Brazilian Journal of
HEALTH AND PHARMACY

Pereskia aculeata Miller (*Cactaceae*): A non-conventional food plant with medicinal potential

Pereskia aculeata Miller (Cactaceae): Uma planta alimentícia não convencional com potencial medicinal

Mariana de Souza Ferreira Maciel[1]; Pedro Henrique Santos de Freitas[1]; Maycon Alcântara de Almeida[1]; Natália Prado da Silva[2]; Guilherme Diniz Tavares[2]; Juliana de Carvalho da Costa[3]; Elita Scio[1]*

1. Laboratory of Bioactive Natural Products, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil.
2. Laboratory of Nanostructure Systems Development, Faculty of Pharmacy, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil.
3. Department of Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil.

***Corresponding author:** Elita Scio (ORCID: 0000-0002-5580-4845)
Address: Rua José Lourenço Kelmer, s/n, Department of Biochemistry, Institute of Biological Science, Laboratory of Bioactive Natural Products, Federal University of Juiz de Fora, CEP 36036-900, Juiz de Fora, MG, Brazil.
Phone: + 55 (32) 21023217. E-mail: elita.scio@ufjf.edu.br

Data de Submissão: 14/09/2022; Data do Aceite: 15/05/2023;

Citar: MACIEL, M.S.F.; FREITAS, P.H.S.; ALMEIDA, M.A.; SILVA, N.P. et al. Pereskia aculeata Miller (Cactaceae): A non-conventional food plant with medicinal potential. **Brazilian Journal of Health and Pharmacy**, v. 5, n. 2, p. 1-14, 2023.

RESUMO

Pereskia aculeata Miller (Cactaceae) é uma planta alimentícia não-convencional (PANC), popularmente denominada de "ora-pro-nobis", encontrada no Brasil. Além do amplo uso alimentar, suas folhas também têm sido utilizadas em distúrbios renais, processos inflamatórios e cicatrização de feridas. Este estudo teve como objetivo avaliar o perfil fitoquímico, o potencial antioxidante, antiglicante e citotóxico de uma fração hexânica (FH) oriunda das folhas de P. aculeata. Trata-se de um estudo no qual foram utilizados os seguintes métodos: cromatografia gasosa acoplada a espectrometria de massas (CG-EM); ensaio de neutralização do 2,2-difenil-1-picril-hidrazil (DPPH•), inibição da produção de óxido nítrico e da oxidação do ácido linoleico; modelo de glicação de proteína induzida por frutose e glicose; e citotoxicidade em fibroblastos (L929) e queratinócitos (HaCaT). A caracterização química revelou a presença de doze compostos, como os fitoesteróis campesterol, estigmasterol e sitosterol, além dos triterpenos, taraxerol e taraxasterol. FH apresentou potencial antioxidante em todos os métodos utilizados e atividade antiglicante na concentração de 1,50 mg/mL, inibindo a formação de produtos finais de glicação avançada. No ensaio de citotoxicidade, FH manteve a viabilidade celular acima de 70 %. Esses resultados sugerem que a FH é uma fonte natural de substâncias bioativas, com predominância de fitoesteróis e triterpenos, com destacado potencial antioxidante e antiglicante, não sendo citotóxico para as células da pele. Este trabalho aprimorou o conhecimento da espécie vegetal, ao caracterizar quimicamente as substâncias presentes e correlacioná-las às atividades biológicas encontradas, além de ampliar novos horizontes para investigações in vivo relacionadas às atividades biológicas descritas.

Palavras-chave: Produtos finais de glicação avançada; antioxidantes; queratinócitos; fibroblastos; fitoesteróis.

ABSTRACT

Pereskia aculeata Miller (Cactaceae) is an unconventional food plant (UFPs), popularly known as "ora-pro-nobis", found in Brazil. In addition to the wide food use, its leaves have also been used in kidney disorders, inflammatory processes, and wound healing. This study aimed to evaluate the phytochemical profile, the antioxidant, antiglycant and cytotoxic potential of the hexane fraction (HF) from the leaves of *P. aculeata*. This is an experimental study in which the following methods

were used: gas chromatography-mass spectrometry (GC-MS); 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) neutralization assay, inhibition of nitric oxide production, and linoleic acid oxidation; fructose and glucose-induced protein glycation model; and cytotoxicity in fibroblasts (L929) and keratinocytes (HaCaT). The chemical characterization revealed the presence of twelve compounds, such as the phytosterols campesterol, stigmasterol, and sitosterol, and the triterpenes taraxerol and taraxasterol. HF showed antioxidant potential in all methods and antiglycant activity at a concentration of 1.50 mg/mL, inhibiting the formation of advanced glycation end products. In the cytotoxicity assay, HF maintained cell viability above 70 %. These results suggest that P. aculeata is a natural source of bioactive substances, with a predominance of phytosterols and triterpenes, outstanding antioxidant and antiglycant potential, and no cytotoxicity to skin cells. This work improved the knowledge of the plant species by chemically characterizing the substances present in HF and correlating them to the biological activities found, in addition to broadening new horizons for in vivo investigations related to the biological activities described.

Keywords: Advanced glycation end products; antioxidants; keratinocytes; fibroblasts; phytosterols.

INTRODUCTION

Pereskia aculeata Miller (Cactaceae), popularly known as “ora-pro-nobis”, found in tropical regions of the American continent. It is a non-conventional food plant used as food in different Brazilian areas, probably due to its high protein and mucilage content (GARCIA *et al.*, 2019). They also contain minerals such as calcium and iron (BARREIRA *et al.*, 2021) and bioactive compounds such as phenolic and carotenoids (PINTO *et al.*, 2020).

Besides the wide food use, its leaves have also been used in traditional medicine for kidney disorders, inflammatory processes, wound healing, and anemia (PINTO, SCIO, 2014). Experimentally, wound healing (PINTO *et al.*, 2016), antinociceptive (PINTO *et al.*, 2015a), anti-inflammatory (PINTO *et al.*, 2015b), antimicrobial (GARCIA *et al.*, 2019), antiproliferative, and antioxidant (PINTO *et al.*, 2012) properties were already reported.

The biological activities of P. aculeata have been correlated with the vast chemical composition of its leaves, with a predominance of phenolic compounds, such as phenolic acids, flavonoids (GARCIA *et al.*, 2019), carotenoids, and phytosterols (PINTO *et al.*, 2020). Furthermore, in the hexane fraction obtained from the crude methanol extract, high amounts of phytosterols, such as sitosterol, campesterol, and stigmasterol, and the triterpenes taraxerol and taraxasterol were also found (PINTO *et al.*, 2015b; PINTO *et al.*, 2020).

Plant extracts containing these bioactive compounds, such as phytosterols and triterpenes, can attenuate oxidative stress, closely related to the glycation process, by favoring the decrease of free radicals (SHENG *et al.*, 2016). Glycation is a non-enzymatic reaction in which reducing sugars, such as fructose and glucose, bind to amino groups of proteins, producing irreversible fluorescent derivatives called advanced glycation end products (AGEs) (MOLDOGAZIEVA *et al.*, 2019).

