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Wound healing properties and mucilage content of *Pereskia aculeata* from different substrates

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Pereskia aculeata Mill., Cactaceae, is a cactus with high mucilage production, well-known for its nutritional properties. Folk use consists on skin injuries, and mucilage is probably involved in the wound healing activity. This work studied some aspects of its cultivation, specifically regarding soil (substrate), to correlate the effects of nutritional content to mucilage production and to the wound-healing property. Plants were grown under five different soil treatment (sand, crude soil, sand and soil, sand and cattle manure, soil and cattle manure), and after eight months extracts were prepared by turbo-extraction to obtain a crude hydroethanolic extract. We evaluated the effects of these extracts on swelling index, cytotoxicity, and *in vitro* wound healing property. The results show that the substrate used in cultivation may interfere with mucilage production, but not with cytotoxicity and wound healing, this shows the safety of its use, despite the soil treatment received along the various biomes where *P. aculeata* is cultivated. Furthermore, morphological studies demonstrated the beneficial effect of the mucilage-containing extract on the fibroblast cell culture, corroborating its folk use for wound healing.

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Introduction

Pereskia aculeata Mill., Cactaceae, also known as *ora-pro-nobis*, is a cactus traditionally used not only for medicinal purposes, but for alimentary and ornamental reasons too (Albuquerque et al., 1991; Gronner et al., 1999; Merce et al., 2001; Duarte and Hayashi, 2005). The fruits show expectorant and anti-syphilis properties, the leaves are used as emollient (Pio-Correa, 1978), anti-inflammatory, wound healing (Almeida and Corrêa, 2012), and auxiliary for post-burning treatment of the skin (Gronner et al., 1999).

Wound healing is a property commonly attributed to the mucilage of the cactus leaves (Aburjai and Natsheh, 2003; Thornfeldt, 2005), however, this property has never been tested under experimental conditions. Wound healing is a complex process involving steps of cellular migration and proliferation, especially, but not only, of fibroblast cells (Krishnan, 2006). Thus, it is expected that the test compound should be effective in promoting both the cellular proliferation and migration of fibroblast as indication of wound healing property. A convenient method to avoid the use of animal experimentation was recently reviewed by Yarrow et al. (2004), where the observation

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of proliferation and migration of fibroblast in culture can be done over a glass plate covered with a matrix, turning the environment closest to the natural conditions.

In addition to cellular events, wound healing is commonly accompanied by an inflammatory process, which plays an important role in the delivery of mediators involved in chemical signaling for immune cells (van Solingen et al., 2014). It is well-known that polyphenolic compounds typically exhibit antioxidant activity, and present the ability to scavenge free radicals produced by cellular metabolism or other exogenous sources (Bianchi and Antunes, 1999; Everette et al., 2010). One of the characteristics of the inflammatory stage of wound healing is the exacerbated production of free radicals, and the content of polyphenolic compounds can break down this process, generating, on one hand, a relief in the inflammatory symptoms, including pain and itch; but disrupting the wound healing process by interference on the delivery of chemical signaling.

P. aculeata is a plant whose medicinal properties are barely studied, and little information is found regarding its cultivation and its effects on the general development and secondary metabolic production. Since this species is widely distributed over the Brazilian territory, growing on different biomes (Rosa and Souza, 2003), this work had two main purposes: 1- Understand the substrate influence, and the nutrient content in consequence, on mucilage and polyphenolic compounds production; and 2- Study the wound healing properties of the leaves in relation to their mucilage content.

Material and methods

Plant material

Plant identification was carried out by Prof. Dr. Marlon Machado, from State University of Feira de Santana; and a voucher specimen was deposited in the Herbarium of the State University of Feira de Santana, under identification number HUEFS 189684.

