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Assessment of mineral substances level and antioxidant potential in leaves of three guava tree varieties

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Guava tree leaves are used empirically for the treatment and prevention of several pathologies, however, few studies report the chemical composition and the biological activity of the species. In this context, this study was conducted with the objective of evaluating the chemical composition and the antioxidant activity of the leaves of Paluma, Pedro Sato and Século XXI cultivars. The leaves were oven dried at 37°C, for five days. After drying they were ground in order to obtain the meal. The parameters analyzed were phenolic compounds, ascorbic acid, dietary fiber, minerals and antioxidant activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and beta-carotene/linoleic acid methods. The Século XXI cultivar presented the highest contents of phenolic compounds, dietary fiber and antioxidant activity by the DPPH method, as well as the contents of the minerals copper and manganese. The contents of ascorbic acid and iron were higher for Paluma and Século XXI cultivars. Cultivar Paluma presented the highest percentages of oxidation inhibition for linoleic acid and for the mineral magnesium. Cultivar Pedro Sato presented the highest iron content. Regarding zinc, there was no significant difference among the cultivars analyzed.

Key words: Phenolic compounds, dietary fiber, minerals.

INTRODUCTION

Since ancient times, man has sought to know the plants and their medicinal properties, based on casual discoveries which have been proven by science. Approximately 25% of the drugs used are of plant origin, 75% synthetic and 50% are related to the isolated principles of medicinal plants (Silva and Mura, 2007).

In every part of the plant, active substances with various types of molecular structures, synthesized from the primary metabolism, named secondary metabolites, can be found. The search for substances with different biological activities has attracted the interest of many researchers. In this context, plants that have antioxidant activity have been studied, since reactive oxygen species can promote the oxidation of proteins, lipids and DNA that contribute to the development of cancer, diabetes, atherosclerosis, inflammation and aging (Fabri et al., 2011).

Guava tree (*Psidium guajava* L.) is a fruit tree belonging

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to the Myrtaceae family (Ital, 1988). Its leaves were introduced into popular therapy to alleviate relieve, cure and prevent various diseases. They have been used for the control of diabetes mellitus, hypertension (Ojewole, 2005), gastrointestinal disorders (diarrhea, stomachache, gastrointeritis, indigestion and dysentery), cardiovascular diseases, anti-inflammatory (Ojewole, 2006), antimicrobial, antioxidant (Thaipong et al., 2006), hepatoprotective, anticancer and anti-malarial actions (Roy et al., 2006).

Thus, the present study aimed to investigate the antioxidant potential and levels of antioxidants and minerals present in the leaves of guava Paluma, Pedro Sato and Século XXI.

MATERIALS AND METHODS

The new leaves of young guava tree cultivars Pedro Sato, Paluma and XXI Century were collected in the city of Lavras in May, 2009. All the leaves of the three cultivars were exposed to the same environmental conditions. The leaves were placed in an oven at 37°C for five days. After this period, they were crushed and ground in a mill, in order to obtain the flour. The leaves were identified by the College of Agriculture Lavras Herbarium where a voucher specimen was deposited which received the voucher number: Paluma No. 26276, No. 26277 Pedro Sato and Século XXI n ° 26278.

A completely randomized design with 3 treatments (tree cultivars Pedro Sato, Paluma and Século XXI) and 7 replicates was used. The results were submitted to analysis of variance using the program Sisvar and when significant, the means for each treatment were compared by the Tukey test at 5% probability. The flours of the three cultivars were submitted the following analyses.

Dietary fiber

The soluble and insoluble dietary fiber content was determined through the Sigma Total Dietary Fiber Kit. This method is based on non-hydrolyzed portion which resists food sequential enzymatic digestion with α-amylase, protease, amyloglucosidase and is insoluble in ethanol and between 78 and 98%. The results were expressed in gram/100 g of sample (Association of Official Analytical Chemists- AOAC, 2005).

Minerals

The minerals analyzed were iron, potassium, copper, magnesium, manganese and zinc. The samples were analyzed in extracts obtained by nitric-perchloric acid digestion and readings were performed in an atomic absorption spectrophotometer (Malavolta et al., 1997).

Phenolic compounds

The extraction was carried out with 50% methanol at a rate of 3 g sample diluted with 250 ml of 50% methanol at reflux for three consecutive times at 80°C and the extracts combined, evaporated to 25 ml (Goldstein and Swain, 1963) and subjected to determination using the Folin-Denis reagent, which is reduced by the phenols, a blue colored complex in alkaline solution and measured at 760 nm (AOAC, 2005). The results were expressed as mg gallic acid, 1 g sample dry.

Determination of antioxidant activity

Four dilutions were made from the extracts used for the determination of phenolic compounds.

