducing sugars; while alkaloids, anthraquinones, coumarins and cyanogenic glycosides were not detected.

The HPLC-UV-PAD fingerprints of the fruit extracts are shown in Figure 1. Despite a few differences in the number of peaks, from our analysis of the UV spectrum and retention times (R_t) of the eluted peaks we suggest that all chromatograms (at 254 nm) are qualitatively similar, with respect to the major compounds. Peaks 1 (R_t 4.2 min), 2 (R_t 5.5 min), 3 (R_t 6.3 min) and those in region 5 (R_t 10.2—28.7 min) exhibited two absorption maxima, around 209 and 260-290 nm, which is indicative of phenolics, without any specific assignment. Peak 4 (R_t) showed intense UV bands at 224 and 284 nm for another phenolic compound. In the region 6 (R_t 29—36 min), the UV spectrum displays bands of maximum absorption at 257 and 359 nm, typical for flavonoids[41].

A general comparison of the hydroalcoholic extracts E70, E90 and E100 suggested that they are similar, probably because of the proximity in polarity of the solvents, which implies that these three solvents were able to extract the same class of metabolites. The most significant difference between these hydroalcoholic extracts appears to be in the concentration of the active phenolics, since the peak areas of E70 suggest higher concentrations in this extract than in the others, leading to the conclusion that 70 vol% ethanol was the solvent that extracted the compounds of interest to this study most efficiently. On the other hand, the chemical fingerprint of EAq showed more pronounced qualitative differences, suggesting that the use of water to extract the guava led to extraction of the same groups of metabolites, but in different amounts.



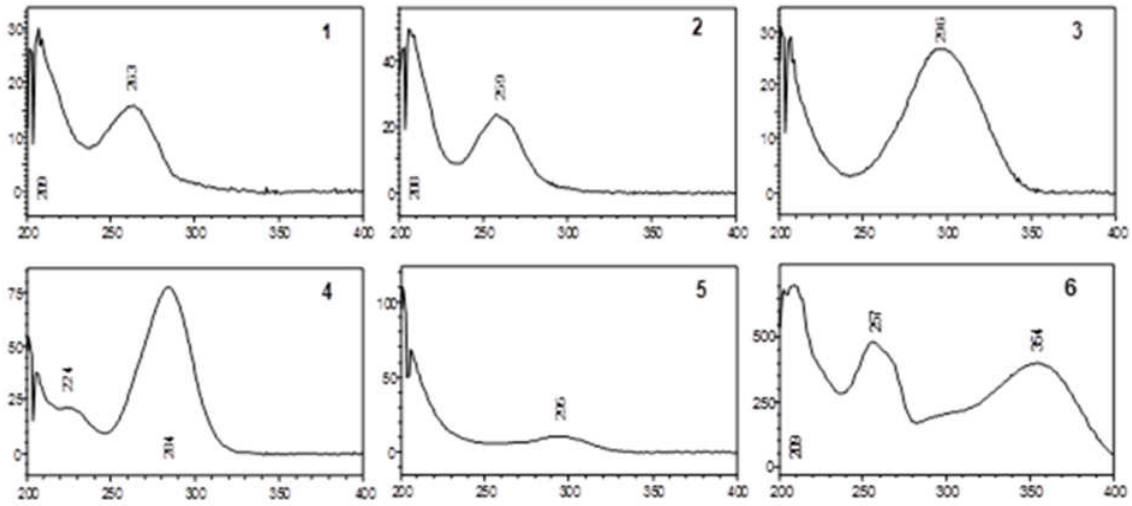


Fig. 1: HPLC-UV/PAD profiles and UV spectra of the representative compounds in the fruit extracts of *P. guajava* L. For chromatography conditions see text.

Total phenolic content

The total phenolic content results (Table 2) showed that the most effective solvent to extract phenolic compounds from the dried and powdered *P. guajava* L. was 70% (v/v) aqueous ethanol, followed by water and finally by 90% ethanol and absolute ethanol, these last two showing the same extraction efficiency. These results are consistent with a study by Shouqin *et al.* (2005)[42], which showed that the amount of flavonoids extracted is strongly influenced by the concentration of ethanol in water, rising with increasing ethanol concentration up to 75 vol%, but falling with increasing ethanol concentration beyond this point.