AGEs can modify the biological properties of macromolecules, promoting oxidative stress and increasing the expression of inflammatory mediators. Thus, the presence of these compounds substantially contributes to the emergence or worsening of various pathological conditions, such as diabetes, cardiovascular diseases, and Alzheimer’s, in addition to playing a crucial role in skin aging (PAPACHRISTOU *et al.*, 2021).

In this context, given the importance of the physiological and pathological effects resulting from oxidative stress and the accumulation of AGEs, especially in skin aging, this study aimed to evaluate the phytochemical profile, the antioxidant, antiglycant and cytotoxic potential of a hexane fraction (HF) from the leaves of P. aculeata.

METHODOLOGY

Chemicals and cell culture reagents

2,2-diphenyl-1-picryl-hydrazyl radical (DPPH•), linoleic acid, β-carotene, calcium carbonate, aluminum chloride, glacial acetic acid, methanol, hexane, dichloromethane, aminoguanidine, quercetin, fructose, glucose, and all the other solvents and reagents of analytical grade were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was purchased from Invitrogen (Carlsbad, CA, USA). Cell lines of murine fibroblasts (L929) and human keratinocytes (HaCaT) were used. Cell medium, penicillin, streptomycin, and all the other cell study reagents were purchased from Gibco (Waltham, MA, USA). All other chemicals used were of analytical grade.

Plant material

P. aculeata leaves were collected in Juiz de Fora, State of Minas Gerais, Brazil (latitude: 21° 66'7775" S, longitude: 43° 29'5569" W). A voucher specimen (Nº 57539) was deposited in the Herbarium Leopoldo Krieger of the Federal University of Juiz de Fora for future evidence. The National System authorized the research for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN; no. ACAC564).

Extract preparation

First, the leaves were dried in the shade at 35 ºC. Once dried, the material (approximately 1 kg) was powdered using a knife mill (Marconi MA048, Piracicaba, SP, Brazil) and then extracted by maceration with methanol (1:10 *m/v*) for 48 h. Next, the extract was concentrated on a rotary evaporator (R-3 Buchi, Flawil, Switzerland) under a monitored temperature (50 ± 2 ºC) to obtain the crude methanol extract (ME – 157.76 g). ME was resuspended in methanol/water (8:2 v/v) and then fractionated with hexane by solvent partition (PINTO *et al*., 2015b). Finally, the hexane fraction (HF – 51.78 g) was stored in a refrigerator at 4 °C until submitted to the following analysis.

Phytochemical characterization by GC-MS analysis

The phytochemical characterization of HF was performed by gas chromatography-mass spectrometry (GC-MS) analysis (Agilent Technologies GC Hewlett Packard 6890 and MS Hewlett Packard P 5972 devices) connected to the system an analytical DB-5MS capillary column (30 m length x 0.25 mm inner diameter; 0.25 mm film thickness). The injector temperature was set to 270 °C, and the oven temperature was programmed as a gradient from 50 °C to 300 °C for 50 min (5 ºC/min). An isotherm at 300 °C was maintained for 15 min more, so the total run time was 65 min. Helium was the carrier gas with a flow rate adjusted to 0.5 mL/min (99 %). The mass spectrometer was operated by electron impact (70 eV) with the ion source at 230 °C. HF was filtered through a 0.22 µm, 13 mm, non-sterile, PTFE membrane filter (Merck) and diluted in HPLC grade hexane (> 95 %). The percentage composition of the HF constituents was expressed as a percentage concerning the peak area. The compounds were identified by mass spectra comparison using the mass spectrometer library database (WILEY 275.1) and available literature.

Antioxidant activity

Scavenging of DPPH• radical

The antioxidant activity was performed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging activity assay (BRAND-WILLIAMS

et al., 1995). The samples, diluted at different concentrations (0.98 – 250 µg/mL), were added to 150 µL of a methanolic DPPH• solution (20 µg/mL). After 30 min of incubation time without light and at room temperature, the absorbance was measured at 517 nm. The experiment was carried out in triplicate. Quercetin was used as the reference standard. IC(50) values were calculated using the GraFit Version 5 (Erithacus Software, Horley, UK) and indicated the concentration of extract required to scavenge 50 % of DPPH• free radicals.

Nitric oxide radical scavenging activity

Nitric oxide (NO•) radical scavenging activity was determined by the Griess reaction, with some modifications, using sodium nitroprusside in a buffer solution to generate nitric oxide through spontaneous decomposition (GREEN *et al.*, 1982). For the experiment, 62.5 µl of sodium nitroprusside (10 mM) in phosphate-buffered saline (PBS) were mixed with the samples (100 µg/mL) and incubated at 25 °C for 60 min. Next, 125 µl of Griess reagent (sulphanilamide 1 %, N-1-naphthyl ethylenediamine dihydrochloride 0.1 %) in phosphoric acid 2.5 % (v/v) were added. Quercetin was used as the reference standard. The absorbance of the chromophore formed was measured at 540 nm. The experiment was carried out in triplicate. The antioxidant activity (AA) was calculated regarding the inhibition percentage (%) relative to the control.

β-Carotene bleaching assay

The β-carotene bleaching method described by MARCO (1968) was performed with slight modifications. A mixture of 30 µL linoleic acid, 265 µL Tween 40, 50 µL β-carotene (10 mg/mL in dichloromethane HPLC grade), and 500 µL dichloromethane was prepared. After removing the dichloromethane using nitrogen gas, 25 mL of oxygenated distilled water was added with vigorous shaking to form a liposome solution. Aliquots of 250 µL of the liposome solution were transferred to a 96-well plate, and then samples (38.46 µg/mL) were added. Quercetin was used as the reference standard. The emulsion system was incubated for 2 h at 45 ºC, and the absorbance read at 470 nm. The antioxidant activity (AA) was calculated regarding the inhibition percentage (%) relative to the control.

Phosphomolybdenum reduction activity

The phosphomolybdenum reduction activity was evaluated as described by PRIETO *et al.* (1999) with slight modification. Quercetin was used as the reference standard. The 300 µL of the samples (2 mg/mL) were mixed with 2 mL of a reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After, the reaction mixture was incubated for 90 min at 95 ºC, and the absorbance was read at 695 nm in a microplate reader. The antioxidant activity (AA) was calculated regarding the inhibition percentage (%) relative to the control.

Antiglycant activity

In vitro antiglycant activity was determined using the fructose/glucose-induced protein glycation model described by FARSI *et al.* (2008). A 96-well plate containing bovine serum albumin (BSA) (10 mg/mL), fructose (1.6 M) or glucose (1.6 M), sodium azide (8 g/L), and samples (0.5 – 1.5 mg/mL) were incubated in 100 mM sodium monophosphate solution (pH 7.4). All solutions were sterilized through a 0.22 µm pore size filter. Aminoguanidine, a well-known AGE formation inhibitor, and quercetin were used as positive controls. After the incubation time at 37 ºC for 7 days, the formation of AGEs was measured by the fluorescence intensity at an excitation wavelength of 350 nm and an emission wavelength of 450 nm with a fluorimeter. The antiglycant activity was expressed as a percentage of fluorescent inhibition using the following formula:

$$\text{Antiglycant activity (\%)} = 1 - (F_{sample}/F_{control}) \times 100$$

where F_{sample} and $F_{control}$ are the mean fluorescence rate of samples (HF and positive controls) and control (vehicle), respectively.

