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Research Article

Biological Activity Survey of *Pereskia aculeata* Mill. and *Pereskia grandifolia* Haw. (Cactaceae)

Adriana Muniz Massocatto[1], Nyéssia Fernanda de Souza Silva[1], Caroline Calixto Kazama[1], Michele Dal Bem Pires[1], Orlando Seiko Takemura[1], Ezilda Jacomassi[2], Ana Lúcia Tasca Gois Ruiz[3], Antonio Laverde Junior[4*]

[1]*Laboratory of Pharmacognosy and Natural Products - Institute of Biological, Medical and Health Sciences - Paranaense University - UNIPAR, Praça Mascarenhas de Moraes, 4282; CEP 87502-210, Umuarama, PR, Brazil.*

[2]*Laboratory of Preclinical Research of Natural Products - Institute of Biological, Medical and Health Sciences - Paranaense University - UNIPAR, Praça Mascarenhas de Moraes, 4282; CEP 87502-210, Umuarama, Brazil.*

[3]*Faculty of Pharmaceutical Sciences - University of Campinas - UNICAMP, R. Cândido Portinari, 200; CEP 13083-871 - Campinas, SP, Brazil.*

[4]*Laboratory of Organic Chemistry and Biomaterials, Department of Chemistry - Federal Technological University of Parana - UTFPR, Av. dos Pioneiros, 3131; CEP 86036-370, Londrina, PR, Brazil.*

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Abstract

Background: *Pereskia aculeata* and *P. grandifolia* are non-traditional Brazilian vegetables with high nutritional value used in traditional medicine. The antioxidant, anticholinesterase, molluscicidal, cytotoxic, and antiproliferative properties of hydroethanolic extracts of *P. aculeata* and *P. grandifolia* leaves (PAL, PGL) and fruits (PAF, PGF) are investigated in this study.

Methods: All extracts were prepared by maceration with ethanol 70%. Their antioxidant properties were assessed through DPPH, ABTS, FRAP, and β-carotene bleaching inhibition assays. A TLC bioautography method was employed to evaluate the inhibiting capacity of the acetylcholinesterase enzyme. The molluscicidal activity was tested against the snail *Biomphalaria glabrata*, which serves as an intermediate host for *Schistosoma mansoni*. The cytotoxic activity was assessed by an *Artemia salina* lethality test and the antiproliferative properties against seven human carcinoma cell lines.

Results: Compounds with anticholinesterase activity were found in all extracts. Polar compounds present in PAF and PGL extracts were the most active (IC_{50} < 25 µg of dry mass) and had an adequate inhibition capacity of the AChE. PGF and PGL were classified as moderate (LC_{50} = 19.2 µg/ml) and modest molluscicidal agents (LC_{50} = 66.6 µg/ml), respectively. All extracts exhibited selective antiproliferative activity against human chronic myeloid leukemia cell lines (K562). PAL, PGL, and PGF presented potent antiproliferative activity (TGI ≤ 5 µg/ml).

Conclusion: Both species exhibited anticholinesterase, cytotoxic and antiproliferative properties. This research supports the potential of these species as sustainable sources of nutraceutical compounds.

Introduction

Pereskia aculeata Mill. and *P. grandifolia* Haw. (Cactaceae), both known as *ora-pro-nobis*, are two native Brazilian non-traditional vegetables with high nutritional value. These species are also grown as ornamental plants and used in traditional medicine.[1]

The succulent leaves of *P. aculeata*, also known as Barbados gooseberry, are high in proteins, amino acids, carotenoids, minerals, vitamins, and total dietary fiber.[2,3] Because it is the primary source of protein available in low-income communities, this species is best-known as "the meat of the poor".[2] The leaves are used in Brazilian cuisine in various preparations (omelets, soups, salads, pies, etc.), as well as the leaf flour (bread, pasta, and cakes).[4] The fruits can go into juices, liquors, jellies, and cakes.[5]

P. aculeata leaves have been employed in traditional medicine to treat kidney disorders, heal skin wounds and inflammatory processes, and as an effective emollient.[1] Scientific studies have covered the biological properties of *P. aculeata* leaf extracts, such as *in vivo* topical anti-inflammatory activity,[6] wound healing activity,[7] and antinociceptive activity.[8] Other properties were attributed to the leaves in *in vitro* experiments as an antioxidant,[5,9] antitumor,[10] trypanocidal,[10] antimicrobial,[5,11] and cytotoxic against cell proliferation, while no attributes were reported against normal cells.[9]

Despite their various nutritional and biological attributions, little is known about this species' chemical

*Corresponding Author: Antonio Laverde Junior, E-mail: aljunior@utfpr.edu.br

composition. Several studies have indicated the presence of alkaloids,[8,12] phenolic compounds,[5,8] carotenoids,[3,13] phytosterols,[1,6] among others.[11] The essential oil extracted from dried leaves is high in phytol (29.4%).[14]

