Antioxidant, mutagenic and cytotoxic screening of *Psidium guajava* L. cultivars for medicinal purposes

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ABSTRACT

The aim of this work is to evaluate the antioxidant activity and the cytotoxic and mutagenic potential of leaf crude infusions of *Psidium guajava* cultivars ‘Paluma’, Pedro Sato and Roxa. The antioxidant activity was measured using the free radical DPPH method. The cytotoxic and mutagenic effects of *P. guajava* cultivars were evaluated by micronucleus test in mice bone marrow. The results showed that the cultivars Paluma and Roxa present higher antioxidant activity in comparison to Pedro Sato cultivar. All cultivars tested do not induce mutagenic or cytotoxic effects. The good antioxidant capacity and the absence of cytotoxic and mutagenic effects suggest that Paluma and Roxa are the cultivar candidate for the development of new drugs and to treat diseases, as well as, can be used against oxidative damage agents.

Key words: *P. guajava* cultivars, crude infusion, antioxidant activity, micronucleus test.

INTRODUCTION

The use of plants by humans to prevent and treat diseases has been in existence since the beginning of the civilizations and is currently present in all cultures till date (Rocha et al., 2015). As a country richest in biodiversity, Brazil presents immense potential for the discovery of new drugs coming from its flora; however, few species are studied for the effect of phytochemicals in human health (Sobrinho et al., 2011; Barbosa et al., 2016); the toxicological potential of medicinal plants is investigated so as to validate its safe use (De Almeida et al., 2006). *Psidium guajava* L. (Myrtaceae), popularly known as guava tree, occur naturally in Brazil and its leaves is used in traditional medicine to prepare infusions that treat intestinal diseases, such as diarrhea and colitis, as well as, to treat gastrointestinal inflammations (De Almeida et al., 2006; Gois et al., 2016). The phytochemistry of Myrtaceae family members have been extensively studied and described in literature. These family members, such as *P. guajava*, presents flavonoids, tannins, sesquiterpenoid alcohols, triterpenoid acids and essential oils as its main phytochemical constituents (Iha et al., 2008). The antioxidant, antimutagenic, antimicrobial and antihypertensive activities exhibited by natural products from this plant family is related to their phytochemical content (Nwinyi et al., 2008), which may vary according to the plant species and / or cultivars.

On the other hand, different classes of chemical agents, including plant secondary metabolites, can induce damages in genetic material (De Souza et al., 2013) and generate mutagenic damage. The mutagenic and carcinogenic effect of genotoxic substances may generate DNA reactive free radicals, which overcharges the endogenous antioxidant defense systems, characterizing oxidative stress (Delarmelina et al., 2014). Thus, the intake of natural products that acts as antioxidant agents can inhibit the development of mutagenesis and carcinogenesis (Ferguson, 1994). Various methods are used to determine the antioxidant and mutagenic activity of natural products. The scavenging activity of the stable free radical 2,2-diphenyl-1-picryl-hidrazila (DPPH) is one of the most common antioxidant tests used to measure antioxidant potential of
natural products and is considered a rapid and reliable test (Huang et al., 2005). Among assays validated by health agencies to access the toxicity of compounds, the in vivo micronucleus assay in mice bone marrow cells is widely accepted and recommended for the evaluation and registration of new chemical and pharmaceutical products (Choy, 2001; Ribeiro et al., 2003). Micronucleus test has been used to evaluate both loss and breakage of chromosomes, aneugenic and clastogenic damage, respectively (MacGregor et al., 1987; Fenech, 2005).

Considering that alterations in the genetic material can initiate a carcinogenic process (Fenech, 2005), the evaluation of antioxidant, mutagenic and cytotoxic activity of medicinal plants constitute an important tool for screening plants for medicinal purposes and is necessary to ensure their safe use. Thus, this work aimed to perform the antioxidant, mutagenic and cytotoxic screening of the aqueous leaf infusion of *P. guajava* cultivars *Paluma*, Pedro Sato and Roxa.

**MATERIALS AND METHODS**

**Crude infusions of *P. guajava* cultivars**

Leaves of *P. guajava* cultivars *Paluma*, Pedro Sato and Roxa were collected at the Universidade Federal do Espírito Santo, Alegre, Espírito Santo-Brazil. The leaves of the three *P. guajava* cultivars were air dried, milled and conditioned in a recipient containing preheated distilled water (80 to 90°C) for 10 min (De Almeida et al., 2006). The crude infusion was filtered and immediately used in the assays.

