academicJournals

Vol. 9(7), pp. 216-222, 17 February, 2015
DOI: 10.5897/JMPR2014.5647
Article Number: EBC0CDF51209
ISSN 1996-0875
Copyright © 2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Pharmaco-toxic characterization of the aqueous extract from *Pereskia grandifolia* leaves

Anderson Assaid Simão*, Angelita Duarte corrêa, Thaís Cristina Lima de Carvalho, Pedro Henrique Souza Cesar, Carlos Henrique de Moura Oliveira and Silvana Marcussi

Chemistry Department, Biochemistry Laboratory, Universidade Federal de Lavras – UFLA, Lavras, MG, Brazil.

Received 15 October, 2014; Accepted 9 February, 2015

Medicinal plants are rich sources of bioactive compounds with potential for therapeutic, cosmetic and food use, and the species *Pereskia grandifolia* is widely used by low-income populations for nutritional and medicinal purposes. The objective of this study was to evaluate the pharmaco-toxic potential of the aqueous extract from *P. grandifolia* leaves, in order to obtain information on the safety of its use. *P. grandifolia* leaves were dried, ground and the obtained flour was subjected to decoction at a 1:25 (wv^{-1}) ratio. The extract was then centrifuged and the supernatant was collected and lyophilized. The genotoxic potential on human leukocytes (comet assay) was evaluated, as well as phospholipase, hemolytic, coagulant and fibrinogenolytic activities, besides potential interactions with isolated hyaluronidases (evaluated in polyacrylamide gel). The evaluated doses of the aqueous extract from *P. grandifolia* leaves did not induce damage to the genetic material of human leukocytes, phospholipase, coagulant and fibrinogenolytic activities, nor altered the electrophoretic migration profile of hyaluronidases in polyacrylamide gel. However, the extract induced *in vitro* hemolysis, in doses higher than 10 µg. Therefore, it is premature to recommend the use of this plant for therapeutic purposes regarding possible risks and benefits to human health and additional studies of toxicity, efficacy and safety are necessary, especially using *in vivo* assays.

Key words: *Pereskia grandifolia*, genotoxicity, hemolysis, coagulation, molecular interactions.

INTRODUCTION

Ora-pro-nobis corresponds to different species of the genus *Pereskia*, cacti known as unconventional vegetables, consumed by rural and urban populations and which contribute to suplement food and family economy (Souza, 2009). *Pereskia aculeata* Miller is the most widely investigated species, and it is consumed in various regions of Brazil (Takeiti et al., 2009). On the other hand, *Pereskia grandifolia* is very little studied regarding its chemical constituents and toxicity.

P. grandifolia is widely distributed throughout Brazil

*Corresponding author. E-mail: andersonufla@hotmail.com, angelita@dqi.ufla.br. Tel: +55-35-3829-1272. Fax: +55-35-3829-1271.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

(Turra et al., 2007). However, the lack of information on their nutritional value and toxicity results in a reduction in their consumption in Brazil (Rocha et al., 2008).

In Malaysia, the leaves of this plant are traditionally used in the treatment of cancer, hypertension, diabetes mellitus and diseases associated with rheumatism, inflammation, headache, atopic dermatitis and hemorrhoids (Sim et al., 2010). They can also be used for gastric pain and ulcer (Goh, 2000); however, for most of these effects, there are no studies which confirm these therapeutic actions, highlighting the importance of research with the objective to increase the pharmacological and toxicological characterization of this plant.

Cacti of the genus *Pereskia* have a high content of proteins with high digestibility, mucilage fiber and minerals such as iron and calcium, thus arousing the interest of pharmaceutical and food industries. Furthermore, different *Pereskia* species have large amounts of bioactive substances, which allow to classify them as functional food (Takeiti et al., 2009). Various secondary metabolites found in cacti, such as alkaloids, terpenoids, anthocyanins, steroids, flavonoids, quinones and lignans are commercially used as pharmacons, natural dyes, flavoring substances and insecticides (Simões et al., 2004).