Seedlings of *Pereskia aculeata* Mill., Cactaceae, were cultured under different types of substrates in the Nursery of Medicinal Plants of the Universidade do Vale do Paraíba, São José dos Campos, SP, Brazil, between the months of December to July. Apical cuttings of 10 cm were kept for 30 days in a 72-cell tray to root, and then were transferred to two-liter polypropylene bags containing the different kinds of substrates. Cuttings (800) were divided in two groups of 400 cuttings each, one for growth and physiological analysis, and other for extraction and later studies.

The plants were randomized into four blocks, and each block was composed of all five different treatments with four repetitions each. Box 1 summarizes the different substrates used at each treatment. The experiment was conducted for eight months, re-fertilizing each box with the same substrate after five months, to assure the correct condition of the experiment.

Physiological parameters data

The measuring of the linear growth of the plant was done in intervals of 15 days, using a measuring tape from the ground to the highest leaf on the top; and the Fresh Matter Weight

Box 1

Treatments and substrates.

Treatment	Substrate
T1	Sand
T2	Crude soil
T3	Sand and soil (1:1)
T4	Sand and cattle manure (5:1)
T5	Soil and cattle manure (5:1)

(FMW) was taken from recently collected seedlings and from each organ separately (leaf, stem and root). Afterwards, the plant material was dried on a stove at 60°C until no changes on weight could be observed for an interval of 24 h, and the final weight was recorded as Dry Matter Weight (DMW).

Extraction

Hydroalcoholic extracts were prepared by turbo-extraction of 55 g of fresh leaves and 100 ml of 95% (v/v) ethanol, for about 5 min then filtered, and the residue was washed with 100 ml of the same solvent. The clear filtrate was left to dry under soft warming and reduced pressure and kept well enclosed and protected from the sunlight.

Phytochemistry

Total phenolic content was determined by the classical Folin-Ciocalteu method (Singleton et al. 1999), employing a diluted solution of 0.1 g of the extract in 100 ml of 95% ethanol (sample solution). The colorimetric reaction was carried out in a 10 ml volumetric balloon, by transferring 0.1 ml of the sample solution, 0.5 ml of 2N Folin-Ciocalteu reagent, 4 ml of 7.5% sodium carbonate in water, and the volume was completed to 10 ml with distilled water. The mixture was kept for 2 h protected from the light before optical measurement at 760 nm (UV/VIS Lambda spectrophotometer, PerkinElmer). The phenolic content was achieved using an analytic curve of gallic acid prepared under the same conditions.

The antioxidant activity was calculated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, measuring the free radical scavenger ability. Sample solution was prepared in 95% ethanol, and diluted to a final test concentration of 0.01% (w/v). The sample (5 ml) was treated with 1 ml of 0.04 mg/ml of DPPH in ethanol and the optical density measured at 515 nm in an UV/VIS Lambda spectrophotometer (PerkinElmer) after 10 minutes.

The Swelling Index (SI) was calculated in accordance with the Brazilian Pharmacopoeia (Farmacopéia Brasileira, 2010), where 1 g of dry leaves powder was transferred to a 25 ml graduated measuring cylinder and 25 ml of distilled water were added. The mixture was agitated every 10 min for one hour, and then let to rest for three more hours at room temperature. The last volume of the plant material was recorded and compared with the first volume of dry material using the equation 1, where SI% is the swelling index given in percentage, and V is the volume of the material:

$$SI\% = \frac{V_{final} - V_{initial}}{V_{initial}} \times 100 \quad \text{Equation 1}$$

Cell Culture

L929 mouse fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM - Gibco), supplemented with 10% fetal bovine serum (FBS) and 1% of penicillin-streptomycin solution. The cells were grown in an incubator at 37°C and 5% CO₂.

Sample Preparation

A stock solution was made by solubilizing 10 mg of the dry crude extract into a volume of 10 ml of growth medium (DMEM). The diluted solution was obtained transferring 10 µl of the stock solution to a 10 ml balloon and the volume completed with growth medium (1 µg/ml), subsequent 1:2 dilutions were performed sequentially using the same medium to yield 0.5 µg/ml, 0.25 µg/ml and 0.125 µg/ml solutions.