**DPH antioxidant activity in vitro**

To evaluate the antioxidant activity of *P. guajava* cultivars, the stable DPPH radical method as described by Rufino et al. (2007) with minor modifications was used. For this purpose, the antioxidant activity of the crude infusions of *P. guajava* cultivars was diluted in ethanol at the concentration of 1000.0, 500.0, 250.0 and 125.0 μg.mL⁻¹. Ascorbic acid was used as the standard and the absorbance value of each sample was measured in a spectrophotometer for microplate (Epoch Microplate Spectrophotometer - BioTek®) at 517 nm. The experiments were performed in triplicate.

The percentage of DPPH inhibition was calculated using the formula (Noipa et al., 2011):

\[
\% \text{ Inhibition DPPH} = \frac{\text{Absolute control} - (\text{Absolute sample})}{\text{Absolute control}} \times 100
\]

Where: Absolute control = Absorbance of the control; Absolute sample = Absorbance of the sample.

**Animals and treatments**

The evaluation of acute mutagenicity of *P. guajava* cultivars infuses was performed by micronucleus test in mice bone marrow cells. 66 male Swiss albino mice (*Mus musculus*), aged 6 to 8 weeks and about 30 g b.w., were supplied by the biotery of the Universidade Federal do Espírito Santo. The mice were housed in polypropylene cages under conditions of controlled light and temperature with free access to water and food.

Mice were randomly divided into 11 groups with six animals [n = 6]. The treatment groups received the crude infusions of *P. guajava* cultivars through gavage at the concentration of 50.0, 250.0 and 500.0 mg.kg⁻¹.b.w; negative control group received saline solution (NaCl 0.9%) through gavage and positive control received cyclophosphamide (50.0 mg.kg⁻¹.b.w.) intraperitoneally. *P. guajava* treatment doses were chosen based on LD₅₀ (Martinez et al., 2001).

The protocols involving animals were performed in accordance with the ethical principles of animal experimentation by the Research Ethical Committee on Animal Use of Universidade Federal do Espírito Santo (CEUA/UFES, 021/2011).

**Micronucleus assay in mice bone marrow cells**

Twenty-four hours after the treatments, the animals were sacrificed, the femurs were removed and the bone marrow cells collected to perform the micronucleus assay, according to the method described by Utesch et al. (2008) and Valadares et al. (2007) with minimal experimental modifications. Two slides per animal were prepared for analysis of bone marrow cells. The bone marrow cells were stained with Leishman to differentiate polychromatric erythrocytes (PCE), normochromatic erythrocytes (NCE) and micronucleated polychromatic erythrocytes (MNPC). For the evaluation of the mutagenicity of *P. guajava* cultivar infuses, 2000 PCEs per animal were analyzed (1000 PCEs per slide), noting the MNPC frequency.

The cytotoxicity of the infuses were evaluated by the ratio of PCE in a total of 400 erythrocytes (PCE + NCE), following the formula PCE/(PCE + NCE). Cell analysis were conducted as described by Krishna and Hayashi. (2000). The slides were analyzed using optical microscopy with increase of 1000x.

**Statistical analysis**

Results are presented as mean ± standard deviation. The data normality was verified by Kolmogorov-Smirnov’s test (p< 0.05). The comparison of antioxidant, mutagenic and cytotoxic activities results were performed by ANOVA post hoc Tukey’s test (p<0.05).
RESULTS AND DISCUSSION

Figure 1 shows the antioxidant activity of *P. guajava* cultivar crude infusions in DPPH assay. *Paluma* and *Roxa* cultivars were more effective in the inhibition of DPPH radical in all concentrations tested and presented results statistically similar to the standard, ascorbic acid. At the concentration of 125 µg.mL⁻¹, *Roxa* cultivar exceeded the ascorbic acid.

Figure 2 shows mutagenicity and cytotoxicity of *P. guajava* cultivar crude infusions. All cultivars of *P. guajava* were not mutagenic at doses tested. *Paluma* cultivar reduced the frequency of micronuclei when compared to negative control in all test doses, as well as, *Roxa* cultivar reduced micronucleus frequency at the dose of 500.0 mg.kg⁻¹.b.w. in comparison to the negative control group. The reduction in the PCE / NCE ratio suggests cytotoxic effect. For the tested doses, the cultivars of *P. guajava* did not induce the decrease of PCE / NCE ratio, in comparison to the control group, suggesting no cytotoxic effects.

Secondary metabolites produced by plants exert different functions in plant organisms and are used by humans for medicinal purposes (Dewick, 2002). These substances are commonly associated with beneficial biological activities for humans, however, some studies demonstrated the plant compounds may increase oxidative stress, the production of free radicals and induce fragmentation of chromosomes and/or damage the mitoticcellular apparatus, causing the loss of whole or part chromosomes (Ribeiro et al., 2003). On the other hand, DPPH assay is considered as a valid method to access scavenging activity of antioxidants from natural products. The DPPH radical reacts with reducing agents, which leads to the electrons pairing off and promotes the discoloration of the DPPH solution – the colour changes from deep violet to light yellow, being widely used to test the ability of plant extract to act as a free radical scavenger (Ayoola et al., 2008; Ch et al., 2018).

