Investigation on the effects of guava (*Psidium guajava* L.) infusions on germination, root tips and meristematic cells of *Latuca sativa*

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ABSTRACT

Guava (*Psidium guajava* L.) is a plant often employed in popular medicine. Recently several studies have alerted about the toxicity of substances present in medicinal plants, which can pose risks to the human health. In this sense, the present work aimed to investigate the phytotoxic, cytotoxic and genotoxic action of three guava varieties – Paluma, Pedro Sato and Roxa ("purple") – on the plant test system *Lactuca sativa* L. Thus, macro- and microscopic evaluations were carried out for five infusion concentrations (2.5, 5.0, 10.0, 20.0 and 40.0 g.L^{-1}) prepared from each variety. Distilled water was used as negative control. Chromatographic and spectroscopic analysis by HPLC-PAD indicated that the chemical composition of the infusion of Roxa is different than that of the infusions of the varieties Paluma and Pedro Sato. It was observed that seed germination and root growth in *L. sativa* exposed to infusions decreased with increasing infusion concentration, regardless of the tested cultivar. For the mitotic index, no statistical differences were observed. On the other hand, a significant increase in the frequency of cell cycle alterations was verified, especially for the highest concentrations tested. The cytogenotoxic effect was significant. Therefore, guava should not be used indiscriminately in popular medicine.

Key words: Citogenetoxicity, root growth, mitotic index, seed germination, IC$_{50}$.

INTRODUCTION

The use of plants as sources of pharmacological substances for the treatment of diseases is cultural in the human species (Teixeira et al. 2003, Akinboro and Bakare 2007, Sousa et al. 2009). The extracts of medicinal plants are effective in the treatment of different diseases (Akinboro and Bakare 2007), and recent studies on phytochemicals and their effects on the human health show that many substances found in medicinal plants are antioxidant, hypoglycemic and anticancer agents (Chen and Yen 2007, Abdel-Hameed et al. 2014, Tlili et al. 2014).

However, it has been also observed that many compounds present in medicinal plants have

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genotoxic and mutagenic effects (Akinboro and Bakare 2007, Sousa and Viccini 2011). Thus, the indiscriminate application of medicinal plants, without knowledge on the cytotoxic, genotoxic and mutagenic potential of their compounds may pose risks to the human health (Teixeira et al. 2003, Sousa et al. 2010). Therefore, it is necessary to carry out biological assays in order to scientifically validate plants with medicinal potential and endorse their use in popular medicine (Malini et al. 2010). This measure has been stimulated in Brazil by the National Policy of Medicinal Plants and Herbal Medicines in force since 2006.

The species *Psidium guajava* L., popularly named guava, belonging to the family Myrtaceae, stands out in a list of 71 medicinal plants of interest published by the Brazilian Unified Health System (SUS), (RENISUS 2009).

A recent review by Gutiérrez et al. (2008) regarding the phytochemistry, pharmacology and traditional use of the guava, reveals the pharma-cological properties of this species. Among others, its antidiarrheal, antimicrobial, antimalarial, analgesic and anti-inflammatory, antihyperglycemic, antioxidant and antiallergic effects can be highlighted. Despite these effects, the authors also indicate some works demonstrating the toxicity of guava extracts, and even antigenotoxic effects of the fruit (Gutiérrez et al. 2008).

Considering the different methodologies available to assess the toxicity and mutagenicity of plant extracts, cytogenetic bioassays using higher plants as models can be emphasized (Sousa et al. 2010). These bioassays enable the detection of the cytogenotoxicity of compounds through evaluation of the cell cycle of root-tip cells (Andrade-Vieira et al. 2012). This way, it is possible to identify the effects of the substances on the chromosomes, assessing the integrity of the genetic material in a real and functional way. In addition, the dimension of the risks to living organisms, brought about by certain compounds, can be established (Lah et al. 2008, Matejczyk et al. 2011).

The use of plant models in cytogenotoxicity bioassays is advantageous and justifiable: they are low-cost, present good correlation with other test models and systems, such as animal ones, and rarely produce false results, being very reliable and therefore constitute as an adequate candidate for genotoxicity monitoring programs. Moreover, the chromosomes of higher plants are large and easy to visualize, which facilitates cytological analyses (Fiskejö 1985, Grant and Owens 2006, Feretti et al. 2007, Dong and Zhang 2010, Andrade-Vieira et al. 2012).

Furthermore, plant bioassays have been proven efficient for prospection of genotoxic agents and are regarded as valid test systems by the United Nations Environmental Program (UNEP), World Health Organization (WHO) and the US Environmental Protection Agency (US-EPA) (Grant 1982, 1999, Ma 1982, 1999).

A novel approach has combined germination and root growth analyses with cytogenetic evaluations. This combination of macroscopic (germination and initial development of the plantlet) and microscopic analyses (mitotic index and cell cycle alterations) has proven to be an important method for investigating the toxic and mutagenic effects of chemical substances (Andrade et al. 2010). This approach is reliable since the microscopic parameters have a direct impact on macroscopic parameters, since the growth of an organ such as the root is intimately dependent on the increase in the number of cells and also on the cell elongation during the development and differentiation process (Harashima and Schnittger 2010).

