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Evaluation of the antimutagenic potential of *Psidium guajava* L. extracts via plant bioassays

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ABSTRACT

DNA damage is considered an important initial event in carcinogenesis. A considerable battery of assays exists to detect antimutagenic potential by exposure to genotoxic agents in combination with a plant compound. Plant systems such as *Lactuca sativa* have been successfully employed in cytogenetic bioassays as models to detect antimutagenic properties. *Psidium guajava* (guava) is an important fruit species with antimutagenic properties. In order to better understand these properties and warrant guava use as an important anticancer and other degenerative diseases agent, the present work aimed to verify the antimutagenic potential of guava trough in vivo assays using *L. sativa* meristematic cells. Three guava cultivars were evaluated (Pedro Sato, Paluma and Roxa) to determine their antimutagenic activity. Guava infusions at a concentration of 2.5 $g \cdot L^{-1}$ were used in the antimutagenic protocols. Protocol combining guava infusion with a mutagenic agent, methyl methanesulfonate (MMS), revealed the antimutagenic potential of the infusions. The percentage of damage observed from MMS exposure was reduced for all three cultivars. The mechanisms by which this antimutagenic effect was achieved were discussed. *L. sativa* was suggested as an efficient model for screening the antimutagenic activity of natural compounds.

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1. Introduction

The activity of mutagens in the organism can activate proto-oncogenes or tumor suppressor genes, causing cell alteration that lead to cancer (Mauro et al., 2014). In this sense, there is a race towards identifying, via studies of natural products, functional foodstuffs able to protect organisms from damage caused by exposure to mutagenic agents.

Guava, *Psidium guavaja* L., is a fruit species form the family *Myrtaceae*. This fruit is prominent on the diet of tropical countries both in its natural state as well as processes as juices or jams. Moreover it has many applications in popular medicine and as a phytotherapic. The species is highly biologically active presenting antioxidative, hepatoprotective, antiallergic, antigenotoxic, antispasmodic, cardioactive, anti-inflammatory properties (Gutiérrez et al., 2008) as well as anti-hyperglycemic, anti-hyperlipidemic effects (Jiao et al., 2017).

Leaves are usually the organ most used for medicine, followed by the bark and the stem. The preparations include juice extraction, decoctions and infusions. Tannins constitute the main component of this species (pedunculagin, guavins, flavonoids (quercetin, quercitrin and glycosidic derivatives), steroids, phenolic acids (gallic acid) and essential oils (bisabolene, cineol) (Flores et al., 2015).

The antimutagenic potential of guava has already been described in prior works. It has been shown that this plant can effectively inactivate the mutagenicity of agents such as sodium azide (NaN_3) in *Salmonella typhirium* strains (Grover and Bala, 1993); has compounds that when isolated from its leaves have anti-mutagenic activity (Matsuo et al., 1994); and present antimutagenic properties when studying the efficiency of SOS-red fluorescent protein (RFP) assay, using genetically modified bacteria as models exposed to plant extracts (Bartolome et al., 2006).

However, the studies so far are still too incipient to suggest beyond doubt the use of guava extracts and components as chemoprotectors. Further researches are needed to validate the use of guava-derived products as functional foodstuffs to prevent DNA damage, and consequently cancer development.

Various biological systems can be used to evaluate the antimutagenic potential of a substance. Often these systems are the same ones used on the studies to evaluate and identify mutagenic agents (Antunes and Araujo, 2000). Plant bioassays are among the possible candidates for this type of studies, as long as the studied compound is evaluated alongside known mutagens. When using plant systems the endpoints are generally evaluated trough analysis of chromosome

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aberrations and disturbances on the normal cell cycle (Mauro et al., 2014; Palmieri et al., 2016a).

Moreover, bioassays using plant systems are robust and reliable, rarely yielding false results, they are also inexpensive and easily repeatable (Fiskesjö, 1985; Grant, 1994). There is also a current effort on the scientific community to reduce mammal-based tests, especially those requiring animal sacrifices, for that purpose plant systems present a great and easily accessible alternative for indicating the potential of antimutagenicity and genotoxicity in complex or isolated substances (Kuhn et al., 2015). Furthermore results obtained using plant cells have been shown to have good correlation to those of human leukocytes (Palmieri et al., 2016b).

Among the various plant bioassays that are commonly used for mutagenic and antimutagenic testing, *Lactuca sativa* (lettuce) presents itself as a great option. It has very fast germination rates, is very easy to procure being found on seed stores and groceries alike, and has been shown to be as reliable than the vastly known and commonly used *Allium cepa* test (Silveira et al., 2017). This work aimed to evaluate the antimutagenic potential of guava (*P. guajava*) leaf infusion via cytogenetic bioassays using *Lactuca sativa* as a model.

2. Material and methods

2.1. Plant material

Leaves from 3 cultivars of *P. guajava* (Paluma, Pedro Sato and Roxa) were obtained from the plant nursery Frucafé at the city of Linhares, North Espírito Santo (ES, Brazil, July 2011). The fresh leaves were dehydrated in an incubator at 60 °C for 12 h (or until constant weight was achieved) and ground in cutting mill. Subsequently, the material was stored in closed glass flasks, at room temperature, in a dry and fresh place.