In vitro wound healing assay

Cellular healing ability was evaluated by the scratch assay, adapted by Liang and col. (2007). The L929 cell line culture was grown until confluence in 24-wells plates. The plates were then treated with 0.5 ml of growth medium (DMEM supplemented with 10% FBS and antibiotics) and 0.5 ml of each diluted sample solution and the cellular monolayer scratched with p200 pipette tip, producing a slash of about 400 µm on it. The plate was placed in an incubator at 37°C, and the thickness of the produced scratch measured every 24 h until complete closure of the injury, for no more than three days. The rate of healing is compared against a control comprising a mixture of 0.5 ml of growth medium and 0.5 ml of water.

Cellular viability

Cytotoxicity of the extracts was evaluated by MTT (3-(4,5-dimethylthiazolone-2-yl)-2,5-diphenyl tetrazolium bromide, SigmaAldrich) method, according to the manufacturer protocol. L929 mouse fibroblast cells (about 10³ to 10⁶ cells per ml) were cultured on 24-wells microtiter plate in Dulbecco's Modified Eagle Medium (DMEM, Gibco), supplemented with 10% fetal bovine serum (FBS) and 1% of penicillin-streptomycin solution

and incubated for 24 h at 37°C and 5% CO₂. A volume of 0.5 ml of the cell culture were treated with 0.5 ml of the diluted solutions of the extract, prepared as described above, to obtain further solution concentrations of 0.5, 0.25, 0.125, 0.0625 µg/ml of the extract per well. Untreated cells were used as positive control. After this period of incubation, the wells were treated with 200 µl of 5 mg/ml of MTT in RPMI-1640 without phenol red, and incubated for 4 h at 37°C. The formazan crystals formed were solubilized by adding 500 µl of dimethylsulfoxide (Synth) and stirred 2 hours, and measured optical density at 570 nm by ELISA (Spectra Count-Parckard®). The assay was made in triplicate.

Statistical analysis

Results were expressed as mean ± SD. The levels of significance between samples were compared by one-way analysis of variance (ANOVA) using Tukey's test as post-hoc test for comparison of means.

Results

The physiologic growth parameters, i.e., height, fresh matter weight and dry matter weight, bring us important observations about how the plant uses the nutrients provided by the surrounding environment. Table 1 shows the measurements obtained from the different substrate treatment. As expected, the influence of the cattle manure is notorious on treatments T4 and T5, leading to the best values of height and total mass. It is well known that manure is an important source of nutrients, improving the humus quantity and water retention capacity of sandy soils (Chang et al., 2007, Santos et al., 2010).

Although both growth and phytomass gain due to cattle manure treatment is notorious, the same is not observed in relation to mucilage content. According to the Brazilian Pharmacopoeia (2010), the mucilage content is estimated from the swelling index of the dry leaves powder in water-medium.

Sugars are commonly the main constituents of mucilage, and considered storage of nutrients and energy; thus, the volume of this storage relates to the total nutrient supply. Thus, although the great growth produced from the use of cattle manure, mucilage accumulation is reversed, in way that the lowest swelling indices are found in treatments T4 and T5, where manure enriched the substrate (Table 2). When the substrate is composed only of sand or soil, the best

Table 1
Physiologic growth parameters according to treatment and vegetal organ.