Despite their beneficial effects, bioactive compounds present in medicinal plants, in certain situations, may not be innocuous and present toxic, genotoxic and carcinogenic effects (Boeira et al., 2010; Osowski et al., 2010), highlighting the need for wide *in vitro* and *in vivo* pharmaco-toxic characterizations of the extracts from these plants in order to evidence their therapeutic effects, as well as the determination of effective and safe conditions for their use. Therefore, the objective of this study was to evaluate the damage-inducing potential of the aqueous extract from *P. grandifolia* leaves to the genetic material of human leukocytes, degradation of phospholipids, hemolytic, coagulant, fibrinogenolytic and interaction with hyaluronidases, in order to investigate the safety of its use by the population and in formulations of new products.

MATERIALS AND METHODS

Preparation of the extract

P. grandifolia leaves were harvested from a garden, namely Horto de plantas Medicinais, at Universidade Federal de Lavras in January, 2014. The leaves were washed in tap water, kept in a 0.1% sodium hypochlorite solution for 1 h, washed in distilled water and dried in an oven for 48 h, at a temperature of 35°C. The dried leaves were grind in a Willey mill and the obtained flour was subjected to decoction in boiling water at a 1:25 (wv^{-1}) ratio. The extract was then centrifuged at 10,000 × g for 10 min (206 BL Fanem Baby®I), the supernatant was collected and the precipitate was subjected twice to the extraction process. The extracts were then lyophilized (FreeZone LABCONCO 4.5 L benchtop lyophilizer), weighed and dissolved in water for the assays. In this study, all reagents were of analytical grade.

Comet assay

Obtention of human blood and preparation of treatments

The experiment was conducted in accordance with the standards of the Ethics Committee on Human Research (COEP) from Universidade Federal de Lavras, and has been approved by this committee (Protocol 545/281). The peripheral blood of five volunteers aged between 21 and 40 years old was used, without disease symptoms, and they declared not to have used prescription drugs for a minimum of 30 days prior to collection, after free informed consent. The blood was collected in vacuum tubes containing the anticoagulant heparin and was immediately protected from light. The aqueous extract from *P. grandifolia* leaves was then added to 300 µl aliquots of blood diluted in 300 µl phosphate buffered saline (PBS) at doses of 10, 30, 60 and 100 µg. Negative controls received water in replacement of the treatments. The concentration 60 µg chosen for the genotoxicity evaluation of the aqueous extract from *P. grandifolia* leaves correspond to the equivalent dose recommended for human consumption of teas, infusions and decoctions, seeking benefits to health, considering the equivalency between the blood volumes of an adult individual and that used in the assay. The blood samples were incubated in an oven at 37°C, in the presence of the treatments for 4 h. The comet assay was then performed.

Obtention of nucleoids and electrophoretic run

The comet assay was performed according to the methodology described by Singh et al. (1988), with modifications. Therefore, an aliquot (15 µl) of each cell suspension containing the treatments was mixed with 100 µl of low melting point agarose (0.5% wv^{-1} in PBS), applied to a microscope slide previously coated with standard agarose solution (1% wv^{-1} in PBS), immediately overlaid with a coverslip and kept at ±4°C for 5 min. For each treatment/volunteer, 3 slides were prepared. The coverslips were then removed and the slides were immersed in lysis solution (2.5 mol L^{-1} NaCl, 100 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA), 10 mmol L^{-1} Tris, 1% Triton X-100, 10% dimethyl sulphoxide (DMSO); pH 10), where they remained for 24 h at 4°C, allowing the removal of anucleate cells and plasma molecules, as well as the lysis of leukocytes, in order to isolate only their nucleoids for analysis. After lysis, the slides were kept at ± 10°C for 25 min in a freshly prepared electrophoresis solution (1 mmol L^{-1} EDTA, 300 mmol L^{-1} NaOH; pH 13), in order to expose alkali-labile sites, and they were then subjected to electrophoresis, conducted at 25 V for 35 min. After electrophoresis, the slides were kept in a neutralization solution (0.4 mol L^{-1} Tris; pH 7.5) for 30 min, dried and fixed with 100% ethanol. All procedures were performed in the dark.