The present work aimed to investigate the effects of leaf infusions, prepared from different guava tree varieties, in order to identify their mutagenic, cytotoxic and genotoxic activity. For this end, bioassays were performed with *Lactuca sativa* L. as plant test system, evaluating the effects of the infusions on germination, root growth (macroscopic tests) and cell cycle (microscopic

tests) of seeds, roots and meristematic cells. Assays using *L. sativa* roots have been largely employed in the detection of mutagenic and genotoxic compounds present in extracts of medicinal plants (Ribeiro et al. 2013). The species stands out for presenting high proliferative activity, large number of seeds, high sensitivity and large chromosomes (Campos et al. 2008). Moreover, it is efficient in exposing phytotoxicity during the first days of development of the treated plantlet, and useful in the determination of the frequency of dividing cells and alterations in the cell cycle (Andrade-Vieira et al. 2014).

MATERIALS AND METHODS

MATERIALS

Leaves from different guava cultivars – Paluma (PL), Pedro Sato (PS) and Roxa ("purple", RX) – were obtained from the plant nursery Frucafé in the city of Linhares, north of Espírito Santo (ES, Brazil), in July 2011. Later, the leaves were dehydrated in incubator at 60°C for approximately 12h (or until the weight remained constant), ground and stored in closed glass flasks at room temperature, in a fresh and dry place.

MODEL SYSTEM

Seeds of *L. sativa* (2n=2x=18) of the commercial cultivar "Americana" (Topseed® seeds) were used as test system material to evaluate the phytotoxic, cytotoxic and genotoxic effects of *P. guajava* infusions.

TREATMENT SOLUTIONS

A concentration of 5 g of ground leaves/L of solution is commonly used to prepare 200 mL of tea (infusion or decoction) from commercial tea bags. Based on this information, five concentrations (2.5, 5.0, 10.0, 20.0 and 40.0 g.L^{-1}) of infusion prepared from different cultivars of *P. guajava* (PL, PS and RX) were tested as to their phyto-, cyto- and genotoxic potential.

The infusions were prepared by heating distilled water (in closed glassware) until boiling, and adding a sufficient amount of ground leaves to prepare 200 mL of solution (m/v) for every concentration of each of the three evaluated cultivars. After a resting time of 15 minutes, the solutions were filtered using paper filter and a funnel, and stored in dark vessels to avoid the contact with light at 4°C until the moment of use (Chen and Yen 2007, Teixeira et al. 2003). Distilled water was used as negative control.

CHROMATOGRAPHIC AND SPECTROSCOPIC ANALYSIS OF INFUSIONS

The infusion concentration of 2.5 g.L^{-1} prepared with the three guava cultivars (PL, PS and RX) was used to determine the qualitative chemical profile of these varieties, by means of High-Performance Liquid Chromatography coupled to a Photodiode Array Detector (HPLC-PAD). The samples were centrifuged at 1,200 rpm for 10 min., the supernatant was filtered through a 0.45 μm nylon filter and aliquots of 20 μL were directly injected into the HPLC-PAD.

The analyses were performed in a Jasco 2000 HPLC (Jasco, Tokyo, Japan) equipped with a PU-2089 Plus pump, a MD-2010 Plus Photodiode Array Detector (PAD), an AS-2055 Plus autosampler and a column oven (CO-2065 plus, 30°C), using a reverse-phase Phenomenex Luna(2) column (C$_{18}$, 250 x 4.6 mm) protected by a RP$_{18}$ guard column (2.5 cm x 3 mm) from Phenomenex, Inc. (Torrance, CA, USA). The elution system used for the HPLC-PAD assay was a binary gradient elution system with solvent A [0.1% trifluoroacetic acid (TFA) in H$_2$O] and solvent B [0.1% TFA in acetonitrile (ACN)] eluted at an initial linear gradient of 95:5 (A:B, v/v), which was changed to 47.5:52.5 (A:B, v/v) after 30 min. The flow-rate was 1.0 mL min^{-1}. The Chrom Nav (Workstation JASCO-Chrom Nav 1.18.03) software was used to control the analytical system and perform the data collection and processing.

The retention times and UV spectra of the peaks on the chromatogram were compared with those of authentic standards of different secondary metabolites classes [gallic acid, protocatechuic acid, ellagic acid, (+)-catechin, quercetrin, quercetin, myricetin and apigenin, all from Sigma Aldrich® (St. Louis, MO, USA)] and also with literature data.

PHYTOTOXIC ANALYSIS

To determine the phytotoxicity of the infusions from the three analyzed guava varieties (PL, PS and RX), germination and root growth assays were carried out in seeds of *L. sativa*. The experiment was designed as randomized blocks (RBD), composed of three blocks, each consisting of three Petri dishes (experimental units) for each treatment. The Petri dishes, each containing 50 seeds, were covered with aluminum foil and maintained in a BOD incubator at 24°C throughout the experiment.

The number of germinated seeds was obtained after 8, 16, 24, 32, 40 and 48 hours of treatment. The germination rate (GR), which is the percentage of germinated seeds, and the speed of germination index (SGI) were determined according to the formulas:

GR = TN x 100/N, where TN represents the total number of germinated seeds and N is the total number of seeds in each dish;

SGI = $(N_{8h} \times 1) + (N_{16h} - N_{8h}) \times \frac{1}{2} + (N_{24h} - N_{16h}) \times \frac{1}{4} + (N_{32h} - N_{24h}) \times \frac{1}{8} + (N_{40h} - N_{32h}) \times 1/16 + (N_{48h} - N_{40h}) \times 1/32$, where N_{xh} is the number of seeds germinated after x hours.

After 48 hours of treatment, the length of roots emitted by each seed from each Petri dish and treatment was evaluated with the aid of a digital caliper. The average root growth was obtained for each Petri dish, and a graph showing the growth curve × infusion concentration was plotted.