The leaves and fruits of *P. grandifolia*, best-known as rose cactus, are also utilized in Brazilian cuisine.[1] In Malaysia, the leaves are employed in traditional medicine to treat cancer, diabetes, hypertension, and diseases associated with inflammation and rheumatism. They are also used for the relief of gastric pain, ulcers, and to rejuvenate the body.[15] In India, for instance, *P. grandifolia* is indicated for swelling reduction.[16] In Brazil the leaves integrate folk medicine as emollients in the treatment of skin rashes, and the fruits were found to have expectorant and antisyphilitic properties.[1] In terms of biological activities, all published research used *P. grandifolia* leaf extracts. Hence, antioxidant properties in extracts and fractions have been demonstrated by some authors.[15,17] Extracts of this species have been described for their cytotoxic activities on various cell lines.[15,18] Furthermore, *P. grandifolia* leaf extracts and different fractions have been reported as antimicrobial agents.[19] On normotensive rats, the hydroethanolic extract had aquaretic and hypotensive effects with direct action on the arginine-vasopressin system.[20]

As with *P. aculeata*, the chemical composition of *P. grandifolia* is still poorly understood. Thus far, the presence of alkaloids,[12] carotenoids[3], phytosterol, and fatty acid esters[15] have been linked. The fruits were found to contain an oleanolic acid saponin[16], and the essential oil extracted from the dried leaves is rich in manool oxide (30.1%) and phytol (25.1%).[14]

Finally, due to some of these two species' properties, they have piqued the interest of the food and pharmaceutical industries. However, there are still other biological properties that may be assessed, particularly in their fruits, which have been little investigated. Therefore, the present study proposed to evaluate the antioxidant, anticholinesterase, molluscicidal, cytotoxic, and antiproliferative properties of leaf and fruit hydroethanolic extracts from *P. aculeata* and *P. grandifolia*.

Materials and Methods

Plant material

The leaves and fruits of *P. aculeata* (23º46'11.8" S; 53º16' 42.5" W) and *P. grandifolia* (23º46'09.2" S; 53º16'42.6" W) were collected in autumn (April-May) at the Botanical Garden of the Paranaense University (Umuarama, Paraná State, Brazil) at 430 m altitude above sea level. Botanist Dr. Ezilda Jacomassi identified the species, and voucher specimens were deposited at the Educational Herbarium of the Paranaense University (campus Paranavaí, Paraná State, Brazil) under exsiccates HEUP-2206 (*Pereskia aculeata* Mill.) and HEUP-2210 (*Pereskia grandifolia* Haw.). Both species were also registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge under registration numbers A578420 and A464E1A, respectively.

Extract preparation

Fresh *P. aculeata* (PAF) and *P. grandifolia* leaves were harvested, washed, dried (ca. 40ºC), and pulverized. Portions of milled leaves (250g each) were exhaustively extracted by maceration (12 days) with ethanol/deionized water (7:3). To obtain crude hydroalcoholic extracts of the *P. aculeata* (PAL) and *P. grandifolia* (PGL) leaves, the filtrates were concentrated using a rotary vacuum evaporator (Technal, Brazil) at 40°C.

The fresh fruits (400g each) were washed, crushed, and subjected to exhaustive maceration for 7 days in ethanol/deionized water (7:3). Under reduced pressure, the filtrates were concentrated, yielding crude hydroalcoholic extracts of *P. aculeata* (PAF) and *P. grandifolia* (PGF) fruits.
All extracts (PAF, PAL, PGF, and PGL) were lyophilized (Liobras, Brazil) and kept frozen at -20°C until use.

Evaluation of in vitro antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The scavenging activity of *Pereskia* extracts (PAF, PAL, PGF, PGL) on DPPH radicals was determined according to a modified Blois method.[21] Aliquots (0.1 ml) of each extract at a range concentration (62.5, 125, 250, 500, 1000, 2000 µg/ml) were mixed with a DPPH (Sigma-Aldrich®) solution in methanol (2.9 ml at 60 µM) and kept in the dark for 30 minutes. After the incubation period, the absorbance values were measured with a spectrophotometer (Femto, 700 plus model; Brazil) at 517 nm. DPPH in a methanol solution (60 µM) was used as a negative control (blank). Three independent experiments were carried out in duplicate (n=6). The radical scavenging ability (%) was calculated according to the following equation:

$$\text{Radical Scavenging Ability (\%)} = [1 - (A_S/A_0)] \times 100 \quad \textbf{Eq. (1)}$$

where A_S and A_0 represent the absorbance of the samples (extracts or standard) and the negative control, respectively. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was employed as the positive reference standard. The radical scavenging activity of the extracts was expressed as an EC_{50} value, which is the effective concentration at which 50% of DPPH radicals were scavenged. This value derived from the plot of radical scavenging (%) against the sample concentrations.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay

The free ABTS radical scavenging assay was carried out according to the above mentioned description with modifications.[22] The ABTS radical cations were produced by mixing ABTS diammonium salt (7.0 mmol/l; Sigma-Aldrich®) with potassium persulfate (2.5 mmol/l). This mixture was kept in the dark at room temperature for 14-16h before use. Next, the ABTS stock solution was diluted to create a solution with an absorbance value of 0.7 at 734 nm (Femto, 700 plus model; Brazil). Finally, 100 µl of each

sample (PAF, PAL, PGF, PGL) at different concentrations (62.5, 125, 250, 500, 1000, 2000 µg/ml) were added to 900 µl of the ABTS radical solution and kept in the dark at room temperature for 10 min. The sample absorbance values were then measured at 734 nm. The radical scavenging activity was calculated according to Eq. 1. Trolox was employed as a positive control, while water was used as a negative control. The samples were examined in triplicate, and the effective concentration of each extract able to reduce 50% of ABTS radicals (EC_{50}) was expressed as µg/ml.