Staining and analysis

The slides were stained with 45 µl of propidium iodide solution (1 mg ml^{-1}), overlaid with a coverslip and analyzed in an epifluorescence microscope (Nikon ECLIPSE E400) at 200× and 400× magnifications. In order to measure damage levels in DNA

molecules of leukocytes, 100 nucleoids of each slide, 3 slides per treatment/individual (totaling 300 nucleoids per treatment/individual) were counted and classified by the same evaluator, using visual score patterns described by Singh et al. (1988). The cells were classified according to the size of the “tail” and the diameter of the “head” in Class 0: no damage (damage < 5%); Class 1: low damage level (5 to 20%); Class 2: intermediate damage level (20 to 40%); Class 3: high damage (40 to 85%); Class 4: totally damaged (damage > 85%). The average damage frequency was calculated from the sum of the percentages of damage 1, 2, 3 and 4.

Phospholipase activity

Phospholipase activity was evaluated using solid media as described by Gutiérrez et al. (1988), replacing agarose with agar. The gel was prepared with 0.01 mol L^{-1} $CaCl_2$; 1:3 vv^{-1} egg yolk in PBS; 0.005% sodium azide; and 1% bacteriological agar, at pH 7.2. After the gel solidified in petri dishes, the aqueous extract from *P. grandifolia* leaves was applied to ~ 0.5 cm gel holes at doses of 1, 5, 10, 25, 50, 100 and 400 µg, and phospholipase activity was evaluated after the plates were kept in a cell culture chamber at 37°C for 24 h. The formation of a clear halo around the orifice in the gel characterized phospholipase activity, which was measured according to the halo diameter.

Hemolysis of human erythrocytes

For the evaluation of hemolytic activity in solid medium, a gel similar to that cited in the methodology for phospholipase activity was prepared, with the replacement of egg yolk by erythrocytes in PBS solution. The blood was collected in vacuum tubes containing the anticoagulant sodium citrate, immediately centrifuged at 400 × g (206 BL Fanem Baby®I) for 10 min and subjected to 3 washes at the same PBS volume; the erythrocytes were then obtained at hematocrit of 100%. After solidification of the gel in a Petri dish, the aqueous extract from *P. grandifolia* leaves was applied to ~ 0.5 cm gel holes at doses of 1, 5, 10, 25, 50, 100 and 400 µg, and hemolytic activity was evaluated after the plates were kept in a cell culture chamber at 37°C for 24 h. The formation of a translucent halo around the gel hole characterized hemolytic activity, which was measured by the diameter of the halo.

Coagulant activity

The coagulant activity was evaluated according to the methodology described by Rodrigues et al. (2000) using citrated human plasma (200 µl), stabilized at 37°C. The aqueous extract from *P. grandifolia* leaves was added to plasma at doses of 1, 5, 10, 25, 50, 100 and 400 µg, with subsequent counting of clotting time, gentle agitation and constant observation until the formation of a solid clot. Incubated plasma and the extracts were observed for 120 min, allowing to confirm the absence of coagulant action.

Proteolytic activity on fibrinogen

In order to evaluate fibrinogenolytic activity, polyacrylamide gel electrophoresis was performed under reducing conditions, as described by Laemmli (1970). Different doses of the aqueous extract from *P. grandifolia* leaves (60, 100, 200 and 400 µg) were incubated with bovine fibrinogen (80 µg) for 60 min at 37°C. The samples were analyzed in a polyacrylamide gel prepared at 12% (wv^{-1}) under denaturing conditions, allowing the observation of α, β, and γ chains of the fibrinogen control, as well as the presence of fibrinopeptides in the samples in which venom-induced proteolysis occurred.