Treatment	Height (cm)	Leaf (g)		Stem (g)		Root (g)	
		FMW	DMW	FMW	DMW	FMW	DMW
T1	14.55 ± 0,66 ^c	3.70 ± 0,47 ^d	0.37 ± 0,06 ^c	4.92 ± 0,04 ^c	1.9 ± 0,12 ^c	5.80 ± 0,53 ^c	1.17 ± 0,15 ^b
T2	14.81 ± 1,02 ^c	9.07 ± 1,35 ^b	0.86 ± 0,31 ^b	6.22 ± 0,71 ^c	1.51 ± 0,18 ^c	2.80 ± 0,43 ^d	0.59 ± 0,08 ^b
T3	16.02 ± 0,96 ^c	6.24 ± 0,21 ^c	0.78 ± 0,10 ^b	6.05 ± 0,21 ^c	2.15 ± 0,26 ^c	3.75 ± 0,52 ^{c,d}	0.8 ± 0,07 ^b
T4	27.15 ± 0,44 ^b	15.78 ± 1,99 ^a	1.56 ± 0,16 ^a	23.08 ± 1,71 ^a	12.42 ± 1,70 ^a	21.66 ± 2,10 ^a	5.68 ± 0,97 ^a
T5	29.04 ± 1,15 ^a	16.06 ± 1,40 ^a	1.65 ± 0,22 ^a	19.48 ± 0,39 ^b	8.13 ± 0,72 ^b	14.40 ± 0,83 ^b	1.4 ± 0,74 ^b

Averages with the same letters are not significantly different according to ANOVA ($p > 0.05$).

Table 2

Percentage of swelling index (SI) of dry leaves powder of *Pereskia aculeata*.

Treatment	SI (%)
T1	273.2 ± 4,17 ^a
T2	286.8 ± 4,34 ^a
T3	153.2 ± 5,00 ^b
T4	146.8 ± 2,19 ^b
T5	120.0 ± 5,55 ^b

Averages with the same letters are not significantly different according to ANOVA ($p > 0.05$).

swelling indices are found (T1 and T2 respectively). Treatment T3 presents an intermediate value, probably due to the best water drainage, and so nutrient availability, produced by sand presence.

Albeit the received substrate treatment influenced the mucilage content, phenolic constituents and antioxidant activity did not change accordingly. The phenolic compounds are the main secondary metabolites produced by plants in response to stress by edafo-climatic conditions or even by predators, as insects or microorganisms (Keutgen and Pawelzik, 2007). According to Moreira and Mancini Filho (2004), much of the biological activity attributed to phenolic compounds regards their free-radical scavenger ability. In this study, no alterations were observed from the different substrates employed for seedling culture (Tables 3 and 4). It is important to notice that our aim was not to prove the antioxidant property of this plant, but to see whether any of the treatments could improve or reduce this expected antioxidant potential. We judged that the information about the antioxidant or phenolic content variance is more important than the absolute value *per se*, since this alteration could imply a positive or negative influence on the wound healing properties, which is the real goal of the present work.

These and subsequent assays were carried out using the dry leaves extracts, obtained by turbo-extraction as previously described, which yields are listed in Table 5.

The antioxidant activity was evaluated from DPPH method due to its simplicity. From Table 4 it is possible to notice that the free-radical scavenging ability does not change significantly between treatments ($p > 0.05$). Equation 2 gives the percentage of scavenging activity (%SA):

$$\%AS = 100 \times \Delta h_x \quad \text{Equation 2}$$

Where: $\Delta h_x = h_c - h_x$; h_c = control absorbance (DPPH + EtOH); h_x = test absorbance (DPPH + sample).

Once phenolic and mucilage content were determined and variations estimated for each treatment, the dry extracts' wound healing potential was evaluated. Unfortunately, no significant variation was observed between the extracts and the control group (water) over a period of three days, which entails that no direct healing action could be, attributed to the hydroethanolic leaves' extracts. However, no cytotoxic

Table 3

Total phenolic content from different substrate treatments given as a gallic acid equivalent (GAE).

Treatment	Concentration (mg of GAC/l)
T1	111.6 ± 12,64
T2	108.2 ± 7,42
T3	139.4 ± 7,64
T4	120.4 ± 8,50
T5	121.1 ± 7,00

No significant differences were observed according to ANOVA ($p > 0.05$).

Table 4

Total phenolic content from different substrate treatments given as a gallic acid equivalent (GAE).