Ferric reducing power antioxidant (FRAP) assay

The reducing power of PAF, PAL, PGF, and PGL extracts was determined by FRAP assay with minor modifications.[23] Aliquots (200 µL) of each extract (1.0 mg/ml) solutions were mixed with 200 µl of ferric chloride (3 mM in 5 mM citric acid). Next, the mixtures were incubated at 37°C for 30 min before receiving 3.6 ml of 2,4,6-tripyridyl-s-triazine (TPZ; Sigma-Aldrich®). The absorbance values were then read at 670 nm (Femto, 700 plus model; Brazil). All samples were analyzed in triplicate. The reducing antioxidant capacity was estimated by a linear regression from the Trolox calibration curve. Finally, the results were expressed as 1 µmol Trolox equivalent per 100g of dried weight (µmol TE/100g DW).

β-carotene bleaching assay

The capacity of the extracts to neutralize free lipophilic radicals by inhibition of oxidative degradation of β-carotene was evaluated according to a β-carotene bleaching assay with slight modifications.[24] A solution of β-carotene (0.5 mg), linoleic acid (25 µl), and Tween 40 (200 µl) in 2 ml of chloroform was prepared under vacuum, and the chloroform was completely evaporated. To form an emulsion, ultrapure water saturated with oxygen (100 ml) was added to the residue and vigorously shaken. Aliquots (2.5 ml) of this emulsion were included to the test tubes and mixed with 350 µl of a methanolic extract solution at concentrations ranging from 62.5 to 2000 µg/ml. The mixtures were incubated for 2h at 50°C after homogenization. Finally, the tubes were immersed in a room-temperature water bath. The absorbance of the samples (extracts, standard, and control) was measured at 470 nm (Femto, 700 plus model; Brazil), against a blank consisting of an emulsion devoid of β-carotene. The measurements were performed in triplicate at 0h (initial time) and at 2h (final time). The antioxidant activity was measured in terms of the percentage of β-carotene oxidation inhibition using the following equation:

$$Inhibition\ (\%) = [1 - (A_{t0} - A_{t2})_{sample}/(A_{t0} - A_{t2})_{control}] \times 100 \quad \textbf{Eq. (2)}$$

where A_{t0} and A_{t2} represent the absorbance of the samples or the control before and after 2h of incubation.

Evaluation of in vitro anticholinesterase activity

The anticholinesterase activity of the *Pereskia* extracts (PAF, PAL, PGF, PGL) was assessed by means of a bioautographic assay[25] as previously described.[21] The acetylcholinesterase enzyme (AChE; Sigma-Aldrich®) was dissolved in a tris-hydrochloric acid buffer (0.05 M, pH 7.8) with bovine serum albumin (1 mg/ml, 98%, Sigma-Aldrich®) and stored at 4°C. Aliquots of different volumes (equivalent to 600, 400, 200, 150, 100, 50 and 25 µg dry mass) of each extract stock solution were loaded on TLC F_{254} plates (10 x 10 cm, 0.2 mm thickness; Merck®). To monitor possible active compounds[21], the plates were eluted with a dichloromethane-methanol solution (9:1 v/v) for chromatographic separation.[21] Dried TLC plates were sprayed with the AChE solution (1U/ml) and incubated at 37°C for 20 min. The plates were then sprayed with a 1-naphthyl acetate (1.5 mg/ml in ethanol 40%; Sigma-Aldrich®) and Fast Blue B (0.5 mg/ml in ultrapure water; Sigma-Aldrich®). After 2 minutes, the presence of enzymatic inhibitors was indicated by the appearance of white spots on the purple-colored dye background. The activity was formally characterized as strong, moderate, and weak according to the intensity of the white spots observed on the plates.

Evaluation of in vivo molluscicidal activity

The molluscicidal activity of the extracts against *Biomphalaria glabrata* (Say, 1818, Gastropoda: Planorbidae) snails was assessed according to World Health Organization (WHO) guidelines[26] with minor changes.[27] A total of ten adult snails were evaluated in preliminary tests (acute toxicity) for each extract solution (100 and 400 µg /ml in dechlorinated water). Two snails were placed in each extract/concentration (100 ml) container and kept in well-ventilated areas for 24h. Next, their heartbeats were verified using a stereo microscope. The surviving snails were placed in dechlorinated water for another 24h for mortality re-evaluation. Positive and negative control experiments were carried out with niclosamide (0.5 µg/ml; UCI-Farma, Brazil) and dechlorinated water, respectively. For lethal extracts at a concentration of 100 µg/ml, additional experiments were carried out to determine the lethal concentration values for 50% of the tested population (LC_{50}) using a dose-response curve (75, 50, 25, 10, and 5 µg/ml) following the same protocol. Each extract was subjected to three separate experiments. A statistical approach (Probit analysis) of lethality data was used to calculate the value of LC_{50}.