Viability assay

Cell cultures

The murine fibroblast cell line (L929) and the human keratinocyte cell line (HaCaT) were cultured in Dulbecco's Modified Eagle Medium (DMEM) and DMEM/Ham's F-12 (1:1), respectively, supplemented with 10 % heat-inactivated fetal bovine serum (FBS) and 1 % penicillin/streptomycin at 37 ºC in an atmosphere of 5 % CO2.

MTT assay cells

Cell viability was assessed using the MTT method with modifications (MOSMANN, 1983). In a 96-well plate, L929 cells were seeded at 5 x 103 cells per well, and HaCaT cells were seeded at 1 x 104 cells per well and incubated for 24 h at 37 ºC in an atmosphere of 5 % CO2. The day after, 90 µL of DMEM or DMEM/Ham's F-12 and 10 µL of serial dilutions of the five samples (6.25 – 100 µg/mL) were added to the cells and incubated 24 h. The culture media were used as a negative control and DMSO (5%) as a positive control. The sample was incubated for 24 h at 37 ºC in an atmosphere of 5% CO2. After that, 10 µL of MTT (5 mg/mL) was added, and the cells were incubated for an additional 150 min at 37 ºC in an atmosphere of 5% CO2. Then, 100 µL of the DMSO was added to the cells, and the absorbance of each sample was detected at 595 nm. The cell viability (%) was calculated compared to the control group.

Statistical analysis

All data were reported as mean ± standard deviation (SD) of triplicate determinations. Analysis of variance (ANOVA) with significant differences between means determined at $p < 0.05$ was applied. The significance of differences between means was calculated by Bonferroni's test using GraphPad Prism software, version 7.0 (San Diego, California, USA).

RESULTS AND DISCUSSION

Phytochemical characterization by GC-MS analysis

GC-MS was performed to detect the compounds present in the HF. Twenty-one signals with retention times ranging from 30.71 to 56.47 were detected. Among them, 12 were identified, as shown in Table 1.

Approximately 41 % of the identified compounds belong to the phytosterol classes (Table 1), with campesterol, stigmasterol, and sitosterol being the most abundant (Figure 1). These data corroborate other studies, as phytosterols have already been reported in the leaves of P. aculeata (PINTO *et al.*, 2015b), Pereskia bleo (MALEK *et al.*, 2009), and Pereskia grandifolia (NURESTRI *et al.*, 2009).

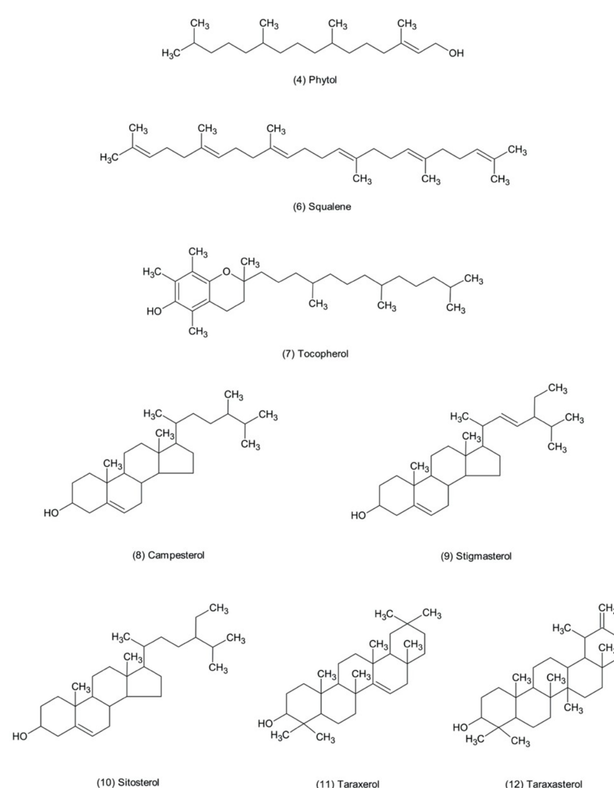

Figure 1: Chemical structure of compounds identified in the hexane fraction of Pereskia aculeata. (4) Phytol; (6) Squalene; (7) Tocopherol; (8) Campesterol; (9) Stigmasterol; (10) Sitosterol; (11) Taraxerol; (12) Taraxasterol. Chemical structures were drawn using the software ACD/ChemSketch Freeware (Advanced Chemistry Development Inc., Toronto, CA).

Table 1: Chemical constituents identified in the hexane fraction of Pereskia aculeata by GC-MS analysis.

Peak	Rt (min)	Compound	Molecular Weight	Area (%)	Main MS fragmentation ions
1	30.71	Trans-pinane[a]	263	4.21	263 → 151, 137, 123, 109, 95, 82, 68, 43
2	31.60	Neophytadiene[b]	278	2.18	278 → 193, 149, 123, 109, 95, 81, 57, 43
3	32.59	Methyl palmitate[a,b]	270	1.10	270 → 227, 207, 181, 157, 143, 129, 100, 87, 74, 57, 44
4	35.96	Phytol[a]	207	6.71	207 → 140, 123, 95, 71, 43
5	38.37	Tributyl acetyl citrate	329	5.39	329 → 259, 213, 185, 157, 129, 112, 84, 67, 43
6	47.10	Squalene[a]	410	3.80	410 → 341, 281, 231, 207, 175, 149, 121, 95, 69, 41
7	51.40	Tocopherol[a]	430	1.65	430 → 386, 355, 281, 207, 165, 107, 75, 44
8	53.04	Campesterol[a]	476	6.43	476 → 400, 367, 341, 315, 281, 255, 231, 207, 176, 145, 105, 81, 44
9	53.51	Stigmasterol[a,b]	412	6.73	412 → 379, 351, 314, 280, 255, 231, 207, 159, 133, 105, 81, 55
10	54.63	Sitosterola,[b]	414	27.60	414 → 381, 354, 329, 303, 281, 255, 231, 207, 173, 145, 105, 81, 43
11	55.14	Taraxerola,[b]	426	9.22	426 → 302, 281, 269, 207, 204, 186, 135, 133, 107, 69, 44
12	56.47	Taraxasterol[b]	426	10.08	426 → 400, 365, 326, 281, 257, 229, 207, 189, 175, 147, 135, 121, 95, 69, 43
Total				**85.10**	

Rt: retention time; a.Identificated by comparison with Wiley 275.1; b.Identificated by comparison with PINTO et al. (2015b).