DPPH radical inhibition observed in our study using crude infusion, has also been reported in studies with ethanolic extracts of *P. guajava* (Cedric et al., 2018; Ch et al., 2018). In this study, the antioxidant activity exhibited was related to the phytochemicals present in these extractive forms. As reported in the literature, the leaf crude infusion of *P. guajava* presents large amounts of aromatic and thermolabile substances, such as saponins (De Almeida et al., 2006), as well as, the ethanolic extract of this plant presents flavonoids, terpenoids, saponins and tannins in its composition (Ayoola et al., 2008; Ch et al., 2018), conferring to extracts of *P. guajava* biological activities such as antioxidant activity.

*In vitro* assay evaluating the cytotoxicity of *P. guajava* crude infusion in mouse peritoneal macrophages showed that the storage of the crude infusion induces cytotoxic damage, which is not observed when the crude infusion is used immediately after being prepared (De Almeida et al., 2006). The aromatic compounds present in plant extracts are commonly inactivated when exposed to high temperatures, even though they may generate toxic compounds as a result of enzymatic processes during their storage (Watkins and Nguyen, 1989). Thus, the toxicity reported by De Almeida et al. (2006) is related to the oxidized substances resulting from the production of free radicals and the production of toxic derivatives in the storage of the crude infusion.

*In vivo* assays, such as the micronucleus test, allow the evaluation of the ability of substances used by human stop remote mutations in DNA, damage the antioxidant systems of cells and induce carcinogenesis (Belcavello et al., 2012). Our *in vivo* results corroborate those presented by Teixeira et al. (2003) and Martinez et al. (2001). The crude infusion of *P. guajava* at different concentrations did not induce...
Figure 2: Mutagenicity and cytotoxicity of *Psidium guajava* cultivars *Paluma*, *Pedro Sato* and *Roxa* leaf crude infusions. (A) Mutagenicity; (B) Cytotoxicity of *Paluma* cultivar; (C) Mutagenicity and (D) Cytotoxicity of *Pedro Sato* cultivar; (E) Mutagenicity and (F) Cytotoxicity of *Roxa* cultivar. Different letters indicate significant differences according to Tukey test at 5% of probability.

Mutagenicity in the bone marrow cells of Winstar rats in human peripheral blood cells and *Allium cepa* cells, suggesting that the crude infusion of *P. guajava* does not cause damage to DNA (Teixeira et al., 2003). Similarly, the administration of dried leaves of *P. guajava* at doses of 500.0, 1000.0 and 2000.0 mg.kg⁻¹.b.w. in *Swiss* mice showed through the micronucleus test in bone marrow that the dry drug does not induce mutagenic damage (Martínez et al., 2001).

Genomic stability in living organisms is directly related to the functioning of DNA maintenance and repair mechanisms (Boiteux and Jinks-Robertson, 2013; De Souza et al., 2013) promoting mutations in the DNA, the first step in the development of carcinogenesis. Thus, the evaluation
of mutagenicity and cytotoxicity induced by natural products are important tools to ensure the use of natural products by the population.

Crude infusion of the *Paluma* and *Roxa* cultivars of *P. guajava* showed good antioxidant activity and were more prominent than the cultivar *Pedro Santo*. In recent years, the antioxidant capacity of natural products has been widely studied and used for the selection of medicinal plants with potential for the development of new drugs, since antioxidant compounds can be used to combat oxidative damage (Ayoola et al., 2008). Natural or semi-synthetic substances with the ability to act on mechanisms that induce oxidative damage to cells are potent ant mutagenic agents. Studies in vivo with hesperidin, a natural phenolic compound suggest that this compound acts on the antioxidant system of the cells, promoting potent ant mutagenic effects, preventing and repairing damages to the DNA (Da Silva et al., 2017).

**Conclusion**

Crude infusion of the three *P. guajava* cultivars did not induce cytotoxic and mutagenic damages. The cultivars *Paluma* and *Roxa* were the ones that present better antioxidant performance compared to *Pedro Santo* cultivar and the ascorbic acid standard. We suggest that *Paluma* and *Roxa* cultivars of *P. guajava* are good candidates for development of new nutraceuticals for treatment of diseases. However, further studies need to be carried out evaluating the toxicological and chemoprotective aspects of different extractive forms of these cultivars, ensuring their use by the population.

**ACKNOWLEDGEMENT**

This work was supported by grants from FAPES (Fundação de Amparo à Pesquisa do Estado do Espírito Santo).

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