Combining these results with the total yield of the extraction, it is possible to infer that the E70 and EAq extracts are of greater interest.

It can be seen that, while both ethanol and water are solvents able to extract phenolic compounds, the mixture of these solvents containing ethanol at 70 vol% has a greater capacity in the extraction of these compounds from *Psidium guajava* L.

Alothman *et al.* (2009)[22] assayed the total phenolic content of the Thai seedless cultivar of guava, using the fresh fruit, and obtained 155 ± 2.12 mg GAE/100 g fresh weight which corresponds to 15.39% of the content found in the E70 extract in this study.

Flavonoid content

The flavonoid content of the extracts was investigated because of the interest in exploiting the power of these compounds to reduce oxidative damage to cells, demonstrated in several studies[43]. The results obtained are shown in Figure 2.

Table 2: Total phenolic content expressed as gallic acid equivalents per 100 milligrams of crude extract (mg GAE/100 mg extract)

Extract	Extraction 1	Extraction 2	Extraction 3	Mean	SD
E70	0.99234	1.162536	1.059676	1.071517 [a]	0.085713
E90	0.746877	0.701582	0.745857	0.731439[b]	0.025862
A100	0.606330	0.709860	0.665709	0.660633[b]	0.051951
EAq	0.859739	0.879807	0.870251	0.869932[c]	0.010038

[a, b, c] Different letters indicate that the phenolic content in the extracts are significantly different ($p<0.05$).

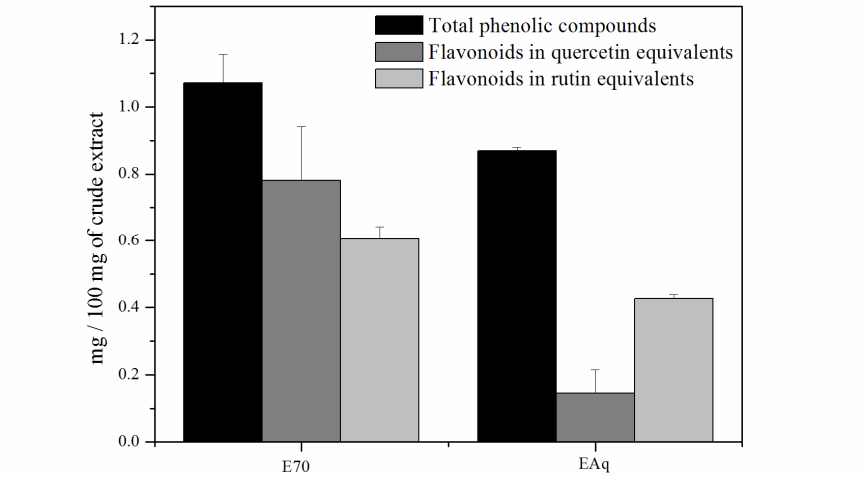


Fig. 2: Concentration of total phenolic compounds in gallic acid equivalents and flavonoids in quercetin and rutin equivalents in the E70 and EAq crude extracts

The results obtained for the extracts in 90 vol% ethanol and absolute ethanol could not be used, since, during the assay to determine the flavonoid content, the solution prepared for absorbance readings in the spectrophotometer was found to be turbid. Some component present in these extracts must have been insoluble in the reaction mixture or some chemical reaction between the constituents led to an insoluble product. Results of a literature search suggest that pectin or hemicellulose, extracted from the cell walls of the fruit, may become insoluble on the addition of sodium hydroxide during the assay to determine the flavonoid content[44].

As was observed in the determination of phenolic compounds, the E70 extract showed a higher concentration of flavonoids than the EAq extract. Probably, if it had been possible to obtain results for the E90 and E100 extracts, they would have contained lower concentrations of these components, since flavonoids are a common class of phenolic compounds.

It was also observed that the concentration of flavonoids in E70 in quercetin equivalents was higher than that measured in rutin equivalents (Figure 2), differently from what is observed in EAq.

In Figure 1, it may be noted that the results are consistent, in that a higher concentration of phenolic compounds than flavonoids was found, as expected, because it is known that the phenolic compounds of guava are mainly represented by flavonoids and tannins.