1,1-diphenyl-2-picrylhydrazyl (DPPH)

The ability to sequester DPPH was performed according to the method described by Thaipong et al. (2006) with modifications. An 0.1 ml aliquots of each dilution were added to 3.9 ml of the DPPH solution in methanol (0.06 mM). At the end of 30 min, the absorbance was measured at 515 nm and the ability to sequester the radical, expressed in percentage, was calculated relatively to the control (no antioxidant), according to the following expression:

$$\text{Sequestration (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance sample}}{\text{Absorbance of control}} \times 100$$

Through the equation of the line, the concentrations (mg/ml) required to inhibit 50% of DPPH• were calculated. The synthetic antioxidant butylated hydroxytoluene (BHT) and quercetin were used as standards, and were subjected to the same conditions.

β-carotenoid/linoleic acid system

The β-carotene/linoleic acid solution system was prepared according to Rufino et al. (2007) with modifications. In test tubes, 5.0 ml of this solution system were added to 0.4 ml of the sample. The synthetic antioxidant BHT and quercetin were used as standard, and subjected to the same conditions. Readings were taken in a spectrophotometer at 470 nm. The tubes were placed in a water bath at 40° C and readings were taken before incubation and after 2 h. The decline in the absorbances read correlated with the system and the percentage of oxidation was set.

$$\text{Oxidation (\%)} = \frac{[(\text{Reduction Abs})\text{sample} \times 100]}{(\text{Reduction Abs})\text{system}}$$

$$\text{Protection (\%)} = 100 - (\text{Oxidation percentage})$$

The synthetic antioxidant BHT and quercetin were used as standards, and were subjected to the same conditions.

Ascorbic acid

The extraction was performed according to Strohecker and Henning (1967), and the extracts were filtered through a 0.22 m Millipore membrane before the chromatographic analysis. A Shimadzu Liquid Chromatograph LC 20A UV-Vis detector was used and the chromatograms were at 254 nm. A C_{18} column was used (250 × 4.6 mm × 5 μm). The mobile phase was buffer, a pH 6.7 containing sodium acetate, 0.004 mol/L ethylenediaminetetraacetic acid (EDTA) 0.05 mol/L, tributyl phosphate, 0.5 mol/L in an isocratic mode with a 0 6 ml/min flow rate, with a run time of 15 min (Silva et al., 2009). An analytical curve with 10 points in duplicate was made.

Table 1. Levels of antioxidants in leaves of three guava tree cultivars.

Cultivar	Phenolic compounds (mg/g DM)	Ascorbic acid (mg/100 g DM)
Século XXI	192.61 (±8.57) a	227 (±19.34)^{ab}
Paluma	174.78 (±6.98) b	256 (±20.70)^{a}
Pedro Sato	130.05 (±12.76) c	200 (±15.78)^{b}
CV (%)	3.20	11.10

Means followed by the same letter in the columns do not differ by the Tukey test at 5% probability. DM: Dry matter.

Table 2. Antioxidant activity of leaves from three guava tree cultivars.

Cultivar	DPPH (IC_{50})	β-carotene/Linoleic ácid (%)
Século XXI	0.0047 (±0.0005)^{c}	77.30 (±1.56)^{c}
Paluma	0.013 (±0.0067)^{b}	85.94 (±0.99)^{a}
Pedro Sato	0.023 (±0.0021)^{a}	82.93 (±1.78)^{b}
CV	4.03%	1.28%

Means followed by the same letter in the columns do not differ by the Tukey test at 5% probability.

Table 3. Content of insoluble and soluble dietary fiber (g 100 g-1 DM) in leaves of three guava tree cultivars.

Cultivar	insoluble dietary fiber	soluble dietary fiber
Século XXI	57.84 (±3.21)^{a}	4.34 (±0.78)^{a}
Paluma	57.10 (±2.67)^{a}	2.67 (±0.24)^{b}
Pedro Sato	42.32 (±1.93)^{b}	4.10 (±0.67)^{a}
CV (%)	4.27	9.56

Means followed by the same letter in the columns do not differ by the Tukey test at 5% probability.

The reagents were high performance liquid chromatography (HPLC) grade and milli-Q water.

RESULTS AND DISCUSSION

The antioxidant compounds present in the plants, include ascorbic acid, vitamin E, carotenoids and polyphenols (Haida et al., 2011). The amount and profile of these phytochemicals vary depending on the type, cultivar and degree of maturity of the plant, as well as weather and soil conditions.