2.2. Plant model system

The tests were performed in *L. sativa* ($2n = 2\times = 18$) (Matoba et al., 2007) seeds of the commercial cultivar "Americana" (Topseed®). The seeds were obtained in local agribusiness stores (city of Alegre – ES, Brazil).

2.3. Treatment solutions

The infusions were prepared by heating distilled water (in close glass) until the boiling point and adding sufficient amount of ground leaves to prepare 200 mL of solution, at the determined concentration, of each of the three evaluated cultivars. After resting for 15 min the solutions were sieved trough filter paper and funnel, and stored in amber-colored vessels at 4 °C until the moment of use (Chen and Yen, 2007).

A Methyl methanesulfonate (MMS – Sigma Aldritch®) solution 4×10^{-4} was used as a DNA damage inducing mutagenic agent (Mauro et al., 2014), being considered the positive control. Distilled water was used as negative control.

2.4. Root growth analysis

To determine the concentrations to be used in the assays, root growth analysis was performed. Five concentrations (2.5; 5.0; 10.0; 20.0 and 40 $g \cdot L^{-1}$) of each guava infusion (Paluma, Pedro Sato and Roxa) were tested in regard to their phytotoxic potential. The experiment was assembled in a randomized block design composed of three blocks. Each block consisted of three Petri dishes (experimental units) for each treatment (each infusion concentration of a given guava cultivar). The Petri dishes, each containing 50 seeds, were covered in aluminum foil, moistened with 5 mL of each treatment solution and maintained in a BOD at 24 °C. After 48 h of exposure the length of the root emitted by each seed was determined by means of a digital caliper.

2.5. Rot exposure for antimutagenicity determination

To evaluate the antimutagenic potential of the infusions, seeds of the experimental model *L. sativa* were placed onto Petri dishes moistened with distilled water to stimulate root emission and maintained in a BOD at 24 °C. After approximately 16 h, seed exhibiting roots of 1–2 mm were transferred to new Petri dishes and subjected to one of the following experiment: (E1) treatment with infusions from different *P. guava* cultivars for 48 h; (E2) pre-treatment with infusions from different *P. guava* cultivars for 24 h and subsequent treatment with MMS for 24 h; (E3) simultaneous treatment with infusions prepared from different *P. guava* with the addition of MMS (1:1); (E4) pre-treatment with MMS for 24 h and post-treatment with infusions from different *P. guava* cultivars for further 24 h. MMS and distilled water were used as positive (C+) and negative (C−) control respectively. After exposure the roots were collected and fixed in Carnoy solution (ethanol, acetic acid solution, 3:1), and stored at −20 °C until use.

2.6. Cytogenetic analysis

For each of the experiments ten slides were prepared for the cytogenetic evaluation of the antimutagenic potential of the infusions. Each slide was prepared from 2 root meristems of *L. sativa* previously collected and fixated on the step above. The roots were hydrolyzed in 5 N HCL at room temperature (20 to 25 °C), squashed onto coverslip and stained with 2% acetic orcein. Between 7000 and 12,000 meristematic cells were evaluated for each established experimental condition (E1, E2, E3 and E4) and control (C− and C+).

The following parameters were evaluated: mitotic index (MI), percentage of alteration in the cell division phases (CA), percentage of micronuclei (MN) and percentage of condensed nuclei (CN). In order to verify the antimutagenic activity of the infusions the percentage of reduction in mitotic index was calculate (DRMI - given by 1- ratio between the MI of the treatment and that of the negative control multiplied by 100) as well as the decrease in damage (DR - calculated via the following formula: DR = [(MN cells in A − MN cells in B) / (MN cells in A − MN cells in C)], where A is the DNA damage induing agent, B is the antimutagenic treatment and C is the negative control (Malini et al., 2010 and in micronuclei occurrence (MR) in relation to the control group.

2.7. Statistical analysis

Data were subject to one-way analysis of variance (ANOVA), and the means were compared by the Kruskal–Wallis test at 5 probability level. The analysis was performed using the open software "R" (R Development Core Team, 2016).

3. Results and discussion

According to Valerio et al. (2007), the parameters assessed during the initial development of lettuce plantlets are efficient to determine the phytotoxicity of a given chemical compound. In addition it is know that tests with higher plants to determine mutagenic and antimutagenic activity are based on the fact that root growth is inhibited when the organ is exposed to a toxic substance (Mauro et al., 2014). Furthermore root growth and development are closely related to cell proliferation, in this sense if a substance is mitodepressive, reducing the frequency of dividing cells, it is thus phytotoxic as it affects root development (Harashima and Schnittger, 2010). Therefore, a root growth test was performed for each of the guava cultivars in order to find a concentration showing no mutagenic potential to be applied in the antimutagenic experiments. The results of this test are presented in Table 1. Only the concentration of 2.5 $g \cdot L^{-1}$ did not present statistical differences in relation to the seeds germinated in distilled water

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