Squalene has already demonstrated antioxidant, emollient, and moisturizing action. It is reported to treat skin conditions, including acne, psoriasis, xerosis-related skin lesions, seborrheic dermatitis, and atopic dermatitis (HON et al., 2018). The triterpenes squalene, taraxerol, and taraxasterol, reported by PINTO et al. (2015b), were also identified in HF (Table 1). Regarding taraxerol and taraxasterol, topical anti-inflammatory activities were found, reducing oxidative stress and the generation of inflammatory mediators (PINTO et al., 2015b). Therefore, both pentacyclic terpenes (Figure 1) can alleviate inflammatory stress and reduce the progression of chronic diseases (YIN, 2015).

Widely distributed in nature, phytol was also detected in HF (Table 1 and Figure 1). Some biological effects such as antioxidant, antinociceptive, anti-inflammatory, immunomodulatory, antimicrobial action, and antipyretic have already been reported for this compound (ISLAM *et al.*, 2018). Other authors have also detected phytol in P. aculeata (PINTO *et al.*, 2015b) and P. bleo (MALEK *et al.*, 2009).

Tocopherol, belonging to fat-soluble vitamins, is recognized for its high antioxidant potential, neutralizing peroxyl radicals and blocking lipid peroxidation, particularly polyunsaturated fatty acids. In addition, it has neuroprotective, anti-inflammatory, and hypocholesterolemic properties (LLORET *et al.*, 2019). Tocopherol has already been reported in other species of the Pereskia genus (MALEK *et al.*, 2009). However, in P. aculeata, it had yet to be identified (Table 1).

Antioxidant activity

Although several methodologies are described to determine the antioxidant activity, it is essential to consider consistent and fast methods, evaluating the advantages and disadvantages of each one. Thus, the need for more than two approaches to trace the antioxidant profile of a sample with different constituents, such as plant extracts, is justified (PINTO *et al.*, 2012). Here, four other methods were used, as shown in Table 2.

The DPPH assay assessed the neutralizing capacity of the free radical DPHH•. Generally, antioxidant compounds promote the donation of a hydrogen atom or transfer an electron to the DPHH• molecule, making it stable and giving rise to the reduced form DPPH-H. Consequently, the color change and the decrease in DPPH• radical absorbance can be monitored in a spectrophotometer at 515-520 nm (BRAND-WILLIAMS *et al.*, 1995).

As shown in Table 2, the antioxidant activity of HF was expressed as the concentration that inhibits 50 % of the free radical DPPH•. The results obtained in the study showed that the IC(50) of HF (5.22 ± 1.45 µg/mL) was significantly ($p < 0.01$) higher than that of quercetin (1.08 ± 0.10 µg/mL). This difference can be attributed to HF having a wide range of compounds (Table 1). At the same time, quercetin is a pure substance used as a reference standard and has proven antioxidant action. Therefore, these results are compatible with PINTO *et al.* (2012) and GARCIA *et al.* (2019).

The NO• radical plays a fundamental role in several biological processes, such as neurotransmission, muscle relaxation, and immune regulation, in addition to being a potent vasodilator agent. However, when produced in excess, it can give rise to oxidative stress, causing cell damage and leading to pathological disorders. In this context, it is interesting to search for plant extracts that can reduce their production or even to sequestered them (CONEGUNDES *et al.*, 2021).

Among the methods described for the detection of NO• radical production inhibition, the most used is the Griess method, in which nitrite reacts with sulfanilamide in an acidic medium. The compound reacts with N-(1-naphthyl)-ethylenediamine hydrochloride (NED), generating an intense red chromophore that can be measured by spectrophotometry at 540 nm (GREEN *et al.*, 1982).

According to Table 2, HF inhibited the production of NO• comparable ($p < 0.0001$) to quercetin. This satisfactory result may be related to the compounds found in HF (Table 1), mainly due to the presence of the triterpenes taraxerol and taraxasterol, which in other studies decreased the production of NO• and inflammatory mediators (PINTO *et al.*, 2015b).

Table 2: Antioxidant activity of the hexane fraction of *Pereskia aculeata*.

Samples	Antioxidant activity			
	DPPH• IC(50) (µg/mL)	NO• (% AA)	BC (% AA)	PMD (% AA)
HF	5.22 ± 1.45**	107.77± 0.30****	72.02 ± 0.03****	36.40 ± 0.22****
Q	1.08 ± 0.10	100.00	100.00	100.00

AA: Antioxidant activity; BC: β-Carotene; DPPH•: 1,1-diphenyl-2-picrylhydrazyl; HF: Hexane fraction; NO•: Nitric oxide; PPMD: Phosphomolybdenum; Q: Quercetin. The values are mean ± standard deviation (SD). ANOVA, followed by Bonferroni's test, was used as a post-hoc. Significant values: ****$p < 0.0001$, **$p < 0.01$, compared to quercetin.

Another assay used to assess antioxidant potential was the β-carotene/linoleic acid co-oxidation method, which determines the ability of a sample to protect a lipid substrate from oxidation based on the inhibition of free radicals generated during the peroxidation of linoleic acid. Therefore, this method is based on the discoloration of β-carotene against linoleic acid degradation products, monitored at 470 nm (SOUZA *et al.*, 2014).

As shown in Table 2, at the concentration of 38.46 μg/mL, HF presented an antioxidant activity of 72.02 %, significantly ($p < 0.0001$) different from quercetin (100 %), used as a reference compound. These values follow the results found by SOUZA *et al.* (2014), with percentages of inhibition of lipid peroxidation of 75.6 %, 81.2 %, and 63.5 % for the aqueous, ethanolic, and acetone extracts of P. aculeata, respectively. Studies with other species of the genus, such as P. bleo, found inhibition values between 40.57 % - 73.31 % for the hexane extract, corroborating the results obtained in this study (NURESTRI *et al.*, 2009).

The phosphomolybdenum reduction method can evaluate the antioxidant activity based on the reduction of molybdenum IV to molybdenum V by an antioxidant agent and its complexation with phosphate in an acidic medium with the formation of phosphomolybdenum V, which has a green coloration. This reaction is monitored at 695 nm and presents, as one of its advantages, the simultaneous evaluation of lipophilic and hydrophilic compounds in complex samples (PRIETO *et al.*, 1999). Also, HF showed a low percentage of molybdenum reduction (36.40 %) compared to the activity of quercetin ($p < 0.0001$), considered as 100 % (Table 2). This result is probably related to the mechanism of action employed by the method, which evaluates compounds capable of reducing molybdenum IV to molybdenum V.

Among the compounds identified in P. aculeata (Table 1), some are noteworthy and may contribute to the antioxidant activity of HF. An example is tocopherol, considered an essential exogenous antioxidant, which plays a fundamental role in the balance of oxidative stress and, consequently, in stabilizing cell membranes. As one of the main lipophilic antioxidants, the topical administration of formulations containing tocopherol helps to protect the skin against oxidative damage caused by ultraviolet radiation and is widely used in the cosmetics industry. Furthermore, in its chemical structure (Figure 1), a phenolic hydroxyl is linked to the benzene ring, which justifies its high antioxidant activity (RIGHI *et al.*, 2021). These biological activities were previously reported in other studies. For example, SONG *et al.* (2021) noted that for leaves and seeds of Dracocephalum moldavica L., the presence of tocopherols, such as γ-tocopherol, with intense antioxidant activity related to the elimination of lipophilic electrophiles, was evidenced.