CYTOGENETIC ANALYSIS

To assess the cytotoxic and genotoxic potential of the guava varieties (PL, PS and RX), at least five roots per Petri dish per treatment were collected after the root length measurements (48h after exposure). The roots were fixed in fresh, cold solution of ethanol and acetic acid (3:1 v/v). Fixed root tips were hydrolyzed in 5 N HCl at room temperature for 18 min, and squashes were prepared with acetic orcein (2%). Nine slides per treatment (each containing two meristems from two roots out of every Petri dish analyzed on section 2.5) were studied under light microscope, and about 10,000 cells per treatment were counted. The following parameters were assessed: (i) mitotic index (MI), defined by the number of dividing cells as a fraction of the total number of cells, and (ii) cell cycle alterations (CCA), expressed as the percentage of chromosome aberrations (c-metaphases, sticky chromosomes, bridges, laggards and fragments) and nuclear alterations (condensed nuclei, micronuclei, lobated nuclei), divided by the total number of analyzed cells.

STATISTICAL ANALYSIS

Data were subjected to one-way analysis of variance (ANOVA), and the averages of all analyzed parameters (GR, SGI, MI and CCA) were compared by Kruskal-Wallis tests at 5% probability level. Statistical analysis was performed with the "R" statistical program (R Development Core Team 2011).

RESULTS AND DISCUSSION

The present work investigated the phytotoxic, cytotoxic and genotoxic potential of three varieties of *P. guajava* (Paluma - PL, Pedro Sato - PS and Roxa - RX) in seeds, roots and meristematic cells of the plant test system *L. sativa*. In addition, the chromatographic profile obtained for the infusions of these varieties is related to their respective effects.

The chromatographic analysis by HPLC–PAD revealed that PL and PS have the same main chemical constituent, a flavonol *O*-heteroside, which can be attested by the UV spectrum with

bands at 240–285 and 300–380 nm (Rodrigues et al. 2008, Merken and Beecher 2000, Mabry et al. 1970) and retention time of approximately 18 minutes (Fig. 1A and 1B). On the other hand, it was possible to observe that RX has a different chromatographic profile (Fig. 1C). The UV spectra of the peaks corroborated the presence of gallic acid derivative (7.2 min), with bands at 210-212 and 270-278 nm, protocatechuic acid derivative (11.2 min), with bands 210-215, 260-270 and 290-295 nm, and flavonol derivatives (18-20 min), with bands at 240–285 and 300–380 nm (Rodrigues et al. 2008, Merken and Beecher 2000).

Phytochemical analyses of fresh leaves of *P. guajava* revealed that flavonoids are the main chemical compound isolated from mature leaves. Different triterpenes and saponins combined with oleanolic acid were also observed (Arima and Danno 2002, Gutiérrez et al. 2008). Different compounds were combined in different amounts in the evaluated varieties. Thus, the chemical constitution of a species depends on various factors, such as geographical origin (Verdeguer et al. 2009) and genetic characteristics (Gilles et al. 2010), and the differences in major chemical constituents between PL and PS in relation to RX reflects these variations.
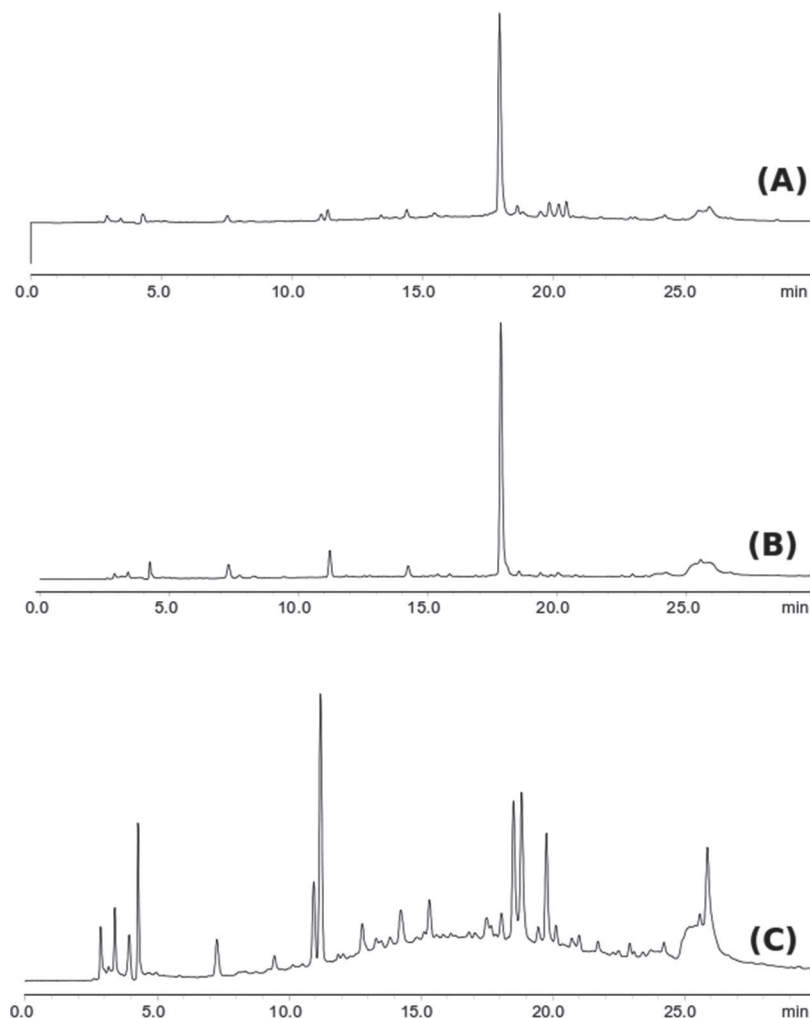

Figure 1 - Chromatographic profile of infusions prepared with three guava varieties: **(A)** Paluma – PL; **(B)** Pedro Sato – PS; and **(C)** Roxa – RX.