Antioxidant activity

The analytical curve (inhibition percent of the DPPH radical *versus* ascorbic acid concentration in µg/mL) produced by measuring the scavenging of DPPH radical by ascorbic acid is described by the equation: y = 20.78 x – 0.63 (R = 0.99947), so that the IC_{50} of ascorbic acid is 2.4365 µg/mL.

The results of the assay of antioxidant activity for the four crude extracts are shown in Figure 3 and Table 3.

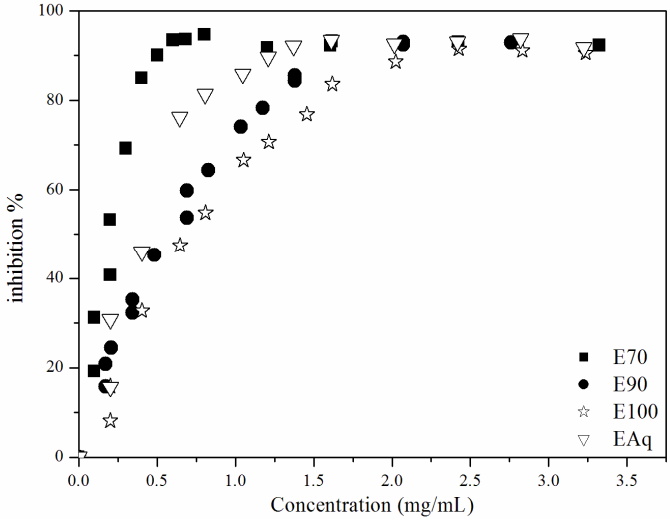


Fig. 3: Antioxidant activity of the crude extracts measured by the capacity to scavenger the DPPH radical

The antioxidant activity of the extracts can be compared with their concentrations of phenolics and flavonoids and it can be observed that a correlation exists between the amounts of these compounds and the antioxidant activity[45], since these components, together with ascorbic acid, also present in *P. guajava* L. extracts, are probably responsible for most of this effect in the extracts.

In Table 3 are listed the IC_{50} values for each of the extracts. To obtain these values, a curve of inhibition percent of DPPH *versus* the concentration of the extract was built. These curves provided enough information to allow the amount of extract needed to quench 50% of the free radicals in the reaction mixture to be calculated. The results were obtained in triplicate for each of the extracts and for three separate extractions.

Table 3: IC_{50} values of the crude extracts obtained (mg of crude extract/mL)

Extract	Extraction 1	Extraction 2	Extraction 3	Mean	SD
E70	0.29319	0.29234	0.28616	0.29056[a]	0.00383
E90	0.68617	0.67415	0.70747	0.68926[b]	0.01687
E100	0.89163	0.93128	0.85919	0.89403[c]	0.03611
EAq	0.7008	0.67036	0.6598	0.67699[b]	0.02129

[a, b, c] Different letters indicate that the IC_{50} values of the extracts are different ($p<0.05$).

The antioxidant activity found in E70 allows several potential uses to be suggested, including: cosmetic use in the prevention of premature skin aging[46,47] and chemoprevention of cancers and other chronic diseases[48-52].

Finally, from this study it may be concluded that guava is a fruit rich in bioactive compounds that might be used in various ways to offer to the population the possibility of preventing certain chronic diseases at low cost.

ACKNOWLEDGMENTS

To Predilecta Food Industry (Brazil) for donating the guava fruits and to FAPESP, CNPq and PADC for the financial support.

REFERENCES

1. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal materials. Science 1985;228:1154-1160.
2. Harvey A. Strategies for discovering drugs from previously unexplored natural products. DDT 2000;5(7):294-300.
3. De Kok TM, Van Breda SG, Manson MM. Mechanisms of combined action of different chemopreventive dietary compounds: a review. Eur J Nutr 2008;47(2):51-59.
4. Lima MAC, Assis JS, Neto LG. Caracterização dos frutos de goiabeira e seleção de cultivares na região do submédio São Francisco. Rev Bras Frutic 2002;24(1):273-276.