Studies suggest that phytosterols, especially campesterol, stigmasterol, and sitosterol, can exert antioxidant properties against the oxidation of linoleic acid and its esters induced by peroxyl radicals in a dose-dependent way (YOSHIDA; NIKI, 2003). Among these compounds, sitosterol acts as a scavenger of free radicals and promotes the stabilization of the liposomal membrane (BABU; JAYARAMAN, 2020). Furthermore, sitosterol has been reported to reduce reactive oxygen species (ROS) and hydrogen peroxide levels and act as a free radical scavenger (PATIL *et al.*, 2018).

Studies with taraxerol isolated from the bark of Manilkara zapota found a DPPH• radical scavenging activity expressed in IC(50) of 16.28 μM, correlated with the probable antioxidant activity of the analyzed plant material (CHUNHAKANT; CHAICHAROENPONG, 2019). Regarding taraxasterol

isolated from Taraxacum, its in vivo ability to attenuate oxidative stress and maximize the activities of the liver's antioxidant enzymes were found to protect hepatocytes from ROS's harmful effects by eliminating lipid peroxides and oxygen free radicals (XU *et al.*, 2018).

These data agree with the results of the DPPH• neutralization, NO• production inhibition, and β-carotene oxidation inhibition assays (Table 2) demonstrated for P. aculeata HF.

Antiglycant activity

The antiglycant activity generally is based on the reaction between BSA and reducing sugars, such as fructose and glucose. AGEs, products of the reaction, are then measured by forming subsets of fluorescent products, such as pentosidine and argipyridine. Thus, it becomes possible to evaluate the inhibition of specific fluorescence generated during glycation and, consequently, inhibit the formation of AGEs (FARSI *et al.*, 2008). As a positive control, aminoguanidine, with proven inhibitory action on forming intermediate carbonyl compounds, and quercetin, a potent antiglycation activity in all glycation stages, was used (ASHRAF *et al.*, 2015).

As shown in Figure 2, according to the BSA-fructose model, HF inhibited glycation at the highest concentrations, with inhibition values between 18.68 and 51.98 %. Notably, at the concentration of 1.50 mg/mL, HF was significantly ($p < 0.0001$) more efficient than aminoguanidine, which is used as an inhibitor of AGEs in the early stages. Concerning the BSA-glucose model, antiglycant activity was found at all concentrations tested, 0.50, 1.00, and 1.50 mg/mL, with inhibition values of 33.42, 45.00, and 57.03 %, respectively. At the 1.50 mg/mL concentration, HF was significantly equal to aminoguanidine. These data are relevant since, in the scientific literature, there are no reports of antiglycant activity for plant extracts of P. aculeata.

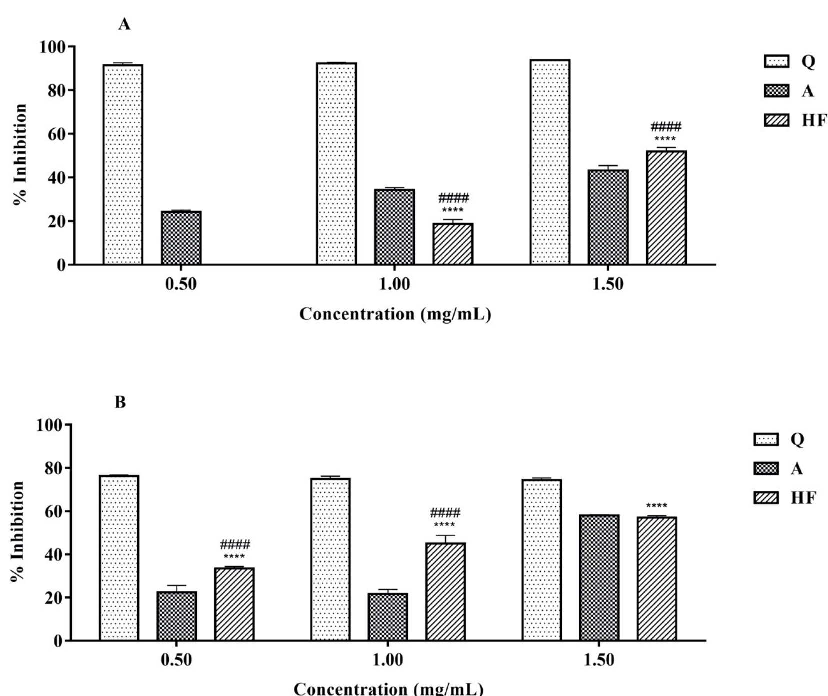

Figure 2: Antiglycant activity of hexane fraction of Pereskia aculeata by fructose (A) and glucose (B). A: Aminoguanidine; HF: Hexane fraction; Q: Quercetin. The values are mean ± standard deviation (SD). ANOVA, followed by Bonferroni's test, was used as a post-hoc. Significant values: ****$p < 0.0001$, compared to quercetin; ####$p < 0.0001$, compared to aminoguanidine.

Studies have shown that natural products can be beneficial as adjuvants in treating diseases associated with the degenerative accumulation of AGEs, mainly due to the combination of multiple biological effects, commonly attributed to the presence of compounds with antioxidant and antiglycant properties. Also, there is evidence that antioxidant agents can also exert antiglycant activity, helping inhibit the formation of AGEs (ASHRAF *et al.*, 2015).

In the present work, the antioxidant activity of HF was previously confirmed by the DPPH•, NO•, and β-carotene methods (Table 2). Subsequently, the in vitro antiglycant activity was also found in the BSA-fructose and BSA-glucose models (Figure 2). Among the compounds reported with both biological effects, tocopherol (Figure 1) stands out for delaying the in vitro glycation of proteins, favoring the decrease in the production of malondialdehyde (MDA), the final product of lipid peroxidation that contributes substantially to the formation of AGEs (PAZDRO; BURGESS, 2012). Furthermore, in vivo studies suggested that tocopherol suppresses NF-κB activation and reduces AGE-induced macromolecule damage (LAL *et al.*, 2002).

SHENG *et al.* (2016) analyzed phytosterols isolated from Musa spp flowers and demonstrated that β-sitosterol significantly inhibited AGE formation in a BSA-fructose model. The compound, isolated and purified from the crude extract of banana flowers, showed an antiglycation capacity of 51.7 %, while aminoguanidine inhibited glycation by 64.0 %. The authors suggested that the mechanism of action is probably related to inhibiting the second phase of glycation that will give rise to AGEs. In HF, most of the identified compounds (41 %) belong to the phytosterol classes (Table 1), with the predominance of sitosterol. Thus, this compound could be responsible for the antiglycant activity of HF, together with campesterol and stigmasterol (Figure 1).

Regarding pentacyclic triterpenes, in vivo and in vitro studies indicated that these compounds could decrease the generation of AGEs by interrupting the interactions between reducing sugars and amino acids through non-enzymatic reactions (RAO *et al.*, 2012). Furthermore, these compounds can also influence the activity and expression of enzymatic proteins involved in the AGE pathway, such as aldose reductase and sorbitol dehydrogenase, potent antiglycant agents (YIN, 2015). Among the pentacyclic triterpenes identified in HF (Table 1), taraxerol and taraxasterol stand out (Figure 1).