Treatment	OD	%SA
T1	0.0740 ± 0,04	19.92
T2	0.1030 ± 0,01	17.03
T3	0.0816 ± 0,01	19.17
T4	0.0991 ± 0,01	17.42
T5	0.0868 ± 0,01	18.65

No significant differences were observed according to ANOVA ($p > 0.05$).

Table 5

Percentage extract yield from 55 g of fresh leaves of *Pereskia aculeata*.

Treatment	% Yield (w/w)
T1	9,2%
T2	8,9%
T3	11,0%
T4	9%
T5	9,1%

effect was observed on the MTT assay. Besides the wound healing experiment have driven to a frustrating result, the non-cytotoxicity activity proves the safe use of this plant as an alimentary source or even for other medicinal purposes, as shown in Fig. 1.

Discussion

As expected, the physiological parameters of growth show the best development of the seedlings when using cattle manure in the substrate, favoring the phytomass development for treatments T4 and T5. However, this improvement in nutrient supply does not reflect an increase in mucilage content, instead, the percentage of swelling index, and greatest mucilage content, was obtained from the less nutritive substrate employed.

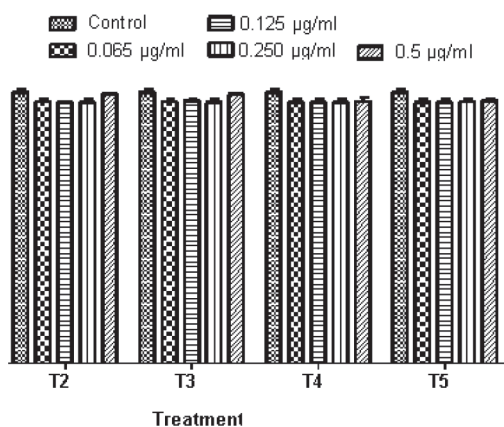


Figure 1 – Percentage of viable L929 fibroblasts treated with crude extracts of *Pereskia aculeata* from MTT assay.

Interestingly, independent to the ground drainage capacity, that results in different water supply to the plants, Table 6 shows the water content of the plants did not change in relation to the treatment ($p > 0.05$), thus showing that *P. aculeata* does not accumulate mucilage to keep water in the organism, and so, the mucilage content is not related to watering regime, but mainly to the nutrient supply.

Besides the differences on mucilage accumulation in the leaves, no difference was observed on the wound healing properties. However, a brief analysis of the fibroblast cells treated with the extracts showed an improvement on morphology in a dose-dependent way, but not due to the treatment that the seedlings received. The photomicrographs from Fig. 2 show the fibroblasts more clearly and with a well-defined form with less intercellular gap when the highest tested concentration (0.5 µg/ml) were applied. The importance of mucilage to fibroblast growth was reported by Aburjai and Natsheh (2003) and Thornfeldt (2005), attributing its presence the function to play as growth factor and to stimulate collagen and elastin production by these cells.

When we compare the action of the extracts over the scratched cell culture and to the cell growth without extract (water control), it is notorious the cytostatic effect of the plant material. It is possible to see from Fig. 3 that the control shows more cell migration, covering almost the entire gap and the

Table 6

Water percentage from each vegetal organ.

Treatment	Leaves	Stem	Root	Total
T1	90.0	61.4	79.8	77.1
T2	90.5	75.7	78.9	81.7
T3	87.5	64.5	78.7	76.9
T4	90.1	46.2	73.8	70.0
T5	89.7	58.3	90.3	79.4

No significant differences were observed according to ANOVA ($p > 0.05$).