5. Duarte MR, Paula FM. Morfodiagnose de *Psidium guajava* L., Myrtaceae. Visão Acadêmica 2005;6(2).
6. Corrêa LC, Santos CAF, Vianello F, Lima GPP. Antioxidant content in guava (*Psidium guajava*) and araçá (*Psidium* spp.) germplasm from different Brazilian regions. Plant Genetic Resources: Characterization and Utilization 2011;9(3):384–391.
7. Padula M, Rodriguez-Amaya DB. Characterization of the carotenoids and assessment of the vitamin A value of Brazilian guavas (*Psidium guajava* L.). Food Chem 1986;20:11-19.
8. Monteiro S. Esperança das goiabas. Rev. Frutas Derivados 2006;3:27–30.
9. Gutiérrez RMP, Mitchell S, Soli RV. *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 2008;117:1–27.
10. Martin KR, Failla ML, Smith Jr JC. β-carotene and lutein protect HepG2 human liver cells against oxidant induced damage. J Nutr 1996;126(9):2098-2106.
11. Martínez A, Ikken Y, Cambero MI, Marín ML, Haza AI, Casas C, Morales P. Mutagenicity and cytotoxicity of fruits and vegetables evaluated by the Ames test and 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) assay. Food Sci Tech Int 1999;5(5):431-437.
12. Etcheverry SB, Ferrer EG, Naso L, Rivadeneira J, Salinas V, Williams PA. Antioxidant effects of the VO(IV) hesperidin complex and its role in cancer chemoprevention. J Biol Inorg Chem 2008;13(3):435-447.
13. Prior RL, Cao G. Antioxidant phytochemicals in fruits and vegetables. Diet and health implication. Hortic Sci 2000;35:588-592.
14. Da Silva EL, Piskula MK, Yamamoto N, Moon JH, Terao J. Quercetin metabolites inhibit copper ion-induced lipid peroxidation in rat plasma. FEBS Lett 1998;430:405-408.
15. Terao J, Piskula MK. Flavonoids and membrane lipid peroxidation inhibition. Nutrition 1999;15:790-791.
16. Bartsch H, Hiroshi O, Pignatelli B. Inhibitors of endogenous nitrosation. Mechanisms and implications in human cancer prevention. Mutat Res 1988;202:307-324.
17. Ames BN, Gold LS. Endogenous mutagens and the causes of aging and cancer. Mutat Res 1991;250:3-16.
18. Agarwal R, Katiyar SK, Khan SG, Mukhtar H. Protection against ultraviolet B radiation-induced effects in the skin of SKH-1 hairless mice by a polyphenolic fraction isolated from green tea. Photochem Photobiol 1993;58(5):695-700.
19. Dani C, Oliboni LS, Vanderlinde R, Pra D, Dias JF, Yoneama ML, Bonatto D, Salvador M, Henriques JAP. antioxidant activity and phenolic and mineral content of rose grape juice. J Med Food 2009;12(1):188–192.
20. Erdogan-Orhan I, Sever-Yılmaz B, Levent Altun M, Saltan G. Radical quenching activity, ferric-reducing antioxidant power, and ferrous ion-chelating capacity of 16 ballota species and their total phenol and flavonoid contents. J Med Food 2010;13(6):1537–1543.
21. Gorinstein S, Zemser M, Haruenkit R, Chuthakorn R, Grauer F, Martin-Belloso O, Trakhtenberg S. Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. J Nutr Biochem 1999;10:367–371.
22. Alothman M, Bhat R, Karim AA. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem 2009;115:785–788.
23. Wagner H, Bladt S, Zgainsky E. Plant drug analysis: a thin layer chromatography atlas. Berlin: Springer Verlag; 1984.
24. Matos FJA. Introdução a fitoquímica experimental. 3th ed. Fortaleza: Edições UFC; 2009.
25. Singleton VL, Rossi Jr JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-158.
26. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999;47:3954-3962.
27. Zhishen H, Mengcheng T, Jianmin W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;64:555-559.
28. Mensor LL, Menezes FS, Leitão GG, Reis AS, Dos Santos TC, Coube CS, Leitão SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001; 15:127–130.
29. Falcão DQ, Costa ER, Alviano DS, Alviano CS, Kuster RM, Menezes FS. Atividade antioxidante e antimicrobiana de *Calceolaria chelidonioides* Humb. Bonpl. & Kunth. Rev Bras Farmacog 2006;16:73-76.