Interaction of the aqueous extract from *Pereskia grandifolia* leaves with hyaluronidases

For the evaluation of possible interactions between constituents of the extract from *P. grandifolia* and hyaluronidases, considering enzyme fragmentation or structure binding, the polyacrylamide gel electrophoresis method described by Leber and Balkwill (1997) was used. The hyaluronidases and the extract was incubated at the ratios 1:0.5, 1:1, 1:5, 1:10, 1:50 and 1:100 (hyaluronidase/extract; ww^{-1}) for 30 min at 37°C. Samples were analyzed in a 10% polyacrylamide gel in the absence of denaturing agents and without boiling to preserve the native structure of the enzymes. After the electrophoretic run, staining in Coomassie Blue was carried out for 30 min and destaining in a 10% acetic acid solution. The analysis of the results was performed visually, and migration profiles were reported.

Statistical analysis

The results of the comet assay were statistically evaluated by analysis of variance and the means were compared using the Scott Knott test (P < 0.05) with the aid of the R software (R Development Core Team, 2011).

RESULTS AND DISCUSSION

In the comet assay, in which fragmentation levels of DNA molecules obtained from human leukocytes were evaluated, there was no statistical difference between the nucleoids treated with extracts from *P. grandifolia* leaves and those untreated (negative control), for all damage classes (Table 1). It was possible to observe the predominance of undamaged nucleoids (class 0), with variation in average percentages from 50.97 to 58.63% for different doses of the extract and with a low damage level (class 1), the averages observed ranged between 37.60 and 41.10%. Nucleoids with intermediate damage levels (class 2) represented low average percentages, ranging between 1.84 and 3.70%, and for all doses evaluated, nucleoids with high fragmentation levels (class 3) or totally fragmented DNA (class 4) were absent (Table 1). The average damage frequency (Figure 1) demonstrates that, in general, there was no significant increase in the amount of damaged nucleoids with increasing doses of the extract, showing the lack of genotoxicity, considering the experimental conditions used, thus suggesting an absence, in the composition of the aqueous extract from *P. grandifolia* leaves, of molecules capable of inducing genotoxicity and possibly mutagenicity to leukocyte DNA.

Table 1. Average converted into percentage of nucleoid number per comet class after treatment with the aqueous extract from *Pereskia grandifolia* leaves.

Treatment	Comet class (%)[1]				
	0	1	2	3	4
C (-)[2]	61.00 ± 10.33[a]	37.67 ± 9.68[a]	1.94 ± 0.68[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
P. grandifolia					
10 µg	56.57 ± 10.14[a]	39.60 ± 9.24[a]	3.60 ± 1.19[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
30 µg	55.17 ± 10.47[a]	40.20 ± 10.96[a]	3.70 ± 1.52[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
60 µg	50.97 ± 10.88[a]	41.10 ± 7.95[a]	3.41 ± 1.51[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
100 µg	58.63 ± 14.23[a]	37.60 ± 14.56[a]	1.84 ± 0.63[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]

Data represent the average values obtained in 300 nucleoids/treatment/volunteer. 5 volunteers (1 volunteer/experiment) were used. Same letters in the columns do not differ by the Scott-Knott test (P <0.05). Classes[1]: (0 = damage<5%); (1 = 5 to 20%); (2 = 20 to 40%); (3 = 40 to 85%) and (4 = damage>85%). C (-)[2] = negative control prepared with the addition of water.