Each cultivar represents a different genotype of the same species, *P. guajava*, which are genetically divergent according to analyses of SSR markers and morphological data presented by Coser et al. (2012). In this work, the analyses of groupings, both for the morphological as well as the molecular data, indicate the genotypes/varieties PS and RX as the closest, whereas PL is the most divergent (Coser et al. 2012).

In the present study, the genetic effects are predominant to explain the divergences encountered, since the plant material was collected from a plant nursery with controlled environment (temperature, humidity and light). These genetic differences result in a distinct chemical compositions reflecting on the germination, root growth and cell cycle of *L. sativa* treated with each variety of guava, as demonstrated and discussed next.

The data regarding the effect of the infusions on seed germination in *L. sativa* are presented in Table I. A dose dependency was observed in the delay in seed germination for the infusions of the three tested varieties. Significant reduction ($p < 0.05$) in the percentage of germinated seeds (GR) was detected for the concentration of 10 g.L^{-1} in the varieties PL and RX. The variety PS significantly reduced GR only at the highest concentration (40 g.L^{-1}), in comparison to the control.

The greatest reduction observed in GR was for the cultivar PS, decreasing the parameter in 46% (Table I). Differently, for SGI, PS was the variety showing the smallest reduction (37%) in relation to the control. Significant differences were observed for RX at the lowest tested concentration (2.5 g.L^{-1}), with approximately 51% of delay in germination, as well as for PL at the concentration of 5 g.L^{-1}, with 43% of reduction in SGI (Table I).

TABLE I

Germination test in seeds of *Lactuca sativa* after 48h of exposure to infusions of different guava varieties (Paluma, Pedro Sato and Roxa).

	Paluma		Pedro Sato		Roxa	
	GR	SGI	GR	SGI	GR	SGI
Control	95.20 ± 4.60 a	16.72 ±1.48 a	95.60 ± 3.28 a	16.90 ± 2.05 a	94.80 ± 4.60 a	15.91 ± 1.31 a
2.5 g.L^{-1}	90.40 ± 2.82 a	16.73 ± 1.31 a	91.20 ± 4.33 a	14.03 ± 1.44 b	94.00 ± 2.82 a	14.14 ± 1.48 b
5 g.L^{-1}	92.00 ± 2.96 a	14.44 ± 1.11 b	94.40 ± 3.34 a	14.79 ± 0.74 b	94.40 ± 2.96 a	13.72 ± 1.11 c
10 g.L^{-1}	86.40 ± 7.12 b	13.99 ± 0.65 c	85.60 ± 6.84 a	12.94 ± 1.58 c	86.80 ± 7.12 b	12.57 ± 0.65 d
20 g.L^{-1}	79.20 ± 5.01 c	11.26 ± 0.77 d	85.60 ± 5.89 a	11.81 ± 1.50 c	79.60 ± 5.01 c	10.98 ± 0.77 e
40 g.L^{-1}	57.20 ± 5.01 d	7.17 ± 0.83 e	51.60 ± 9.20 b	6.19 ± 1.20 d	64.80 ± 5.01 d	7.82 ± 0.83 f

Results followed by the same letter (in the columns) did not present statistically significant difference according to the Kruskal-Wallis test ($p < 0.05$).

Germination and root growth bioassays are simple, rapid, reliable and very low-cost, as they do not require high-tech equipment (Valerio et al. 2007). These tests enable the evaluation of the adverse effects of a toxic compound on germination and root growth, already at the initial stages of seed development, which makes the process considerably simpler and faster (Dutka 1989, Lewis 1995). Moreover, when associated with studies of allelopathy or natural products originated from

plants, as in the present work, they are commonly applied in the screening of the phytotoxic potential of the components present in the plant extracts (Sousa et al. 2009).

In this sense, the regressions curves presented on Figure 2, based on the evaluation of root growth under effect of guava infusions, complements the germination analysis. The root growth analysis (Fig. 2) corroborates the dose dependency observed in the presented germination data (Table I). The higher

the concentration, the lower the root growth (Fig. 2A, 2B and 2C). Growth inhibition was higher than 50% at the concentration of 40 g.L^{-1} in all varieties. Reductions of 53% (in PS) and 58% (in RX) in root size were observed. For all evaluated infusion concentrations, RX appeared as the most phytotoxic, being more efficient in reducing the length of roots.