The levels of antioxidants are presented in Table 1. It is observed that cultivar Século XXI showed the highest levels of phenolic compounds and Pedro Sato presented the lowest content. Cultivar Paluma stood out by ascorbic acid (256 mg 100 g^{-1} dry matter). Chen et al. (2007) found 154.36 mg/g of phenolic compounds, similar to this study. However, higher values (575.3 mg/g) were obtained by Qian and Nihorimbere (2004). This variation may be due to differences in varieties, climate and extraction method, because high temperatures (80°C) may degrade these compounds. Phenolic compounds have different chemical structures, but have at least one aromatic ring, with at least one hydrogen replaced by hydroxyl. They exhibit various biological effects such as anti-inflammatory, antimicrobial, hypolipidemic, anticancer, including antioxidant activity (Wojdylo et al., 2007).

The antioxidant activity of the plant is related to the levels of its antioxidants. The DPPH method assesses the ability of the sample to inactivate the DPPH radical by donating hydrogen, thus the IC_{50} percent is inversely proportional to the antioxidant activity. Phenolic compounds and ascorbic acid are capable of donating hydrogen to the radical (Volp et al., 2008). Among the cultivars, Século XXI showed a higher antioxidant activity (0.0047 mg/ml) and higher content of phenolic compounds (192.61 mg/g dry matter) in relation to the other cultivars analyzed (Table 2). Boscolo et al. (2007) studied the antioxidant activity of Pitangueira leaves, which belongs to the Myrtaceae family and found the IC_{50} percent to be 0.245 mg/ml, antioxidant activity lower than those found in the leaves of the three cultivars studied.

The IC_{50} values for the standards percent BHT and quercetin were 0.00002 and 0.01501 ± 0.00198 ± 0.00002 mg/ml. Only the leaves of Século XXI and Paluma cultivars showed a higher antioxidant activity than BHT levels. Regarding quercetin, no cultivar stood out.

The β-carotene/linoleic acid method evaluates the ability of the sample to protect the peroxidation of linoleic acid, thus the percentage of inhibition is directly proportional to the antioxidant activity. From Table 2, it is observed that cultivar Paluma showed the highest antioxidant activity. The percentage of inhibition patterns BHT and quercetin standards were 11.23% (± 0.06) and 9.14% (± 0.14), respectively. All cultivars showed inhibition percentage above the standards.

Table 3 shows that Paluma and Século XXI showed the highest levels of insoluble dietary fiber, soluble fiber has stood out for Século XXI and Pedro Sato, thus suggesting that their leaves are more efficient for the control of hypercholesterolemia. Data from literature (Martinella, 2006; Lima, 2008) show that content of dietary fiber similar to those found for cultivars Século XXI and Pedro Sato played a hypocholesterolemic effect.

The Food Drug Administration (FDA) recommends the consumption of 25 g of total fiber per day on a 2000 calorie diet. Thus, the administration of 100 g of flour in the form of leaf tea or capsules is providing more than the necessary daily recommended intake. Dietary fibers are important for the body and are constituted by insoluble fractions containing cellulose, lignin and some

Table 4. Mineral content for leaves of three guava tree cultivars.

Cultivar	Iron (mg/kg)	Potassium (g/kg)	Copper (mg/kg)	Magnesium (g/kg)	Manganese (mg/kg)	Zinc (mg/kg)
Century XXI	111.07 (±6.78)[a]	13.80 (±1.35)[b]	37.93 (±5.87)[a]	2.28 (±0.34)[b]	122.30 (±10.52)[a]	32.38 (±6.90)
Paluma	95.5 (±10.07)[a]	16.03 (±2.76)[a]	16.50 (±2.56)[c]	2.37 (±0.12)[b]	232.48 (±12.02)[c]	28.80 (±9.34)
Pedro Sato	66.18 (±8.56)[b]	8.73 (±2.34)[c]	28.45 (±3.15)[b]	3.0 (±0.19)[a]	85.4 (±5.89)[b]	30.88 (±5.68)
CV (%)	10.99	5.57	13.34	2.45	6.84	25.56

Means followed by the same letter in the columns do not differ by the Tukey test at 5% probability.

hemicelluloses, and the soluble fraction of pectins, gums and mucilages. Insoluble fibers accelerate intestinal transit, increase the weight of feces, contributing to reducing the risk of gastrointestinal diseases. Soluble fiber slows gastric emptying, glucose absorption and reduces serum cholesterol (Lima et al., 2008).

Minerals are essential to the human body, so they must be obtained through the diet. Calcium is essential to building and maintaining bones and teeth, neurotransmitter release, transfer of ions across membranes. Iron has the function of transporting oxygen. The ions magnesium, manganese and zinc are enzymatic cofactors. Manganese protects cells from free radicals. Copper promotes the mobilization of iron. Potassium has a diuretic effect (Nelson and Cox, 2004).