Viability assay

The leaves of P. *aculeata* are traditionally recognized for their nutritional properties, such as their high content of proteins, minerals, vitamins, and fibers (BARREIRA *et al.*, 2021). However, there are few studies on their toxicity. Some studies reported the non-toxicity of P. *aculeata* extracts when administered orally, such as the one by SILVA *et al.* (2017). Studies were also carried out with the liver PLP2 cell lines (GARCIA *et al.*, 2019), human promyelocytic leukemia HL60, and MCF-7 human breast adenocarcinoma cells (PINTO *et al.*, 2012). However, none were related to the topical toxicity of plant extracts of P. *aculeata*.

Despite the scarcity of data, there is a study by PINTO *et al.* (2015b) in which a hexane-partitioned formulation of P. *aculeata* was evaluated for acute skin irritation, showing that the formulation did not irritate. Another study demonstrated that formulations with hexane and the hydromethanolic partition of P. *aculeata* accelerated the healing process in mice (PINTO *et al.*, 2016). To corroborate these results and investigate the cytotoxic potential of P. aculeata in skin cells, since HF had a significant antiglycant action (Figure 2) and this action is closely related to skin aging, this study evaluated the behavior of HF against cell lines found in the skin, such as keratinocytes and fibroblasts, as shown in Figure 3.

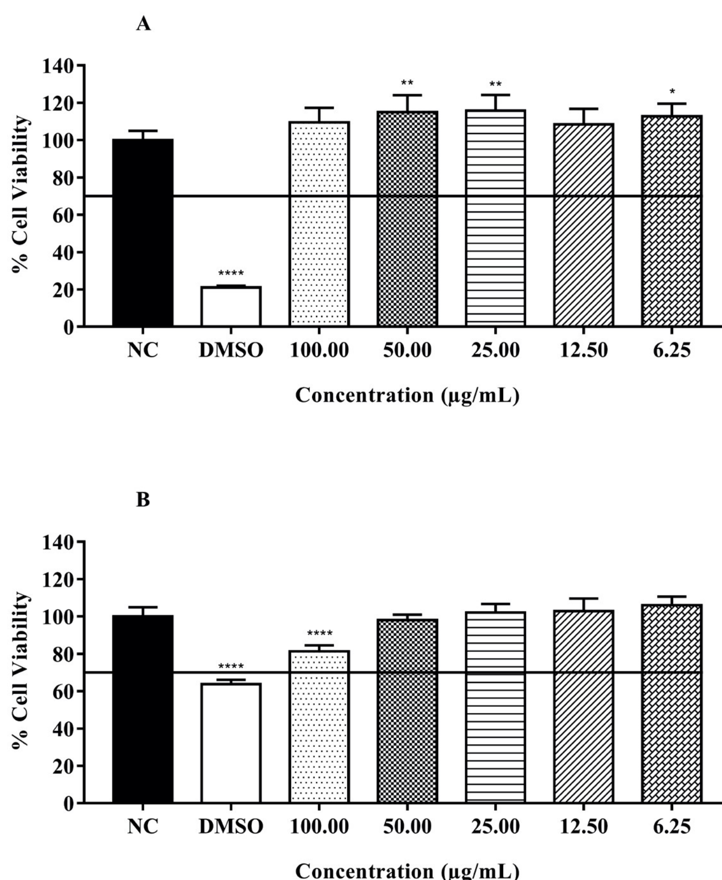

Figure 3: Viability of the HaCaT (A) and L929 (B) cell lines. The concentration range of HF was 100 to 6.25 µg/mL. DMSO: Dimethylsulphoxide; NC: Negative control. The values are represented as mean ± standard deviation (SD) of the concentration (µg/mL). Significant values: ****$p < 0.0001$, **$p < 0.01$, *$p < 0.1$ compared to the negative control. ANOVA, followed by Bonferroni's test, was used as a post-hoc.

According to ISO 10993-5 (2009), the cytotoxic effect occurs when there is a reduction in cell viability by more than 30 %, which was not observed in this experiment, as in the presence of HF, at all concentrations, cell viability was higher 70 % (Figure 3). This effect is directly related to the healing action since keratinocytes and fibroblasts restore the typical composition of the cellular and extracellular matrix of the skin and are involved in different stages of healing (HEGDE *et al.*, 2021). In addition, it is suggested that HF has a proliferative action since it presented results equal to or greater than the negative control, corroborating studies carried out by PINTO *et al.* (2016).

CONCLUSIONS

HF showed phytosterols, such as campesterol, stigmasterol, and sitosterol, and triterpenes, such as taraxerol and taraxasterol, among their constituents. Tocopherol, already reported in other species of the genus, was identified for the first time in P. aculeata. In general, HF presented antioxidant activity in all methods, emphasizing the DPPH• neutralization assay, inhibition of NO• production, and inhibition of β-carotene oxidation. Regarding the antiglycant potential, HF showed efficacy at a concentration of 1.50 mg/mL, inhibiting the formation of AGEs from the BSA-glucose and BSA-fructose models, respectively. In the toxicity assay against HaCaT

and L929 cell lines, HF maintained cell viability above 70 % at all concentrations tested. Given the above, it is possible to conclude that this work significantly enhances the knowledge and study of P. aculeata and opens new horizons for in vivo investigations related to the described biological activities. Notably, there are no reports in the scientific literature that plant extracts of P. aculeata have in vitro glycation inhibitory activity, making HF a promising candidate in treating pathologies associated with the accumulation of AGEs.

FUNDING

This work was supported by a grant from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG CEX APQ 00487-16 and 1087-18).

ACKNOWLEDGMENTS

The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Universidade Federal de Juiz de Fora (UFJF) for scholarships, to Analytical Platform of Farmanguinhos (FIOCRUZ) for the GC-MS analysis, Elton Fontes for collecting the plant material, and to Delfino Antônio Campos for technical assistance.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors ORCID Number

Mariana de Souza Ferreira Maciel
(0000-0003-3537-9236)

Pedro Henrique Santos de Freitas
(0000-0002-6635-0471)

Maycon Alcântara de Almeida (0000-0001-8066-3741)

Natália Prado da Silva (0000-0002-4257-784X)

Guilherme Diniz Tavares (0000-0001-6276-0994)

Juliana de Carvalho da Costa (0000-0002-2336-7361)

Elita Scio (0000-0002-5580-4845)

REFERENCES

ASHRAF, J.M.; SHAHAB, U.; TABREZ, S.; LEE, E.J.; CHOI, I.; AHMAD, S. Quercetin as a finer substitute to aminoguanidine in the inhibition of glycation products. **International Journal of Biological Macromolecules**, v. 77, p. 188-192, 2015. DOI: https://doi.org/10.1016/j.ijbiomac.2015.03.021.