Figure 2 further illustrates important parameters that can be estimated, such as the inhibition limit, which is the lowest concentration necessary to obtain an observable inhibition (2.5 g.L^{-1}, in this case); and IC$_{50}$ (growth inhibition 50%), which corresponds to the concentration of the tested agent in which the size of the roots in the plant model is 50% smaller than
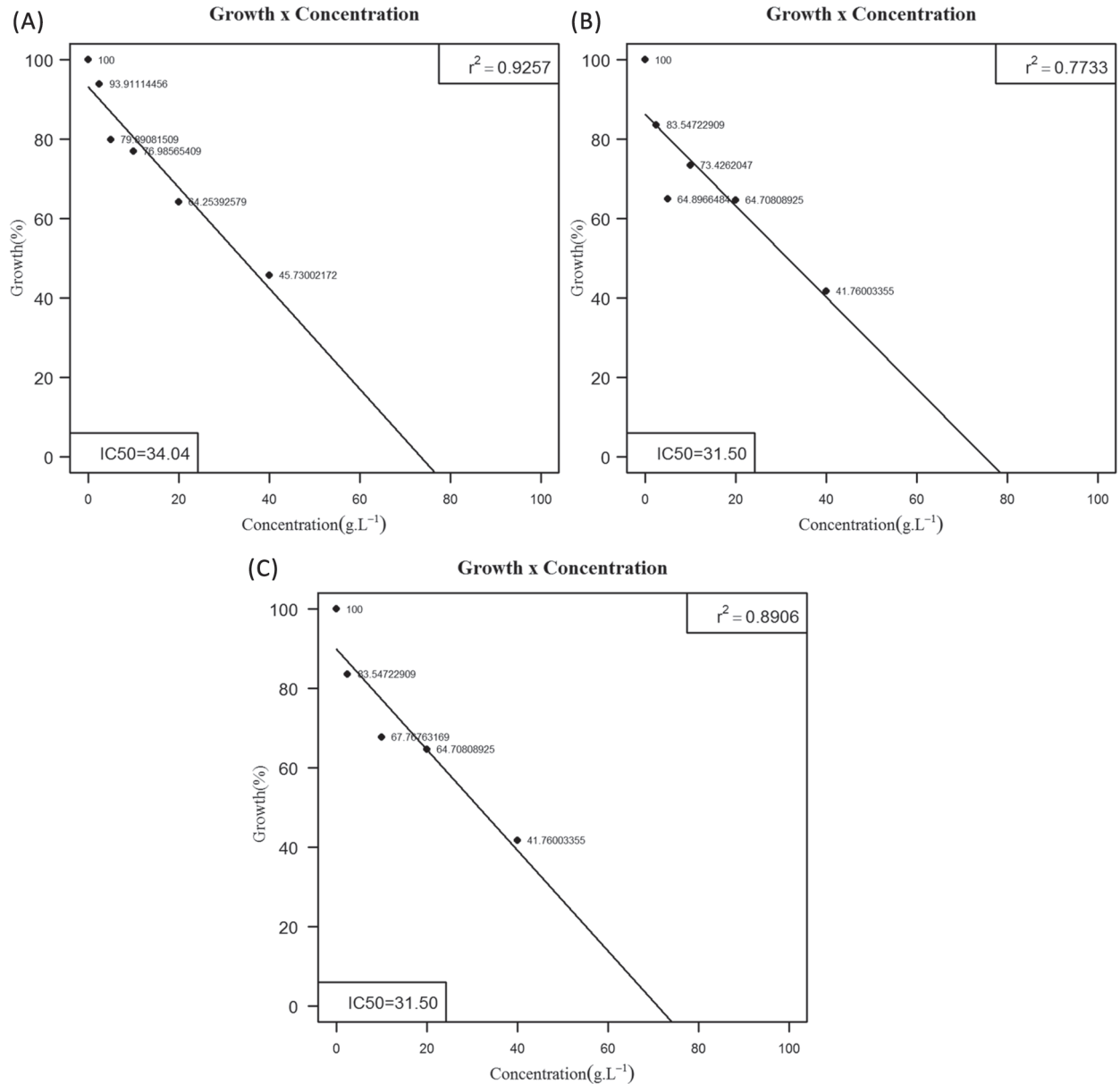

Figure 2 - Root growth curves for *Lactuca sativa* observed after 48h of treatment with diverse infusion concentrations of three guava varieties: **(A)** Paluma – PL; **(B)** Pedro Sato – PS; and **(C)** Roxa – RX. The dots represent the observations and the line designates the regression curve: (a) y = 931754 – 1,2682x; (b) y = 912406 – 1,1108x; and (c) y = 862554 – 11504x The data on growth are in comparison to the control, where the concentration 0 corresponds to the control (water) with growth of 100%.

in the control treatment (Narwal et al. 2009). From the cytogenetic point of view, this concentration (IC$_{50}$) is highly representative, as it reflects very clearly the toxic effects of the tested agent without the inhibition being too severe, which would result in very fragile roots, and/or too small for assembly on slides for cell cycle evaluation (Palmieri et al. 2014). Such concentration value were 31.5 g.L^{-1} for PS and RX and 34 g.L^{-1} for PL (Fig. 2).

The microscopic parameters evaluated in this work, such as MI and CCA, have been applied to assess the cytogenotoxic activity of different compounds *in vivo* and *in vitro* (Pugliesi et al. 2007). The cytotoxic level of the agent can be determined both by the increase as well as by the decrease in MI (Lubini et al. 2008). The first could be a consequence of disordered cell proliferation, potentially leading to the formation of tumors (Campos et al. 2008). On the other hand, the

reduction in MI could be explained by the arresting of the cell division at interphase, as well as by death of the interphase nucleus, hindering the beginning of the prophase and thus the cell division (Solomon et al. 1999).

In this context, the percentages of cells in division (MI) and CCAs (chromosome or nuclear alterations, such as micronuclei) are assessed, defining whether the substance or compound evaluated is genotoxic (CCA), cytotoxic (MI, CCA and micronuclei), or mutagenic (micronuclei) (Leme and Marin-Morales 2009). Therefore, the data presented in Table II demonstrates that the evaluated extracts of *P. guajava* did present cytotoxic effect, since statistical difference was observed among the different infusion concentrations tested, and the negative control for all three varieties. This indicates that the infusions disrupt the cell cycle.