Brine shrimp lethality assay

Using *Artemia salina* (Leach, 1819, Crustacea: Branchiopoda) as a model, the brine shrimp lethality bioassay was performed to investigate the *in vitro* toxicity of PAF, PAL, PGF, and PGL extracts.[28] After 48h of incubation in artificial seawater at room temperature in the dark, live nauplii hatched from *A. salina* eggs were migrated to the illuminated area of the container (positive phototropism). To achieve different concentrations (1000, 500, 250, 125, 62,5 µg/ml), stock solutions of each extract were prepared

in DMSO followed by serial dilution in artificial seawater. Groups of 10 nauplii were then captured and transferred to assay tubes containing 5 ml of artificial seawater and extracts at different concentrations.[21] After 24h, the number of dead nauplii for each treatment was counted. A parallel series of tests with standard potassium dichromate (0.5 mM) and artificial seawater (DMSO 1%) were carried out. Each bioassay was performed in triplicate. The Probit method was used to calculate the lethal concentration for 50% of mortality (LC_{50}). Finally, the extracts were classified as active ($LC_{50} \leq 1000$) or inactive ($LC_{50} > 1000$).[28]

Evaluation of in vitro antiproliferative activity

Following the NCI-60 protocol[29] with minor modifications[30], the antiproliferative effect of the PAF, PAL, PGF, and PGL extracts was evaluated against seven human tumor cell lines kindly provided by the National Cancer Institute (Frederick, MA, USA): 786-0 (renal adenocarcinoma); K562 (chronic myeloid leukemia); NCI-ADR/RES (multidrug resistant ovarian adenocarcinoma); NCI-H460 (large cell carcinoma of the lung); OVCAR-3 (ovarian adenocarcinoma); PC-3 (adenocarcinoma of the prostate), and UACC-62 (melanoma). The cell culture conditions were RPMI 1640 (GIBCO BRL) and 1% of penicillin: streptomycin solution (1000 U/ml: 1000 mg/ml) (Vitrocell®) (complete medium) at 37°C in humidified air supplemented with 5% of CO_2, both for maintenance and experiments. The cells were subjected to a 48-hour serial dilution (0.25, 2.5, 25, and 250 µg/ml for extracts; 0.025, 0.25, and 25 µg/ml for doxorubicin, positive control) of each sample diluted in DMSO/complete medium (100 µl /compartment, in triplicate, DMSO final concentration ≤ 0.25%). The sulforhodamine B assay was used to measure cell growth. The results were plotted as a concentration-cell growth curve, and two effective concentrations, GI_{50} and TGI (sample concentration required to elicit 50% and total growth inhibition, respectively) were calculated by means of a non-linear regression analysis using the software ORIGIN 7.5. (Origin LabCorporation, Northampton, MA, USA).

Statistical analysis

The data were presented in the form of mean ± standard deviation. A one-way analysis of variance (ANOVA) was then performed, allowing the significance to be estimated. A *p-value* ≤ 0.05 was considered statistically significant.

Results and Discussion

P. aculeata and *P. grandifolia* are two non-traditional species with enormous nutraceutical potential, representing an important and inexpensive source of high-quality nutrition, especially for the underprivileged population. Despite the economic and technological aspects of these species, there is still a great necessity to expand studies on the subject to uncover new biological properties. In this context, we aimed to carry out an unedited study on certain biological activities, such as anticholinesterase, molluscicidal, and antiproliferative properties in addition to *in vitro* toxic effect evaluation, with the view of expanding the current body of knowledge on the potentialities of these non-conventional food plants. There are very few reports available in the literature on the biological properties of fruits from *Pereskia* species. Hence, we seek to extend our preliminary studies to assess the antioxidant properties of extracts from leaves and, in particular, the fruits of both *Pereskia* species.

Plants produce a wide range of antioxidant compounds that can protect against molecular damage caused by reactive oxygen species and free radicals generated in our bodies during the biochemical oxidation process. Due to the complex nature of phytochemicals, four tests were selected to evaluate the antioxidant capacity of the *Pereskia* extracts. Initially, DPPH and ABTS assays were used to evaluate the ability of the extracts to inhibit hydrophilic radicals, and the FRAP assay to reduce radical generation reaction. Finally, the lipophilic radical inhibition of the extracts was assessed using the β-carotene bleaching inhibition assay. The antioxidant activities determined by these methods are shown in Table 1.

According to the data in Table 1, the radical scavenging capacity of different extracts was higher when measured by ABTS assay than when assessed by DPPH assay. Garcia *et al.*[5] also reported this difference in both methodologies when analyzing the antioxidant activity of *P. aculeata* leaf extracts. However, the results obtained by different assays are not directly comparable due to differences in the mechanism of radical capture in each test.