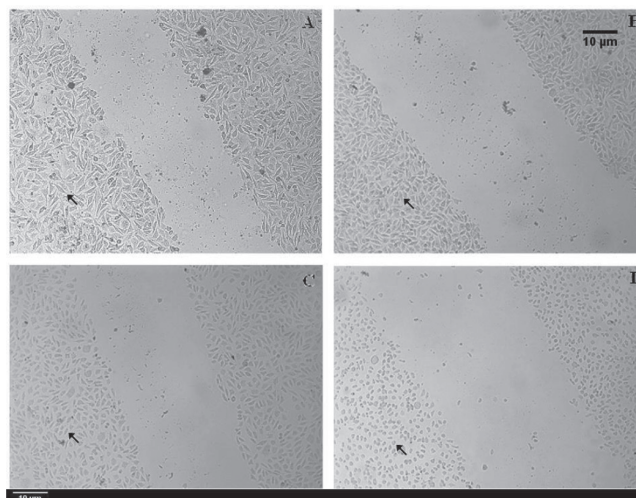


Figure 2 – PL929 fibroblast cell observed 72 h after injury to the cellular monolayer from the *in vitro* scratch assay. The arrows point the differences in the morphological aspect of the cells in accordance to the extract concentration used. A. 0.5 µg/ml; B. 0.25 µg/ml; C. 0.125 µg/ml; D. 0.0625 µg/ml.

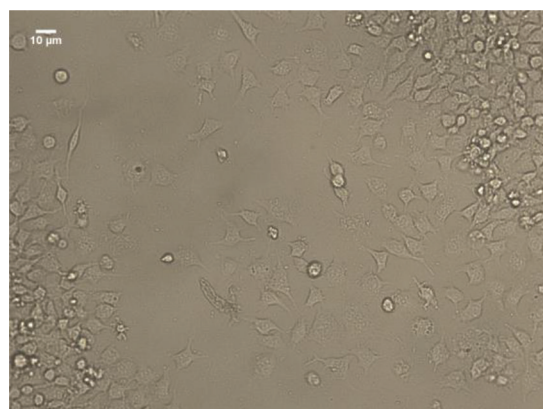


Figure 3 – L929 fibroblasts observed 72 h after injury to the cellular monolayer in the absence of crude extract of *Pereskia aculeata*.

same effect is not noticed when the extracts are added on the culture. This effect is somewhat paradoxical, and another study on the influence of extract components should be carried out to achieve a complete understanding regarding why mucilage might be responsible for cell morphology meliorate while there is a pronounced cytostatic effect not explained by the viability cell assay, that proves that a decrease of mitochondrial function does not happen even at the highest concentration of crude extract.

Due to the following observations: the change on mucilage content from each submitted treatment; the low tax of cell migration in the presence of any tested extract; the cell morphology improvement in a dose-dependent way; and no observed cytotoxic effect on the fibroblast cell culture, we can infer that *P. aculeata* is able to produce high amounts of mucilage even under the less favorable condition for mucilage

production (sand/soil and cattle manure), which guarantees the efficacy of the treatment under a variety of culture conditions, but that this content is raised when cultivated in a poorly nutritive soil (sand and/or soil), with no influence on the total phenolic content nor on antioxidant potential. Even those extracts that elicited worst swelling index (poor mucilage content) were able to produce a significant benefit to fibroblast function showing that the usage of *P. aculeata* for wound healing is completely justified by its production of appropriate conditions for a rapid curative process, probably providing energy, nutrient and physical protection due to the mucilage presence, with no cytotoxic effects on fibroblasts.

Authors' contributions

EGC (MSc student) contributed in collecting plant samples, confection of herbarium, running the laboratory work and analysis of the data. CPS contributed to biological studies and data analysis. LB contributed in plant extraction, phytochemical studies and critical reading of the manuscript. RFM and WMJ designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