BABU, S.; JAYARAMAN, S. An update on β-sitosterol: a potential herbal nutraceutical for diabetic management. **Biomedicine & Pharmacotherapy**, v. 131, 110702, 2020. DOI: https://doi.org/10.1016/j.biopha.2020.110702.

BARREIRA, T.F.; PAULA FILHO, G.X.; PRIORE, S. E.; SANTOS, R.H.S.; PINHEIRO-SANT'ANA, H.M. Nutrient content in ora-pro-nóbis (Pereskia aculeata Mill.): Unconventional vegetable of the Brazilian atlantic forest. **Food Science and Technology**, v. 41, n. 1, p. 47-51, 2021. DOI: https://doi.org/10.1590/fst.07920.

BRAND-WILLIAMS, W.; CUVELIER, M.E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **Lwt - Food Science and Technology**, v. 28, n. 1, p. 25-30, 1995. DOI: http://doi.org/10.1016/s0023-6438(95)80008-5.

CHUNHAKANT, S.; CHAICHAROENPONG, C. Antityrosinase, Antioxidant, and Cytotoxic Activities of Phytochemical Constituents from *Manilkara zapota* L. Bark. **Molecules**, v. 24, n. 15, 2798, 2019. DOI: http://doi.org/10.3390/molecules24152798.

CONEGUNDES, J.L.M.; SILVA, J.M.; MENDES, R.F.; FERNANDES, M.F.; PINTO, N.C.C.; ALMEIDA, M.A.; DIB, P.R.B.; ANDRADE, R.O.; RODRIGUES, M.N.; CASTAÑON, M.C.M.N.; MACEDO, G.C.; SCIO, E. Anti-inflammatory and antinociceptive activity of *Siparuna guianensis* Aublet, an amazonian plant traditionally used by indigenous communities. **Journal of Ethnopharmacology**, v. 265, 113344, 2021. DOI: http://doi.org/10.1016/j.jep.2020.113344.

FARSI, D.A. HARRIS, C.S.; REID, L.; BENNETT, S.A.L.; HADDAD, P.S.; MARTINEAU, L.C.; ARNASON, J.T. Inhibition of non-enzymatic glycation by silk extracts from a Mexican land race and modern inbred lines of maize (*Zea mays*). **Phytotherapy Research**, v. 22, n. 1, p. 108-112, 2007. DOI: http://doi.org/10.1002/ptr.2275.

GARCIA, J.A.A.; CORRÊA, R.C.G.; BARROS, L.; PEREIRA, C.; ABREU, R.M.V.; ALVES, M.J.; CALHELHA, R.C.; BRACHT, A.; PERALTA, R.M.; FERREIRA, I.C.F.R.. Phytochemical profile and biological activities of 'Ora-pro-nobis'

leaves (*Pereskia aculeata* Miller), an underexploited superfood from the Brazilian Atlantic Forest. **Food Chemistry**, v. 294, p. 302-308, 2019. DOI: http://doi.org/10.1016/j.foodchem.2019.05.074.

GREEN, L.C.; WAGNER, D.A.; GLOGOWSKI, J.; SKIPPER, P.L.; WISHNOK, J.S.; TANNENBAUM, S.R. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. **Analytical Biochemistry**, v. 126, n. 1, p. 131-138, 1982. DOI: http://doi.org/10.1016/0003-2697(82)90118-x.

HEGDE, A.; ANANTHAN, A.S.; KASHYAP, C.; GHOSH, S. Wound Healing by Keratinocytes: A cytoskeletal perspective. **Journal of the Indian Institute of Science**, v. 101, n. 1, p. 73-80, 2021. DOI: http://doi.org/10.1007/s41745-020-00219-9.

HON, K.L.; KUNG, J.S.C.; NG, W.G.G.; LEUNG, T.F.. Emollient treatment of atopic dermatitis: Latest evidence and clinical considerations. **Drugs in Context**, v. 7, p. 1-14, 2018. DOI: http://doi.org/10.7573/dic.212530.

ISO 10993-5:2009 Biological evaluation of medical devices Part 5: Tests for cytotoxicity: *in vitro* methods. ISO, 2022. Available from: https://www.iso.org/standard/36406.html. Accessed on: 22 Aug 2022.

LAL, M.A.; BRISMAR, H.; EKLÖF, A.C.; APERIA, A. Role of oxidative stress in advanced glycation end product-induced mesangial cell activation. **Kidney International**, v. 61, n. 6, p. 2006-2014, 2002. DOI: http://doi.org/10.1046/j.1523-1755.2002.00367.x.

LLORET, A.; ESTEVE, D.; MONLLOR, P.; CERVERA-FERRI, A.; LLORET, A. The Effectiveness of Vitamin E Treatment in Alzheimer's Disease. **International Journal of Molecular Sciences**, v. 20, n. 4, 879, 2019. DOI: http://doi.org/10.3390/ijms20040879.

MALEK, S.N.A.; SHIN, S.K.; WAHAB, N.A.; YAACOB, H. Cytotoxic Components of Pereskia bleo (Kunth) DC. (Cactaceae) Leaves. **Molecules**, v. 14, n. 5, p. 1713-1724, 2009. DOI: http://doi.org/10.3390/molecules14051713.

MARCO, G.J. A rapid method for evaluation of antioxidants. **Journal of The American Oil Chemists' Society**, v. 45, n. 9, p. 594-598, 1968. DOI: http://doi.org/10.1007/bf02668958.

MOLDOGAZIEVA, N.T.; MOKHOSOEV, I.M.; MEL'NIKOVA, T.I.; POROZOV, Y.B.; TERENTIEV, A.A. Oxidative Stress and Advanced Lipoxidation and Glycation End Products (ALEs and AGEs) in Aging and Age-Related Diseases. **Oxidative Medicine and Cellular Longevity**, v. 2019, p. 1-14, 2019. DOI: http://doi.org/10.1155/2019/3085756.

MOSMANN, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. **Journal of Immunological Methods**, v. 65, n. 1-2, p. 55-63, 1983. DOI: http://dx.doi.org/10.1016/0022-1759(83)90303-4.

NURESTRI, A.M.S.; SIM, K.S.; NORHANOM, A.W. Phytochemical and Cytotoxic Investigations of Pereskia grandifolia Haw. (Cactaceae) Leaves. **Journal of Biological Sciences**, v. 9, n. 5, p. 488-493, 2009. DOI: http://doi.org/10.3923/jbs.2009.488.493.

PAPACHRISTOU, S.; PAFILI, K.; PAPANAS, N. Skin AGEs and diabetic neuropathy. **Bmc Endocrine Disorders**, v. 21, n. 1, 28, 2021. DOI: http://doi.org/10.1186/s12902-021-00697-7.

PAZDRO, R.; BURGESS, J.R. Differential effects of α-tocopherol and N-acetyl-cysteine on advanced glycation end product-induced oxidative damage and neurite degeneration in SH-SY5Y cells. **Biochimica et Biophysica Acta (Bba) - Molecular Basis of Disease**, v. 1822, n. 4, p. 550-556, 2012. DOI: http://doi.org/10.1016/j.bbadis.2012.01.003.