The antioxidant activity of the *Pereskia* extracts was classified into four categories based on their EC_{50} values: highly active ($EC_{50} < 50$ µg/ml); moderately active (EC_{50} = 50-100 µg/ml); weakly active (EC_{50} = 100-200 µg/ml) or inactive ($EC_{50} > 200$ µg/ml).[5] Our results (Table 1) reveal that active compounds are present in each extract, although

Table 1. Evaluation of the antioxidant activity of *P. aculeata* and *P. grandifolia* leaf (PAL and PGL) and fruits (PAF and PGF) extracts measured via different inhibition assays.

Extracts/ standard	DPPH	ABTS	FRAP	β-carotene assay
	EC_{50} (µg/ml)	EC_{50} (µg/ml)	µM TE/100 g DW	EC_{50} (µg/ml)
PAF	1612.9 ± 50.2^{b}	1209.8 ± 61.1^{b}	5.9 ± 1.1^{d}	2516.8 ± 101.9^{b}
PAL	3351.5 ± 109.1^{c}	2851.7 ± 101.4^{c}	17.7 ± 1.7^{b}	3523.4 ± 189.8^{d}
PGF	4132.3 ± 138.4^{d}	3305.6 ± 123.7^{d}	9.8 ± 1.4^{c}	5330.6 ± 217.4^{e}
PGL	4950.2 ± 150.5^{e}	3712.5 ± 135.4^{e}	24.3 ± 1.6^{a}	2701.0 ± 105.7^{c}
Trolox	101.2 ± 10.1^{a}	74.5 ± 5.5^{a}	-	188.7 ± 3.5^{a}

Values are means ± standard deviation. $^{a-e}$ the same letters within the same colunm were not significantly different.

Table 2. Inhibition of the acetylcholinesterase enzyme in the presence of different concentrations of *P. aculeata* and *P. grandifolia* extracts by TLC bioautographic analyses.

Observed spots	R_f spot	Dry mass						
		600 µg	400 µg	200 µg	150 µg	100 µg	50 µg	25 µg
				***P. aculeata* leaves**				
A1	0.64	+++	+++	+++	++	+	+	-
B1	0.87	+++	+++	+++	++	+	+	-
				***P. aculeata* fruits**				
A2	0.00	+++	+++	+++	+++	++	+	+
B2	0.80	+++	+++	+++	++	+	+	-
				***P. grandifolia* leaves**				
A3	0.00	+++	+++	+++	++	++	+	+
B3	0.89	++	++	+	+	-	-	-
				***P. grandifolia* fruits**				
A4	0.00	++	++	++	+	+	-	-
B4	0.64	+	+	-	-	-	-	-

AChE inhibition was characterized by legends according to the intensity of the white spots observed on the plates as: (-) no activity; (+) low; (++) moderate; (+++) strong activity.

not in sufficient concentrations to classify them as active extracts. These data are consistent with previous reports in the literature for this genus.[11,17,31] Other authors have described moderate results for *P. aculeata*[5] and *P. bleo*.[32]

A promising treatment for Alzheimer's disease is the inhibition of the acetylcholinesterase enzyme, which catalyzes the hydrolysis of the neurotransmitter acetylcholine. Most of the anticholinesterase medications used for treatments have side effects such as hepatotoxicity, gastrointestinal disorders, low bioavailability, and a narrow therapeutic index.[33] Therefore, the study of new AChE inhibitors is of paramount interest for the treating this disease.

The TLC bioautographic analysis is a useful technique for detecting the presence of bioactive compounds in plant extracts.[25] Through bioautography, a small modification to the methodology allowed for a preliminary assessment of the minimum amount of extract in which each component would be able to inhibit the AChE.[21] In this study, this technique was employed to detect anticholinesterase substances in *P. aculeata* and *P. grandifolia* extracts (Table 2). All extracts exhibited at least two spot zones with AChE inhibitory capacity. The most polar compounds found in extracts of *P. aculeata* fruits and *P. grandifolia* leaves (Table 2: A2 and A3, respectively) were the ones with the highest inhibiting capacity (25 µg of dry mass) of AChE. Considering that these compounds presented a similar minimum activity for the same extract mass (Table 2: A2 and A3 spots), their inhibiting concentration is lower due to the complexity of the extract composition. As a result, the inhibition concentration can be considered lower than the lowest concentration tested in this experiment (IC < 25 µg of dry mass). *P. aculeata* extracts were more active (PAF > PAL), while *P. grandifolia* fruit extract was less active. El-Hawary *et al.*[34] have recently reported that *Opuntia ficus-indica* extracts had substantial neuroprotective activity against $AlCl_3$-induced neurotoxicity. According to this study, rats treated with *O. ficus-indica* extracts had a significant reduction in AChE levels, indicating promising neuroprotective activities. Docking results with polar polyphenolic compounds identified in this species suggested that these compounds could act as AChE and SERT (serotonin transporter) inhibitors. Finally, phenolic compounds may be related to the anticholinesterase activity observed in *Pereskia* extracts. To the best of our knowledge, this is the first time this activity is documented in the literature for a *Pereskia* species.