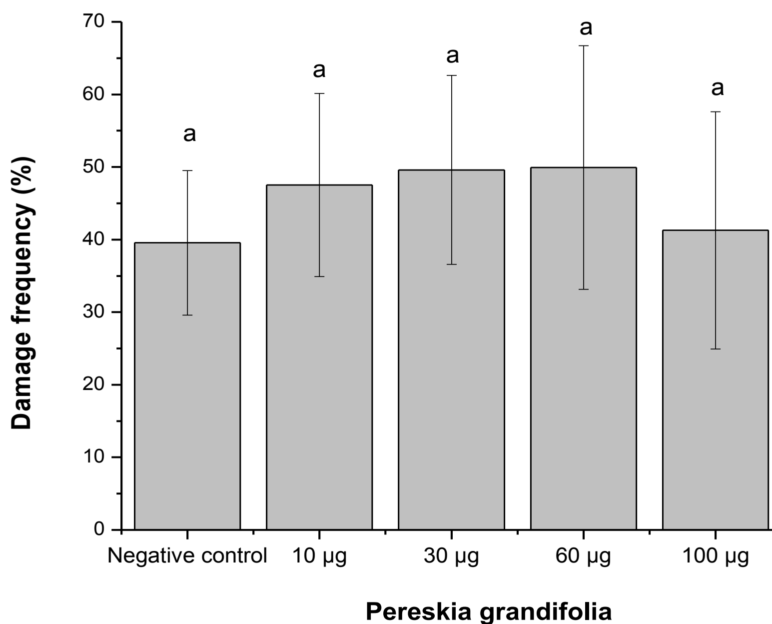

Figure 1. Damage frequency (DF) in the DNA of human leukocytes treated with different doses of the aqueous extract from *Pereskia grandifolia* leaves. DF: sum of damage from class 1 to 4. Same letters in the columns do not differ by the Scott-Knott test (P < 0.05).

Some researchers have used the comet assay to complement the toxicological characterization of plant extracts, thus investigating the genotoxic effects of secondary metabolites (for example, phenolic compounds, alkaloids and terpenes). Pereira et al. (2012) investigated the genotoxic potential of white bean flour, containing tannins and other phenolic compounds, on the genetic material of human leukocytes, and described the predominance of low fragmentation levels of DNA molecules in their results, under the evaluated conditions. Wan Ibrahim et al. (2010) evaluated 20 aqueous plant extracts and found that only two of them, *Vitex Pinnata* L. and *Quercus infectoria* Oliver, caused damage higher than 50% to the DNA of human leukocytes, and they are attributed to phenolic content, more specifically to tannic acid. Strange et al. (2009) analyzed natural products such as guaco (*Mikania glomerata* Spreng), espinheira-santa (*Maytenus ilicifolia* Mart. ex Reiss) and salvia (*Lippia alba* Mill. NE), and observed cytotoxic and genotoxic activity induced by metabolites such as coumarins, tannins and terpenes, suggesting the need for a toxicological characterization of plant metabolites, since

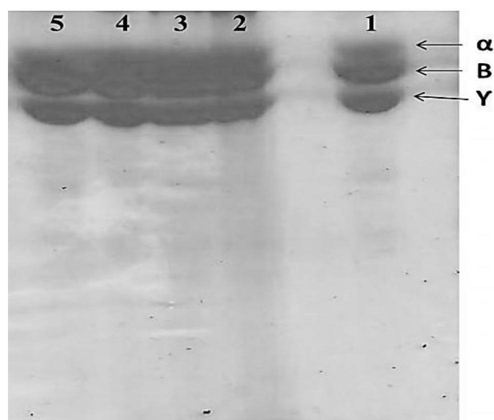

Figure 2. PAGE for visualization of fibrinogenolytic activity. Evaluation of the interaction between fibrinogen and the aqueous extract from *Pereskia grandifolia* leaves (AEPGL). Samples: 1- Control, Fibrinogen (80 µg); 2- Fibrinogen (80 µg) + AEPGL (60 µg); 3- Fibrinogen (80 µg) + AEPGL (100 µg ml^{-1}); 4- Fibrinogen (80 µg) + AEPGL (200 µg); 5- Fibrinogen (80 µg) + AEPGL (400 µg ml^{-1}).