TABLE II

**Mitotic index (MI) and frequency of cell cycle alterations (CCA)
in *Lactuca sativa* meristematic cells after 48 hours of exposure to
infusions of different guava varieties (Paluma, Pedro Sato and Roxa).**

	Paluma		Pedro Sato		Roxa	
	MI	CCA	MI	CCA	MI	CCA
Control	7.31 ± 1.60 a	0.50 ± 0.52 a	7.65 ± 2.01 a	0.38 ± 0.18 a	7.98 ± 1.72 a	0.24 ± 0.20 a
2.5 g.L^{-1}	6.27 ± 1.22 a	0.67 ± 0.47 a	7.55 ± 2.04 a	0.40 ± 0.24 a	7.38 ± 1.66 a	0.45 ± 0.19 a
5 g.L^{-1}	5.99 ± 1.06 a	1.21 ± 0.34 b	6.51 ± 2.06 a	0.43 ± 0.26 a	6.56 ± 1.62 a	0.69 ± 0.24 b
10 g.L^{-1}	5.97 ± 1.31 a	0.92 ± 0.23 b	6.49 ± 1.61 a	0.42 ± 0.14 a	6.85 ± 1.77 a	0.69 ± 0.32 b
20 g.L^{-1}	6.03 ± 0.97 a	1.02 ± 0.46 b	6.97 ± 1.54 a	0.40 ± 0.13 a	7.98 ± 1.72 a	0.59 ± 0.20 b
40 g.L^{-1}	7.03 ± 2.13 a	1.55 ± 0.31 c	7.15 ± 1.81 a	1.1 ± 0.54 b	7.38 ± 1.66 a	0.94 ± 0.38 c

Results followed by the same letter (in the columns) did not present statistically significant difference according to the Kruskal-Wallis test (p < 0.05).

According to Andrade et al. (2010), the decrease in root growth is closely related to reduction in MI. However, even though reduction in root growth was observed, the MI was not reduced. This fact can be explained by another chemical compound commonly found in leaves of *P. guajava*, namely tannins. These substances act in plants against herbivory, but it also inhibits germination (Teixeira et al. 2003). Indeed, it was observed that infusions of all varieties

negatively affected germination. Thus, reduction in the root elongation may be a consequence of the delay in germination, detected by decrease of SGI. In this sense, roots started growing after germination, without the cell proliferation being affected by the infusions.Considering CCAs, significant differences were observed for the varieties PL and RX at the concentration of 5 g.L^{-1}, whereas for PS a difference was only observed at the highest tested concentration

(40 g.L^{-1}) (Table II). Chromosome aberrations are characterized by alterations in any chromosome structure or in the total number of chromosomes, which may occur either spontaneously or as a result of exposure to physical or chemical agents (Russel 2002). Overall, chromosome fragments and bridges demonstrate a clastogenic mechanism of action of the tested substance, being related to its genotoxicity; differently, lost, sticky and c-metaphase chromosomes are related to aneugenic mechanisms of action and a cytotoxic effect of the substance (Leme and Marin-Morales 2009, Andrade-Vieira et al. 2012).

PS did not present significant cytogenotoxic effect in the common use concentration in folk medicine (5 g.L^{-1}). This result is corroborated by Teixeira et al. (2003), who evaluated the effect of *P. guajava* infusions on *Allium cepa* (onion) cells, Wistar rat bone marrow and human lymphocytes, and demonstrated that there was no cytotoxic or genotoxic activity of the infusion on these three test systems. This fact was related to the presence of chemical compounds such as flavonoids, the main chemical compound present in infusions of the three guava varieties (Fig. 1), which are indicated as being responsible for antimutagenic properties in the species.

Significant cytotoxic and genotxic effects were observed on the 5 g.L^{-1} concentration for RX and PL RX toxicity could be related to the predominance of gallic acid derivatives as its main chemical component. However for PL a clear relation between its toxicity and its chemical composition could not be established. This cito- and genotoxicity is cause for concern and suggest caution when using these varieties in phytotherapy. In addition other studies have shown that medicinal plants used by the population can be toxic and therefore should not be used indiscriminately (Sousa and Viccini 2011, Sousa et al. 2010).

Therefore, this study concluded that the evaluated varieties of guava presented a phytotoxic effect, inhibiting germination and root growth, as well as cyto- and genotoxic effects leading to the formation of CCA in *L. sativa*. All these parameters were dose dependent Thus, our results recommend caution when using guava as a medicinal and phytotherapic plant. Furthermore *L. sativa* has proven to be an adequate model for evaluation of toxic effects. Even so, other researches utilizing specific test systems targeting humans are necessary to perfectly evaluate how this toxicity translates to the physiology of an animal organism. Studies as the one presented here are important to alert about the indiscriminate use of plants in popular medicine.