30. Singh S, Garg V, Yadav D, Beg M, Sharma N. *In-vitro* antioxidative and antibacterial activities of various parts of *Stevia rebaudiana* (Bertoni). *Int J Pharm Pharm Sci* 2012;4(3):468-473.
31. Medina MLB, Pagano FG. Characterization of guava pulp (*Psidium guajava* L.) "Criolla Roja". Rev Fac Agron 2003;20:72-86.
32. Franco G. Tabela de Composição Química dos Alimentos. 9th ed. São Paulo: Ed. Atheneu; 2003.
33. Córdova KRV, Gama TMMTB, Winter CMG, Kaskantzis Neto G, De Freitas RJS. Características físico-químicas da casca do maracujá amarelo (*passiflora edulis* flavicarpa degener) obtida por secagem. B.CEPPA Curitiba 2005;23(2):221-230.
34. Michael HN, Salib JY, Isaac MS. Acylated flavonol glycoside from *Psidium guajava* L. seeds. Pharmazie 2002;57:859–860.
35. Salib JY, Michael HN. Cytotoxic phenylethanol glycosides from *Psidium guajava* seeds. Phytochem 2004;65:2091–2093.
36. Mandal V, Mohan Y, Hemalatha S. Microwave assisted extraction – an innovative and promising extraction tool for medicinal plant research. Pharmacognosy Reviews 2007;1(1):7-18.
37. Andreo D, Jorge N. Antioxidantes naturais: técnicas de extração. B.CEPPA Curitiba 2006;24(2):319-336.
38. Wang L, Weller CL. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci Technol 2006;17:300–312.
39. Mylonaki S, Kiassos E, Makris DP, Kefala P. Optimisation of the extraction of olive (*Olea europaea*) leaf phenolics using water/ethanol-based solvent systems and response surface methodology. Anal Bioanal Chem 2008;392:977–985.
40. Kassing M, Jenelten U, Schenk J, Strube J. A new approach for process development of plant-based extraction processes. Chem Eng Technol 2010;33(3):377–387.
41. Mabry TJ, Markham KR, Thomas MB. The systematic identification of flavonoids. New York: Springer-Verlag; 1970.
42. Shouqin Z, Jun X, Changzheng W. High hydrostatic pressure extraction of flavonoids from própolis. J Chem Technol Biotechnol 2005;80:50–54.
43. Prior RL. Fruits and vegetables in the prevention of cellular oxidative damage. Am J Clin Nutr 2003;78:570S–8S.
44. Renard CMGC, Voragen AGJ, Thibault J-E, Pilnik W. Studies on apple protopeetin. IV: apple xyloglueans and influence of pectin extraction treatments on their solubility. Carbohydrate Polymers 1991;15:387-403.
45. Choudhury KD, Choudhury D, Paul SB. Antioxidant activity of leaf extracts of *Lasianthus lucidus* Blume. Int J Pharm Pharm Sci 2012; 4(3):533-535.
46. Ropke CD, Sawada TC, Silva VV, Michalany NS, Barros SBM. Photoprotective effect of *Pothomorphe umbellata* root extract against ultraviolet radiation induced chronic skin damage in the hairless mouse. Clin Exp Dermatol 2005;30:272-276.
47. Almeida RL, Da Silva VV, Rivelli DP, Miranda DV, Sawada TCH, Barros SBM, Ropke CD. Padronização e determinação da fotoestabilidade do extrato de folhas de *Pothomorphe umbellata* L. Miq (pariparoba) e avaliação da inibição *in vitro* de metaloproteinases 2 e 9 na pele. Rev Bras Ciênc Farm 2008;44(1):43-50.
48. Steinmetz KA, Affiliations
49. K. A. Steinmetz is a scientific officer for the World Cancer Research Fund, London, England, and a consulting nutrition epidemiologist in Forchheim, Germany.
50. Address correspondence to: Kristi A. Steinmetz, PhD, RD, Kersbacher Strasse 33A, 91301 Forchheim, Germany.
51. Potter JD. Vegetables, fruit, and cancer prevention: a review. J Am Diet Assoc 1996;96(10):1027-1039.
52. Adom KK, Liu RH. Antioxidant activity of grains. J Agric Food Chem 2002;50(21):6182–6187
53. Sun J, Chu Y, Wu X, Liu RH. Antioxidant and antiproliferative activities of common fruits. J Agric Food Chem 2002;50(25):7449–7454.
54. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004;74(17):2157-2184.
55. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr 2004;134:3479S-3485S.