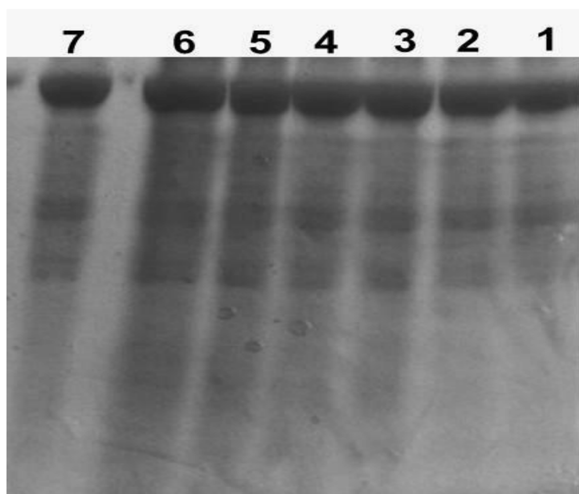

Figure 3. Effect of the aqueous extract from *Pereskia grandifolia* leaves on the structure of isolated hyaluronidases. Samples: 1- pure hyaluronidase (10 µg); 2- hyaluronidase + aqueous extract from *Pereskia grandifolia* leaves (AEPGL) (1:0.5, w/w), 5 µg extract; 3 hyaluronidase + AEPGL (1:1, w/w), 10 µg extract; 4- hyaluronidase + AEPGL (1:5, w/w), 50 µg extract; 5- hyaluronidase + AEPGL (1:10, w/w), 100 µg extract; 6- hyaluronidase + AEPGL (1:50, w/w), 500 µg extract; 7- hyaluronidase + AEPGL (1:100, w/w), 1000 µg extract.

they can cause adverse and/or therapeutic effects depending on factors such as dose and duration of use. Studies conducted with *P. grandifolia* leaves showed the presence of some metabolites with pharmacological properties such as saponins (Almeida et al., 2014), tannins, flavonoids (Turra et al., 2007) and alkaloids (Doetsch et al., 1980). However, some scientific reports have demonstrated that these compounds can be toxic to mammalian cells, even at low concentrations (Boeira et al., 2010; Osowski et al., 2010). In the present study, the conditions for the preparation of the extract, the evaluated doses and the parameters used in the assays resulted in the lack of genotoxicity, even considering the presence of compounds from secondary metabolism in the extract from *P. grandifolia* leaves. At the tested doses, the aqueous extract from *P. grandifolia* leaves did not induce lysis of phospholipids (results not shown); however, it was hemolytic at doses higher than 10 µg (results not shown), possibly due to the presence of interactions of extract constituents with different structures present in erythrocyte membranes, namely, proteins or steroids.

From the metabolites reported in the literature to be present in *P. grandifolia* leaves, saponins have hemolytic potential. The ability of saponins to interact with sterols, present in the plasma membrane of erythrocytes, increases the permeability of this membrane, allowing ions and water into the cells, resulting in their rupture and release of hemoglobin (Karabaliev and Kochev, 2003). Although surfactant and hemolytic properties are striking features of saponins, they are not common to all saponins and, therefore, it is not possible to conclude that they are responsible for the hemolysis observed in present study. Thus, additional studies should be conducted to verify the hemolytic potential of the aqueous extract from *P. grandifolia* leaves.

Regarding coagulant activity, none of the tested doses was able to induce coagulation, thus suggesting the absence of molecules capable of cleaving or interacting with components of the coagulation cascade (results not shown) in the composition of the evaluated aqueous extract. The aqueous extract from *P. grandifolia* leaves was not proteolytic on the fibrinogen molecule or with binding properties of this protein in the evaluated concentrations (60, 100, 200 and 400 µg), since the electrophoretic profile of α, β and γ chains resulting from reduced fibrinogen molecules remained unchanged after incubation in the presence of the extract for a period of 60 min (Figure 2). This result corroborates the lack of the coagulation effect of the extract on citrated human plasma, since one of the coagulation-inducing mechanisms is related to the cleavage of fibrinogen molecules for the formation of fibrin networks.

At the evaluated ratios and incubation conditions used, no change was observed in the migration profile of the enzyme hyaluronidase, commercially obtained, which characterizes binding of enzymes to constituents of the extract or hyaluronidase fragmentation (Figure 3). In the

samples containing higher doses of the extract, it is possible to observe darkened bands in the migration profile, which correspond to a higher concentration of non-protein compounds, probably secondary metabolites and/or carbohydrates present in the extract, but without any changes in the electrophoretic profile of hyaluronidase molecules.

Hyaluronidases are enzymes present in our organism responsible for the degradation of hyaluronic acid, which is part of the composition of interstitial spaces (Stern and Jedrzejas, 2006). These enzymes have been widely used by cosmetic (mainly to increase skin elasticity, allowing a better hydration) and pharmaceutical industries (to loosen the tissues, facilitating drug administration), besides being a valuable tool for research, since its inhibition results in changes in inflammatory response (Dunn et al., 2010; Menzel and Farr, 1998; Pirrelo et al., 2007).