Another biological activity addressed in this study was molluscicidal activity against *Biomphalaria glabrata* snails. In Brazil, these snails are intermediate hosts for *Schistosoma mansoni*, the trematode responsible for schistosomiasis,[35] which is considered one of the most widespread parasites in the world, second only to malaria. One of the most effective methods of control against this snail is the use of molluscicides that eliminate or reduce its population. The control of the population of *B. glabrata* has been done with synthetic molluscicides, which are harmful to the ecosystem because they do not have a specific target, affecting not only the mollusk but also the plankton and animals that rely on it, often causing a trophic imbalance.[36] In the search for new substances to control mollusks that are intermediate hosts of *Schistosoma mansoni*, plants come up as alternative sources. In this sense, to complement the study on the potential biological properties of *P. aculeata* and *P. grandifolia*, the molluscicidal activity of all extracts was also assessed due to their water solubility. Among all extracts that were tested in this study, only those of *P. grandifolia* showed activity in the preliminary concentrations tested (Table 3: 400 and 100 µg/ml) against adult *B. glabrata* snails.

According to the recommendations of WHO[26], only aqueous or alcoholic extracts of vegetal materials that cause the death of 90% of the malacological population at concentrations lower than 20 µg/ml in laboratory testing are considered potentially active and viable for field assays. Based on the results for *P. grandifolia* (Table 3), the fruit extract (LC_{95} = 31.2 µg/ml and LC_{50} = 19.2 µg/ml) can be classified as a moderate molluscicidal agent and the leaf

Table 3. Evaluation of molluscicidal activity of extracts of *P. aculeata* and *P. grandifolia* leaves (PAL and PGL) and fruits (PAF and PGF) against *Biomphalaria glabrata* snails with 24 h exposure and recovery time.

Concentrations (µg/ml)	% lethality against *B. glabrata*				
	PAL	PAF	PGL	PGF	Niclosamide*
400.0	0.0	0.0	100.0	100.0	nt
100.0	0.0	0.0	100.0	100.0	nt
75.0	nt	nt	70.0	100.0	nt
50.0	nt	nt	10.0	100.0	nt
25.0	nt	nt	0.0	80.0	nt
10.0	nt	nt	0.0	0.0	100.0
5.0	nt	nt	0.0	0.0	100.0
LC_{95} (µg/ml)	> 400.0	> 400.0	90.4	31.2	< 5.0
LC_{50} (µg/ml)	> 400.0	> 400.0	66.6	19.2	< 5.0
LC_{5} (µg/ml)	> 400.0	> 400.0	49.0	11.8	< 5.0

*Niclosamide: LC_{95} ≤ 0.5 µg/ml[36]; nt: no tested.

extract (LC_{95} = 90.4 µg/ml and LC_{50} = 66.6 µg/ml) as a weak molluscicidal agent. The leaves and fruits of *P. aculeata* were inactive. This is the first report on the molluscicidal potential of a Cactaceae species in the literature.

Several studies have attempted to correlate toxicity against *A. salina* with biological activities such as antitumor, antimicrobial, parasiticide, among others.[37] Due to its simple methodology, this bioassay is widely employed to monitor extracts and fractions as an attempt to streamline the process of separation and identification of bioactive compounds. According to Meyer *et al.*,[28] the activity is considered significant when LD_{50} ≤ 1000 µg/ml. Given the preliminary assessment of the toxic potential of *P. aculeata* and *P. grandifolia*, lethality tests were carried out on nauplii of *A. salina* experiments for this purpose.

The toxic activity of the cactus extracts is shown in Table 4. All extracts were deemed toxic, with LC_{50} ≤ 1000 µg/ml. Among the evaluated extracts, the most toxic was the *P. grandifolia* leaf extract (LC_{50} = 95.3 ± 1.2 µg/ml). The remaining extracts (PAL, PAF, and PGF) had a moderate toxic activity against *A. salina* (LC_{50} = 372.4 ± 1.3, 266.2 ± 1.3, and 218.9 ± 1.3 µg/ml, respectively).

These findings are noteworthy as they corroborate antiproliferative studies in cancer cell lines of some species of *Pereskia*.[9,11,15,18,38] To the best of our knowledge, this is the first report on the cytotoxic activity of the *P. aculeata* and *P. grandifolia* fruits.

The antiproliferative properties of *P. aculeata* and *P. grandifolia* extracts were assessed using a protocol developed by the National Cancer Institute (NCI/EUA). The concentration-response curves of PAL, PAF, PGL, PGF extracts, and doxorubicin (positive control) against seven human tumor cell lines [UACC-62 (melanoma), NCI-ADR/RES (adriamycin-resistant ovarian cancer), 786-0 (kidney), NCI-H460 (lung), PC-3 (prostate) OVCAR-3 (ovary), and K562 (leukemia)] were calculated and summarized in Table 5.