RESUMO

A goiaba (*Psidium guajava* L.) é uma planta bastante utilizada na medicina popular. Recentemente alguns trabalhos tem alertado acerca da toxicidade de substâncias presentes em plantas medicinais, o que pode trazer riscos à saúde humana. Neste sentido, o presente trabalho objetivou investigar a ação fitotóxica, citotóxica e genotóxica de três variedades de goiaba - Paluma, Pedro Sato e Roxa - no sistema teste vegetal *Lactuca sativa* L. Assim, foram realizadas avaliações macro- e microscópicas para cinco concentrações de infusões (2,5, 5,0, 10,0, 20,0 e 40,0 g.L^{-1}) preparadas a partir de cada variedade. A água destilada foi usada como controle negativo. As análises cromatográficas e espectroscópicas por HPLC-PAD indicaram que a composição química da infusão da Roxa é diferente das infusões das variedades Paluma e Pedro Sato. Foi observado que a germinação das sementes e o crescimento da raiz em *L. sativa* expostas às infusões diminuem com o aumento da concentração da infusão, independentemente da cultivar testada. Para o índice mitótico, diferenças estatísticas não foram observadas. Por outro lado, foi verificado um aumento significativo na frequência de alterações do ciclo celular, especialmente para as maiores concentrações testadas. O efeito citogenotóxico foi significativo. Portanto, a goiaba não deve ser utilizada indiscriminadamente na medicina popular.

Palavras-chave: Citogenotoxicidade, crescimento radicular, índice mitótico, germinação de sementes, IC$_{50}$.

REFERENCES

ABDEL-HAMEED ESS, NAGATY MA, SALMAN MS AND BAZAID SA. 2014. Phytochemicals, nutritionals and antioxidant properties of two prickly pear cactus cultivars (*Opuntia ficus indica* Mill.) growing in Taif, KSA. Food Chem 160: 31-38.

AKINBORO A AND BAKARE AA. 2007. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. J Ethnopharmacol 112: 470-474.

ANDRADE LF, DAVIDE LC AND GEDRAITE LS. 2010. The effect of cyanide compounds, fluorides, aluminum, and inorganic oxides present in spent pot liner on germination and root tip cells of *Lactuca sativa*. Ecotoxicol Environ Saf 73: 626-663.

ANDRADE-VIEIRA LF, BOTELHO CM, LAVIOLA BG, PALMIERI MJ AND PRAÇA-FONTES MM. 2014. Effects of *Jatropha curcas* oil in *Lactuca sativa* root tip bioassays. An Acad Bras Cienc 86: 373-382.

ANDRADE-VIEIRA LF, FERREIRA MFS, BERNARDES PM AND OLIVEIRA WBS. 2012. Toxicidade de agrotóxicos: uma abordagem citogenética e molecular. In: Pratossoli D, Junior WCJ, Zago HB, Alves FR, Viana UR, Junior HJGS and Rodrigues C (Eds), Tópicos Especiais em produção Vegetal III, Editora UFES, Vitória, ES, Brasil, p. 39-79.

ARIMA H AND DANNO G. 2002. Isolation of antimicrobial compounds from guava (*Psidium guajava* L). Biosc Biotech Bioch 66: 727-730.

CAMPOS JMS, DAVIDE LC, SOARES GLG AND VICCINI LF. 2008. Mutagenic effects due allelopathic action of fern (Gleicheniaceae) extracts. Allelopath J 22: 143-152.

CHEN HY AND YEN GC. 2007. Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L) leaves. Food Chem 101: 686-694.

COSER SM, FERREIRA MFS, FERREIRA A, MITRE LK, CARVALHO CR AND CLARINDO WR. 2012. Assessment of genetic diversity in *Psidium guajava* L using different approaches. Sci Hortic-Amsterdan 148: 223-229.

DONG Y AND ZHANG J. 2010. Testing the genotoxicity of coking wastewater using. *Vicia faba* and *Hordeum vulgare* bioassays. Ecotox Environ Saf 73: 944-948.

DUTKA BJ. 1989. Short-term root elongation toxicity bioassay. In: Methods for toxicological analysis of waters, wastewaters and sediments National Water Research Institute (NWRI), Environment Canada, Burlington, Ontario, Canada.

FERETTI D, ZERBINI I, ZANI C, CERRETI E, MORETTI M AND MONARCA S. 2007. *Allium cepa* chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. Food Addit Contam 24: 561-572.

FISKESJÖ G. 1985. The *Allium* test as a standard in environmental monitoring. Hereditas 102: 99-112.

GILLES M, ZHAO J, AN M AND AGBOOLA S. 2010. Chemical composition and antimicrobial properties of essential oils of three Australian *Eucalyptus* species. Food Chem 119: 731-737.

GRANT WF. 1982. Chromosomal aberration assays in Allium. A report of the US Environmental Protection Agency Gene-Tox program. Mutat Res 99: 273-291.

GRANT WF. 1999. Higher plant assays for the detection of chromosomal aberrations and gene mutations a brief historical background on their use for screening and monitoring environmental chemicals. Mutat Res 624: 107-112.

GRANT WF AND OWENS ET. 2006. *Zea mays* assays of chemical/radiation genotoxicity for the study of environmental mutagens. Mutat Res 613: 17-64.

GUTIÉRREZ RMP, MITCHELL S AND SOLIS RV. 2008. *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 117: 1-27.

HARASHIMA H AND SCHNITTGER A. 2010. The integration of cell division, growth and differentiation. Curr Opin Plant Biol 13: 66-74.

LAH B, VIDIC T, GLASENCNIK E, CEPELJNIK T, GORJANC G AND MARINSEK-LOGAR R. 2008. Genotoxicity evaluation of water soil leachates by Ames test, comet assay, and preliminary Tradescantia micronucleus assay. Environ Monit Assess 139: 107-118.

LEME DM AND MARIN-MORALES MA. 2009. *Allium cepa* test and environmental monitoring: A review on its application. Mutat Res 682: 71-81.

LEWIS MA. 1995. Algae and vascular plant tests In: Rand G (Ed), Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment Taylor and Francis Publishers, USA, p. 135-169.