Studies conducted with medicinal plants and compounds isolated from these plants show that they have potential inhibition of hyaluronidases. Flavonol kaempferol competitively inhibits this enzyme (Middleton et al., 2000). Polyphenols found in wine also inhibit hyaluronidase (Weisse, 1995). Machiah et al. (2006) observed a hyaluronidase inhibitor, purified from medicinal plants (*Withania somnifera*). Choi et al. (2006) also studied the inhibition of hyaluronidase using plants (*Schisandra*), and found that the extract of this plant was effective in the inhibition of hyaluronidase. These studies show that medicinal plants have compounds with properties to interact or inhibit hyaluronidases in their composition, highlighting the need for an investigation of the interaction of plant extracts and their metabolites with hyaluronidase. Therefore, the results obtained in the present study suggest that the constituents of aqueous extract from *P. grandifolia* leaves have neither bound, nor induced cleavage in hyaluronidase molecules, under the evaluated conditions, since have not influence the electrophoretic migration profile of hyaluronidase.

Conclusion

The aqueous extract from *P. grandifolia* leaves has no genotoxic, phospholipase, coagulant, fibrinogenolytic potential and interaction and/or cleavage on isolated hyaluronidases at the evaluated concentrations. However, it is premature to recommend the use of this plant regarding possible risks and benefits to human health, mainly due to the hemolytic activity observed, and additional studies of toxicity, efficacy and safety are necessary for defining doses, formulations and an appropriate usage for human consumption.

ACKNOWLEDGMENTS

The authors would like to thank CNPQ for the post-doctoral grant and FAPEMIG for the financial support and undergraduate research grant.

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

REFERENCES

Almeida MFE, Junqueira AMB, Simão AA, Corrêa AD (2014). Chemical characterization of the non-conventional vegetable known as ora-pro-nobis. Biosci. J. 30(1):431-439.

Boeira JM, Fenner R, Betti AH, Provensi G, Lacerda LA, Barbosa PR (2010). Toxicity and genotoxicity evaluation of *Passiflora alata* Curtis (Passifloraceae). J. Ethnopharmacol. 128(2):526-532.

Choi SI, Park SR, Heo TR (2006). Matrix Degradation Inhibitory Effect of Schisandra Fructus on Human Articular Cartilage and Chondrocytes. J. Ethnopharmacol. 106(2):279-284.

Doetsch PW, Cassady JM, Mclaughlin JL (1980). Cactus alkaloids. XL. Identification of mescaline and other β- phenethylamines in *Pereskia*, *Pereskiopsis* and *Islaya* by use of fluorescamine conjugates. J. Chromatogr. 189(1):79-85.

Dunn A, Heavner JE, Recz G, Day M (2010). Hyaluronidase: a review of approved formulations, indications and off-label use in chronic pain management. Expert Opin. Biol. Ther. 10(1):127-131.

Goh KL (2000). Malaysian herbaceous plants millennium. Malaysia: Advanco, 2000. 142 p.

Gutiérrez JM, Avila C, Rojas E, Cerdas L (1988). An alternative *in vitro* method for testing the potency of the polyvalent antivenom produced in Costa Rica. Toxicon. 26(4):411-413.

Karabaliev M, Kochev V (2003). Interaction of solid supported thin lipid films with saponin. Sens. Actuators B, 88:101-105.

Laemmli UK (1970). Cleavage of structural proteins during the assembly to the head of bacteriophage T4. Nature. 227:680-685.

Leber TM, Balkwill FR (1997). Zymography: a single-step staining method for quantification of proteolytic activity on substrate gels. Anal. Biochem. 249:24-28.

Machiah DK, Girish KS, Gowda TV (2006). A glycoprotein from a Folk Medicinal Plant, *Withania somnifera*, Inhibits Hyaluronidase Activity of Snake Venoms. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 143(2):158-161.