PATIL, M.; PATIL, C.; MAHAJAN, R. Phytochemical investigation and validation of antioxidant potential of β-sitosterol from tubers of *Eulophia herbacea* and *Eulophia ochreata*. **International Journal of Pharmacognosy and Phytochemical Research**, v. 10, n. 9, p. 309-316, 2018. DOI: http://doi.org/10.25258/phyto.10.9.1.

PINTO, N.C.C.; SANTOS, R.C.; MACHADO, D.C.; FLORÊNCIO, J.R.; FAGUNDES, E.M.S.; ANTINARELLI, L.M.R.; COIMBRA, E.S.; RIBEIRO, A.; SCIO, E. Cytotoxic and antioxidant activity of *Pereskia aculeata* Miller. **Pharmacology online**, v. 3, p. 63-69, 2012.

PINTO, N.C.C.; CASSINI-VIEIRA, P.; SOUZA-FAGUNDES, E.M.; BARCELOS, L.S.; CASTAÑON, M.C.M.N.; SCIO, E. *Pereskia aculeata* Miller leaves accelerate excisional wound healing in mice. **Journal of Ethnopharmacology**, v. 194, p. 131-136, 2016. DOI: http://doi.org/10.1016/j.jep.2016.09.005.

PINTO, N.C.C.; DUQUE, A.P.N.; PACHECO, N.R.; MENDES, R.F.; MOTTA, E.V.S.; BELLOZI, P.M.Q.; RIBEIRO, A.; SALVADOR, M.J.; SCIO, E. *Pereskia aculeata*: A plant food with antinociceptive activity. **Pharmaceutical Biology**, v. 53, n. 12, p. 1780-1785, 2015a. DOI: http://doi.org/10.3109/13880209.2015.1008144.

PINTO, N.C.C.; MACHADO, D.C.; SILVA, J.M.; CONEGUNDES, J.L.M.; GUALBERTO, A.C.M.; GAMEIRO, J.; CHEDIER, L.M.; CASTAÑON, M.C.M.N.; SCIO, E. *Pereskia aculeata* Miller leaves present *in vivo* topical anti-inflammatory activity in models of acute and chronic dermatitis. **Journal of Ethnopharmacology**, v. 173, p. 330-337, 2015b. DOI: http://doi.org/10.1016/j.jep.2015.07.032.

PINTO, N.C.C.; MACIEL, M.S.F.; REZENDE, N.S.; DUQUE, A.P.N.; MENDES, R.F.; SILVA, J.B.; EVANGELISTA, M.R.; MONTEIRO, L.C.; SILVA, J.M.; COSTA, J.C. Preclinical studies indicate that INFLATIV, an herbal medicine cream containing *Pereskia aculeata*, presents potential to be marketed as a topical anti-inflammatory agent and as an adjuvant in psoriasis therapy. **Journal of Pharmacy and Pharmacology**, v. 72, n. 12, p. 1933-1945, 2020. DOI: http://doi.org/10.1111/jphp.13357.

PINTO, N.C.C.; SCIO, E. The Biological Activities and Chemical Composition of *Pereskia Species* (Cactaceae) - A Review. Plant Foods for Human Nutrition, v. 69, n. 3, p. 189-195, 2014. DOI: http://doi.org/10.1007/s11130-014-0423-z.

PRIETO, P.; PINEDA, M.; AGUILAR, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. **Analytical Biochemistry**, v. 269, n. 2, p. 337-341, 1999. DOI: http://doi.org/10.1006/abio.1999.4019.

RAO, A.R.; VEERESHAM, C.; ASRES, K. *In Vitro* and *In Vivo* Inhibitory Activities of Four Indian Medicinal Plant Extracts and their Major Components on Rat Aldose Reductase and Generation of Advanced Glycation End Products. **Phytotherapy Research**, v. 27, n. 5, p. 753-760, 2012. DOI: http://doi.org/10.1002/ptr.4786.

RIGHI, F.; PITINO, R.; MANUELIAN, C.L.; SIMONI, M.; QUARANTELLI, A.; MARCHI, M; TSIPLAKOU, E. Plant Feed Additives as Natural Alternatives to the Use of Synthetic Antioxidant Vitamins on Poultry Performances, Health, and Oxidative Status: A review of the literature in the last 20 years. **Antioxidants**, v. 10, n. 5, 659, 2021. DOI: http://doi.org/10.3390/antiox10050659.

SHENG, Z.; DAI, H.; PAN, S.; AI, B.; ZHENG, L.; ZHENG, X.; PRINYAWIWATKUL, Wi.; XU, Z. Phytosterols in banana (*Musa* spp.) flower inhibit α-glucosidase and α-amylase hydrolysations and glycation reaction. **International Journal of Food Science & Technology**, v. 52, n. 1, p. 171-179, 2016. DOI: http://doi.org/10.1111/ijfs.13263.

SILVA, D.O.; SEIFERT, M.; NORA, F.R.; BOBROWSKI, V.L.; FREITAG, R.A.; KUCERA, H.R.; NORA, L.; GAIKWAD, N.W. Acute Toxicity and Cytotoxicity of *Pereskia aculeata*, a Highly Nutritious Cactaceae Plant. **Journal of Medicinal Food**, v. 20, n. 4, p. 403-409, 2017. DOI: http://doi.org/10.1089/jmf.2016.0133.

SONG, E.; CHOI, J.; GWON, H.; LEE, K.Y.; CHOI, S.G.; ISLAM, M.A.; CHUN, J.; HWANG, J. Phytochemical profile and antioxidant activity of *Dracocephalum moldavica* L. seed extracts using different extraction methods. **Food Chemistry**, v. 350, 128531, 2021. DOI: http://doi.org/10.1016/j.foodchem.2020.128531.

SOUZA, R.; LIRA, C.; RODRIGUES, A.; MORAIS, S.; QUEIROZ, C.; CHANG, R.; AQUINO, F.; MUNOZ, R.; OLIVEIRA, A. Antioxidant activity of ora-pro-nobis (*Pereskia aculeata* Mill.) leaves extracts using spectrophotometric and voltametric assays in vitro. **Bioscience Journal**, v. 30, p. 448-457, 2014.

XU, L.; YU, Y.; SANG, R.; LI, J.; GE, B.; ZHANG, X. Protective Effects of Taraxasterol against Ethanol-Induced Liver Injury by Regulating CYP2E1/Nrf2/HO-1 and NF-κB Signaling Pathways in Mice. **Oxidative Medicine and Cellular Longevity**, v. 2018, p. 1-11, 2018. DOI: http://doi.org/10.1155/2018/8284107.

YIN, M.C. Inhibitory effects and actions of pentacyclic triterpenes upon glycation. **Biomedicine**, v. 5, n. 3, 2015. DOI: http://doi.org/10.7603/s40681-015-0013-x.

YOSHIDA, Y.; NIKI, E. Antioxidant Effects of Phytosterol and Its Components. **Journal of Nutritional Science and Vitaminology**, v. 49, n. 4, p. 277-280, 2003. DOI: http://doi.org/10.3177/jnsv.49.277.