The contents of minerals are shown in Table 4. Only for zinc, no significant difference among cultivars was observed. The values of Recommended Daily Intake (RDI) for potassium, magnesium, iron, zinc, manganese, copper and calcium are 4.6 g/day 260 mg/day, 14 mg/day, 7 mg/day, 2.3 mg/day and 900 mg/day and 1.3 g/day, respectively, for adults. The intake of 100 g of leaves in the dry form per day which may be in the form of tea or capsule will provide 34.85, 79.34 and 46.11% of the RDI of the respective minerals: potassium, iron and zinc, which are higher than the levels provided by the fruit. The consumption of 100 g leaves provides more than that indicated by the RDI for the minerals magnesium, manganese and copper.

Conclusion

The leaves of cultivar Século XXI were more efficient to sequester the DPPH radical due to a higher content of phenolic compounds and showed the highest levels of dietary fiber. According to the β-carotene/linoleic acid method cultivar Paluma was more significant to prevent lipid peroxidation and had the highest content of ascorbic acid. All cultivars showed a significant mineral content for daily needs.

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REFERENCES

AOAC (2005). Official methods of Analysis (7 ed). Association of Official Chemistry. Washington, DC, USA.

AOAC (2005). Official methods of Analysis (12 ed). Association of Official Chemistry. Washington, DC, USA.

Boscolo OH, Mendonça-Filho RFW, Menezes FS, Senna-Valle L (2007). Potencial antioxidante de algumas plantas de restinga citadas como medicinais. Rev. Bras. Plantas Med. 9(1):8-12.

Chen HY, Lin YC, Hsieh CL (2007). Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. Food Chem. 104(4):1418-1424.

Fabri RL, Nogueira MS, Dutra LB, Bouzada MLM, Scio E (2011). Potencial antioxidante e antimicrobiano de espécies da família Asteraceae. Rev. Bras. Plantas Med. 13(2):183-189.

Goldstein JL, Swain T (1963). Changes in tannins in ripening fruits. Phytochemistry 2(4):371-383.

Haida KS, Baron A, Haida KS, Faci, Haas J, Silva FS (2011). Compostos fenólicos totais e atividade de antioxidante de duas variedades de goiaba e arruda. Revista Brasileira de Ciências da Saúde 9(28):11-19.

ITAL (1988). Goiaba: cultura, matéria-prima, processamento e aspectos econômicos. (2.ed.) Campinas: Instituto de Tecnologia de Alimentos p. 244.

Lima AJB, Corrêa AD, Alves APC, Abreu CMP, Dantas-Barros AM (2008). Caracterização química do fruto jabuticaba (*Myrcyaria cauliflora* Berg) e de suas frações. Arch. Latinoam. Nutr. 58(4):416-421.

Lima HM (2008). Farinha da casca do maracujá associada ao exercício físico no controle da lipidemia em ratas. Tese, Universidade Federal de Lavras.

Malavolta E, Vitti GC, Oliveira SA (1997). Avaliação do estado nutricional das plantas. Piracicaba: Potafós p. 319.

Martinella F (2006). Avaliação da atividade biológica do extrato hidroalcoólico de Tamarindus indica L. sobre o metabolismo lipídico e na carcinogênese coloteral em hamster. Tese, Universidade de São Paulo.

Nelson DL, Cox MM (2004). Princípios de bioquímica de Lehninger. São Paulo: Sarvier p. 1304.

Ojewole JA (2005). Hypoglycemic and hypotensive of *Psidium guajava* Linn, (Murtaceae) leaf aqueous extract. Meth. Find. Exp. Clin. Pharmacol. 27:689-695.

Ojewole JA (2006). Antiinflamatory and analgesic effects of *Psidium guajava* Linn (Myrtaceae) leaf aqueous extract in rats and mice. Meth. Find. Exp. Clin. Pharmacol. 28:441-446.

Qian H, Nihorimere V (2004) Antioxidant power of phytochemicals from *Psidium guajava* leaf. J. Zhejiang Univ. Sci. 5(6):676-683.

Roy CK, Kamath JV, Asad M (2006). Hepatoprotective activity of *Psidium guajava* Linn leaves extracts. Indian J. Exp. Biol. 44(4):305-311.

Rufino MSM, Alves RS, Brito ES, Morais SM (2007). Determinação da atividade antioxidante total em frutas pelo método beta-caroteno/ácido linoléico. Fortaleza: EMBRAPA.

Silva SMS, Mura JDP (2007). Tratado de alimentação, nutrição e dietoterapia. São Paulo: Roca p. 1256.

Strohecker R, Henning HM (1967). Análises de vitaminas: Métodos comprovados. Madrid: Paz Montolvo p. 428.
Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH (2006). Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compost Anal. 19:669-671.
Volp ACP, Renhe IRT, Barra K, Stringueta PC (2008). Flavonóides anthocyanins: Características e propriedades na nutrição e saúde. Revista Brasileira de Nutrição e Saúde 23(2):141-149.
Wojdylo A, Oszmianski J, Czemerys R (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 105(3):940-949.