Menzel EJ, Farr C (1998). Hyaluronidase and its substrate hyaluronan: biochemistry, biological activities and therapeutic uses. Cancer Lett. 131(1):3-11.

Middleton E, Kandaswami C, Theoharides C (2000). The effects of Plant Flavonoids on Mammalian Cells: Implications for Inflamamation, Heart Disease, and Cancer. Pharmacol. Exp. Ther. 52:673-751.

Osowski A, Pietrzak M, Wieczorek Z, Wieczorek J (2010). Natural compounds in the human diet and their ability to bind mutagens prevents DNA-mutagen intercalation. J.Toxicol. Environ. Health: Part A, 73(17-18):1141-1149.

Pereira LLS, Marcussi S, Sátiro LC, Pereira CA, Andrade LF, Davide LC, Santos CD (2012). Application of Comet assay to assess the effects of white bean meal on DNA of human lymphocytes. Braz. J. Pharm. Sci. 48 (1):103-108.

Pirrelo RD, Ting-Chen C, Thomas SH (2007). Initial experiences with subcutaneous recombinant human hyaluronidase. J. Palliat. Med. 10(4):861-864.

Rocha DRC, Pereira-Júnior GA, Vieira G, Pantoja L, Santos AS, Pinto NAVD (2008). Macarrão adicionado de ora-pro-nobis (*Pereskia aculeata* Miller). Alim. Nutr. 19(4):459-465.

Rodrigues V, Soares AM, Guerra-Sá R, Rodrigues V, Fontes MRM,

Giglio JR (2000). Structural and Functional Characterization of Neuwiedase, a Nonhemorrhagic Fibrin(ogen)olytic Metalloprotease from *Bothrops neuwiedi* Snake Venom. Arch. Biochem. Biophys. 381:213-224.

R Development Core Team. R: A language and environment for statistical computing (2011). Viena: R Foundation for Statistical Computing. http://www.R-project.org/.

Sim KS, Sri-Nurestri AM, Sinniah SK, Kim KH, Norhanom AW (2010). Acute oral toxicity of *Pereskia bleo* and *Pereskia grandifolia* in mice. Pharmacogn. Mag. 6(21):67-70.

Simões CMO, Schenkel EP, Gosman G, Mello JCP, Mentz LA, Petrovick PR (2004). Farmacognosia: da planta ao medicamento. 5. ed. Porto Alegre/Florianópolis: Editora da UFRGS, 2004.

Singh NP, Mccoy MT, Tice RR, Scheider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175(1):184-191.

Souza MRR (2009). O potencial do ora-pro-nobis na diversificação da produção agrícola familiar. Rev. Bras. Agroecol. 4(2):3550-3554.

Stern R, Jedrzejas MJ (2006). The Hyaluronidases: Their Genomics, Structures, and Mechanisms of Action. Chem. Rev. 106(3):818-839.

Strange VS, Gomes TDUH, Andrade MA, Batitucci MCP (2009). Avaliação do efeito mutagênico do extrato hidroalcoólico bruto, por meio de bioensaios *in vivo* e prospecção fitoquímica de *Cecropia glaziovii* Sneth (embaúba), Cecropiaceae. Rev. Bras. Farmacogn. 19(2):637-642.

Takeiti CY, Antonio GC, Motta EMP, Collares-Queiroz FP, Park KJ (2009). Nutritive evaluation of non-conventional leafy vegetable (*Pereskia aculeata* Miller). Int. J. Food Sci. Nutr. 60(1):148-160.

Turra AF, Marçal FJB, Baretta IP, Takemura OS, Laverde JRA (2007). Avaliação das propriedades antioxidantes e susceptibilidade antimicrobiana de *Pereskia grandifolia* Haworth (Cactaceae). Arq. Cienc. Saúde Unipar. 11(1):9-14.

Wan-Ibrahim WI, Sidik K, Kuppusamy UR (2010). A high antioxidant level in edible plants is associated with genotoxic properties. Food Chem. 122(4):1139-1144.

Weisse ME (1995). Wine as a digestive aid: comparative antimicrobial effects of bismuth salicylate and red and white wine. Br. Med. J. 311: 1657-1660.