According to the US National Cancer Institute's antiproliferative activity classification criteria, one extract with a mean log GI_{50} greater than 1.5 is considered inactive.[39] Among all extracts evaluated, only the PGF extract showed an antiproliferative activity (mean log GI_{50} = 1.5) with the ability to moderately inhibit leukemic cell growth (K562, GI_{50} = 0.54 µg/ml) as well as a weak cytostatic effect against almost all other tumor cells, except for melanoma cells (inactive) (Table 5). Interestingly, even inactive extracts were able to inhibit K562 cells. Fouche *et al.*[40] also classified the antiproliferative activity of screened extracts into four categories based on the second effective concentration TGI: inactive (TGI > 50 µg/ml), weak activity (15 µg/ml < TGI < 50 µg/ml), moderate activity (6.25 µg/ml < TGI < 15 µg/ml and potent activity (TGI < 6.25 µg/ml). According to this second criterion, all extracts tested were only active against K562 cells, with PAL, PGL,

Table 4. Percentage of lethality against *Artemia salina* induced by extracts of *P. aculeata* and *P. grandifolia* leaves (PAL and PGL) and fruits (PAF and PGF).

Concentrations (µg/ml)	% lethality against *A. salina*			
	PAL	PAF	PGL	PGF
1000.0	96.7 ± 0.5	100.0 ± 0.0	96.7 ± 0.5	96.7 ± 0.5
750.0	73.3 ± 1.9	96.7 ± 0.5	96.7 ± 0.5	96.7 ± 0.5
500.0	73.3 ± 2.1	60.0 ± 3.3	96.7 ± 0.5	93.3 ± 0.9
250.0	20.0 ± 1.3	36.7 ± 2.5	96.7 ± 0.5	60.0 ± 1.4
100.0	3.3 ± 0.5	13.3 ± 1.3	73.3 ± 0.9	6.7 ± 0.9
75.0	0.0	0.0	30.0 ± 1.4	3.3 ± 0.5
50.0	0.0	0.0	0.0	0.0
LC_{50} (µg/ml)	372.4 ± 1.3	266.2 ± 1.3	95.3 ± 1.2	218.9 ± 1.3

Standard: $K_2Cr_2O_7$ (LC_{50} = 20.1 ± 1.0 µg/ml)

Table 5. Antiproliferative activity of extracts of *P. aculeata* and *P. grandifolia* leaves (PAL and PGL) and fruits (PAF and PGF) expressed as concentration required for 50% (GI_{50}) and total (TGI) cell growth inhibition.

Human tumor cell lines	PAL		PAF		PGL		PGF		Dox	
	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
UACC-62	224.59	>250	>250	>250	>250	>250	77.22	107.64	0.04	0.20
NCI-ADR/RES	>250	>250	>250	>250	>250	>250	32.28	153.13	0.45	8.45
786-0	129.86	>250	>250	>250	215.96	>250	32.75	91.16	0.04	6.12
NCI-H460	>250	>250	>250	>250	250.00	>250	32.40	104.39	0.016	0.22
PC-3	185.77	>250	>250	>250	202.18	>250	33.33	75.63	0.12	2.66
OVCAR-3	161.00	>250	>250	>250	>250	>250	34.51	133.60	0.21	2.89
K562	0.89	3.00	5.27	39.51	1.96	3.77	0.54	5.09	<0.025	<0.025
Mean log GI_{50}	2.2	--	2.4	--	2.3	--	1.5	--	-0.9	--

Concentration range: 0.25-250 µg/ml; exposition time: 48 h; TGI: sample concentration required to total cell growth inhibition (expressed in µg/ml); GI_{50}: sample concentration required to elicit 50% of cell growth inhibition (expressed in µg/ml); mean log GI_{50}: mean cytostatic activity; Dox.: doxorubicin. Human tumor cell lines: UACC-62 (melanoma), NCI-ADR/RES (multi-drug resistant ovarian adenocarcinoma), 786-0 (renal adenocarcinoma), NCI-H460 (large cell carcinoma of lung), PC-3 (adenocarcinoma of prostate), OVCAR-3 (ovarian adenocarcinoma), and K562 (chronic myeloid leukemia).

and PGF extracts exhibiting potent activities (TGI = 3.00, 3.77, 5.09 µg/ml, respectively), and PAF extract displaying weak activity (TGI = 39.51 µg/ml) (Table 5). This selective effect can be investigated in future studies.