LUBINI G, FACHINETTO JM, LAUGHINGHOUSE HD, PARANHOS JT, SILVA ACF AND TEDESCO SB. 2008. Extracts affecting mitotic division in root-tip meristematic cells. Biologia 63: 647-651.

MA TH. 1982. Vicia cytogenetic tests for environmental mutagens. A report of the US Environmental Protection Agency Gene-Tox program. Mutat Res 99: 257-271.

MA TH. 1999. The international program on plant bioassays and the report of the follow-up study after the hands-on workshop in China. Mutat Res 426: 103-106.

MABRY TJ, MARKHAM KR AND THOMAS MB. 1970. The systematic idenfication of flavonoids. Springer-Verlag Berlin Heidelberg, 354 p.

MATEJCZYK M, PŁAZA GA, NAŁĘCZ-JAWECKI G, ULFIG K AND MARKOWSKA-SZCZUPAK A. 2011. Estimation of the environmental risk posed by landfills using chemical, microbiological and ecotoxicological testing of leachates. Chemosphere 82: 1017-1023.

MALINI M, MARIN-MORALES MA, MANTOVANI MS, JAMAL CM, NATI N, PASSOS TS AND MATSUMOTO ST. 2010. Determination of the antimutagenicity of an aqueous extract of *Rhizophora mangle* L (Rhizophoraceae), using in vivo and in vitro test systems. Genet Mol Biol 33: 176-181.

MERKEN HM AND BEECHER GR. 2000. Measurement of food flavonoids by high-performance liquid chromatography: a review. J Agr Food Chem 48: 577-599.

NARWAL SS, SAMPIETRO DA AND CATALÁN CAN. 2009. Chapter 1: Laboratory Bioassays in Allelopathy. In: Narwal SS, Catalán CAN, Sampietro DA, Vattuone MA and Politycka B (Eds), Plant Bioassays, Studium Press, Houston, Texas, p. 1-20.

PALMIERI MJ, LUBER J, ANDRADE-VIEIRA LF AND DAVIDE LC. 2014. Cytotoxic and phytotoxic effects of the main chemical components of spent pot-liner: A comparative approach. Mutat Res-Gen Tox En 763: 30-35.

PUGLIESI GC, ANDRADE SF, BASTOS JK AND MAISTRO EL. 2007. *In vivo* clastogenicity assessment of the *Austroplenckia populnea* (Celastraceae) leaves extract using micronucleus and chromosomes aberration assay. Cytologia 72: 1-6.

R DEVELOPMENT CORE TEAM. 2011. R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing.

RENISUS. 2009. http://portalsaudegovbr/portal/arquivos/pdf/RENISUSpdf (Accessed on 03/28/2014).

RIBEIRO LR, SANTOS MF, SILVA QM, PALMIERI MJ, ANDRADE-VIEIRA LF AND DAVIDE LC. 2013. Cytogenotoxic effects of ethanolic extracts of *Annona crassiflora* (Annonaceae). Biologia 68: 433-438.

RODRIGUES CM, RINALDO D, SANNOMIYA M, DOS SANTOS LC, MONTORO P, PIACENTE S, PIZZA C AND VILEGAS W. 2008. High-performance Liquid Chromatographic Separation and Identification of Polyphenolic Compounds from the infusion of *Davilla elliptica* St Hill. Phytochem Analysis 19: 17-24.

RUSSELL PJ. 2002. Chromosomal Mutation. In: Cummings B (Ed), Genetics, Pearson Education Inc, San Francisco, p. 595-621.

SOLOMON M, BELENGHI B, DELLEDONNE M, MENACHEM E AND LEVINE A. 1999. The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in plants. Plant Cell 11: 431-443.

SOUSA SM, SILVA PS, CAMPOS JMS AND VICCINI LF. 2009. Cytotoxic and genotoxic effects of two medicinal species of Verbenaceae. Caryologia 62: 326-333.

SOUSA SM, SILVA PS AND VICCINI LF. 2010. Cytogenotoxicity of *Cymbopogon citratus* (DC) Stapf (lemon grass) aqueous extracts in vegetal test systems. An Acad Bras Cienc 82: 305-311.

SOUSA SM AND VICCINI LF. 2011. Cytotoxic and genotoxic activity of *Achillea millefolium* L, Asteraceae, aqueous extracts. Rev Bras Farmacogn 21: 98-104.

TEIXEIRA RO, CAMPAROTO ML, MANTOVANI MS AND VICENTINI VEP. 2003. Assessment of two medicinal plants, *Psidium guajava* L and *Achillea millefolium* L, in *in vitro* and *in vivo* assays. Genet Mol Biol 26: 551-555.

TLILI N, MEJRI H, YAHIA Y, SAADOUI E, REJEB S, KHALDI A AND NASRI N. 2014. Phytochemicals and antioxidant activities of *Rhus tripartitum* (Ucria) fruits depending on locality and different stages of maturity. Food Chem 160: 97-103.

VALERIO ME, GARCIA JF AND PEINADO FM. 2007. Determination of phytotoxicity of soluble elements in soils, based on a bioassay with lettuce (*Lactuca sativa* L). Sci Total Environ 378: 63-66.

VERDEGUER M, BLÁZQUEZ MA AND BOIRA H. 2009. Phytotoxic effects of *Lantana camara*, *Eucalyptus camaldulensis* and *Eriocephalus africanus* essential oils in weeds of Mediterranean summer crops. Biochem Syst Ecol 37: 362-369.