- Aburjai, T., Natsheh, F., 2003. Plants used in cosmetics. *Phytother. Res.* 17, 987-1000.
- Albuquerque, M.G.P.T., Sabaa-Srur, A.U.O., Freiman, L.O., 1991. Composição centesimal e score de aminoácidos em três espécies de *Ora-pro-nobis* (*Pereskia aculeata* Mill. *P. bleu* de Candolle e *P. pereskia* (L) Karsten). *Bol. Soc. Bras. Ciênc. Tecnol. Aliment.* 25, 7-12.
- Almeida, M.E.F., Corrêa, A.D., 2012. Utilização de cactáceas do gênero *Pereskia* na alimentação humana em um município de Minas Gerais. *Ciênc. Rural.* 42, 751-756.
- Bianchi, M.L.P., Antunes, L.M.G., 1999. Radicais livres e os principais antioxidantes da dieta. *Rev. Nutr.* 12, 123-130.
- Chang, Ed-H., Chung, R.S., Tsai, Y.H., 2007. Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. *Soil Sci. Plant Nutr.* 53, 132-140.
- Duarte, M.R.; Hayashi, S.S., 2005. Estudo anatômico de folha e caule de *Pereskia aculeata* Mill. (Cactaceae). *Rev. Bras. Farmacogn.* 15, 103-109.
- Everette, J.D., Bryant, Q.M., Green, A.M., Abbey, Y.A., Wanglia, G.W., Walker, R.B., 2010. Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent. *J. Agric. Food Chem.* 58, 8139-8144.
- Farmacopeia Brasileira, 2010, 5ª Ed. Agência Nacional de Vigilância Sanitária, Brasília, DF.
- Gronner, A., Silva, V.D., Maluf, W.R., 1999. *Ora-Pro-Nobis* (*Pereskia aculeata*): a carne de pobre. Lavras:UFLa (Boletim Técnico de Hortaliças, 37).
- Keutgen, A.J., Pawelzik, E., 2007. Modifications of strawberry fruit antioxidant pools and fruit quality under NaCl stress. *J. Agric. Food Chem.* 55, 4066-4072.
- Krishnan, P., 2006. The scientific study of herbal wound healing therapies: Current state of play. *Curr. Anaesth. Crit. Care.* 17, 21-27.
- Liang, C.C., Park, A.Y., Guan, J.L., 2007. *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration *in vitro*. *Nat. Protoc.* 2, 329-333.
- Merce, A.L.R., Landaluze, J.S., Mangrigh, A.S., Szpoganicz, B., Sierakowski, M.R., 2001. Complexes of arabinogalactan of *Pereskia aculeata* and Co²⁺, Cu²⁺, Mn²⁺, and Ni²⁺. *Bioresour. Technol.* 76, 29-37.
- Moreira, A.V.B., Mancini-Filho, 2004. Influência dos compostos fenólicos de especiarias sobre a lipoperoxidação e o perfil lipídico de tecidos de ratos. *Rev. Nutr.* 17, 411-424.
- Pio-Correa, M., 1978. *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*. Rio de Janeiro: Ministério da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal.
- Rosa, S. M., Souza, L.A., 2003. Morfo-anatomia do fruto (hipanto, pericarpo e semente) em desenvolvimento de *Pereskia aculeata* Miller (Cactaceae). *Acta Scientiarum. Biological Sciences*, 25, 415-428.
- Santos, C.M.G., Cerqueira, R.C., Fernandes, L.M.S., Rodrigues, J.D., Ono, E.O., 2010. Efeito de substratos e boro no enraizamento de estacas de pitaya. *Rev. Ceres.* 57, 795-802.
- Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 299, 152-178.
- Thornfeldt, C., 2005. Cosmeceuticals containing herbs: fact, fiction, and future. *Dermatol. Surg.* 31, 873-880.
- van Solingen, C., Araldi, E., Chamorro-Jorganes, A., Fernandez-Hernando, C., Suarez, Y., 2014. Improved repair of dermal wounds in mice lacking microRNA-155. *J. Cell. Mol. Med.* 18, 1104-1112.
- Yarrow, J.C., Perlman, Z.E., Westwood, N.J., Mitchison, T.J., 2004. A highthroughput cell migration assay using scratch wound healing, a comparison of image-based readout methods. *BMC Biotechnol.* 4, 21-30.