The antiproliferative effect has been described for different *Pereskia* species in the literature.[1,40] For instance, the ethanolic extract of *P. sacharosa* leaves promoted cell death via apoptosis induction and changes in cell cycle checkpoints in two leukemic human cell lines (K562 and MV4-11).[41] Furthermore, the methanol extract of *P. bleo* leaves significantly reduced the viability of human nasopharyngeal epidermoid cells (KB; IC_{50} = 6.5 µg/ml)[38] and breast carcinoma cells (T-47D cell line; IC_{50} = 2.0 µg/ml).[42] In T-47D cells, the methanol extract of *P. bleo* leaves induced apoptosis by promoting activation of caspase-3 and c-myc genes.[42] According to Siew *et al.*[43], *P. bleo* leaf extracts were found to have strong or moderately strong antiproliferative activity against breast (T-47D), cervical (C33A), colon (HCT116), liver (SNU-182, SNU-449, HepG2), ovarian (PA-1) and uterine (MES-SA/Dx5) cancer cell lines. Furthermore, dihydroactinidiolide, 2,4-di-*tert*-butylphenol, and α-tocopherol isolated from *P. bleo* leaf extracts exhibited a cytotoxic effect on the viability of human tumor cell lines.[44] 2,4-di-*tert*-butylphenol showed a remarkable cytotoxic activity against KB (IC_{50} = 0.8 µg/ml), MCF7 (IC_{50} = 5.75 µg/ml; breast), A549 (IC_{50} = 6.0 µg/ml; lung) and CasKi cells (IC_{50} = 4.5 µg/ml; cervix) whereas dihydroactinidiolide significantly reduced the viability of HCT116 cells (IC_{50} = 5.0 µg/ml; colon) and α-tocopherol presented a pronounced cytotoxicity against CasKi (IC_{50} = 6.0 µg/ml) and A549 (IC_{50} = 6.0 µg/ml) cells.

The methanolic extract of *P. grandifolia* leaves exhibited moderate cytotoxic activity against KB (IC_{50} = 34 µg/ml; nasopharyngeal epidermoid), CasKi (IC_{50} = 50 µg/ml; cervical) and HCT116 (IC_{50} = 53 µg/ml; colon) human tumor cell lines.[15] The fractionation of this extract resulted in more active fractions such as the hexane (IC_{50} = 5.0 µg/ml for KB cells) and ethyl acetate (IC_{50} = 16.0 and 20.0 µg/ml, for KB and MCF-7 cells, respectively). As observed for *P. bleo* extracts, 2,4-di-*tert*-butylphenol was identified in the active ethyl acetate fraction. According to Liew *et al.*[18], crude methanol extract of *P. grandifolia* leaves also exhibited cytotoxicity against human Saos-2 osteosarcoma cells. When compared to normoxic conditions, the cell viability reduction induced by *P. grandifolia* leaf extracts in Saos-2 cells increased under hypoxic conditions. There was one report in the literature on the activity of methanol extract of *P. aculeata* leaves against human breast adenocarcinoma cells (MCF-7 cell line) and human promyelocytic leukemia cells (HL60 cell line) showing modest cytotoxic effects.[9] Given the present findings and the literary context, the antiproliferative activity observed for PGF should be further investigated in studies involving fractionation and chemical analysis to better understand the activity observed.

Moreover, experimental studies have shown that *P. aculeata* and *P. grandifolia* extracts or active compounds have biological activities such as antiproliferative, anti-inflammatory, antinociceptive, antimicrobial, and diuretic properties.[1,20,40] Thus the current study contributes to the body of knowledge by demonstrating that *P. aculeata* and *P. grandifolia* exhibit *in vitro* anticholinesterase, molluscicidal and antiproliferative activities.

Conclusion

The findings of this study indicate that two non-traditional food plants have new biological properties that have not been previously reported. *P. aculeata* and *P. grandifolia* leaves and fruits contained compounds with acetylcholinesterase inhibitor capacity, with the most polar being the most active (25 µg/ml of dry mass). Molluscicidal activity was found in *P. grandifolia* fruit and leaf extracts. PGF and PGL extracts were found to be moderate (LC_{50} = 19.2 µg/ml) and modest (LC_{50} = 66.6 µg/ml) molluscicidal agents, respectively.

In antiproliferative evaluation, all extracts demonstrated selective activity against human chronic myeloid leukemia cell lines (K562), with PAL, PGL, and PGF presenting potent antiproliferative activity (TGI ≤ 5 µg/ml). To the best of our knowledge, these specific activities are being

reported for the first time on species of the genus *Pereskia* and family Cactaceae, respectively. Moreover, all extracts exhibited moderate cytotoxic activity against *A. salina* (PGL > PGF > PAF > PAL) and were deemed bioactive. On the other hand, no antioxidant activity was detected in any of the extracts of these two Cactaceae. Nonetheless, because these extracts are a complex mixture of compounds, fractionation may result in specific mixtures or pure compounds with potential activity at low concentrations. Therefore, the present study on the leaves and fruits of *P. aculeata* and *P. grandifolia* encourages further research to elucidate the *in vivo* activities and chemical composition, reinforcing their potential as sustainable sources of nutraceutical compounds.

Author Contributions

ALJ, ALTGR, and OST contributed to the conception and design of the experiments. AMM, NFS, CCK, MDBP, EJ, and ALTGR contributed to the acquisition of data. ALJ, ALTGR, and OST contributed to the analysis and interpretation of the data. ALJ supervised the project and wrote the manuscript with input from all authors. All authors read and agreed to the published version of the article.

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Conflict of Interest

The authors report no conflicts of interest.

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