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

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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 (IC₅₀ < 25 μg of dry mass) and had an adequate inhibition capacity of the AChE. PFG and PGL were classified as moderate (IC₅₀ = 19.2 μg/ml) and modest molluscicidal agents (LC₅₀ = 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.

**Keywords:**
- Anticholinesterase activity
- Anti-proliferative activity
- Molluscicidal activity
- Nutraceutical food
- Ora-pro-nobis

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 ³ 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).³ The fruits can go into juices, liquors, jellies, and cakes.⁴

*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,⁵ wound healing activity,⁶ and antinociceptive activity.⁷ Other properties were attributed to the leaves in *in vitro* experiments as an antioxidant,⁸ antimicrobial,⁹ antitumor,⁹ trypanocidal,¹⁰ antinociceptive,¹¹ 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...
composition. Several studies have indicated the presence of alkaloids, phenolic compounds, carotenoids, phytochemicals, and others. The essential oil extracted from dried leaves is rich in phytosterols.

The leaves and fruits of *P. grandifolia*, best-known as rose cactus, are also utilized in Brazilian cuisine. 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. In India, for instance, *P. grandifolia* is indicated for swelling reduction. 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. In terms of biological activities, all published research used *P. grandifolia* leaf extracts. Hence, antioxidant and anticholinesterase properties in extracts and fractions have been demonstrated by some authors. Extracts of these species have been described for their cytotoxic activities on various cell lines. Furthermore, *P. grandifolia* leaf extracts and different fractions have been reported as antimicrobial agents. On normotensive rats, the hydroethanolic extract had aquaretic and hypotensive effects with direct action on the arginine-vasopressin system.

As with *P. aculeata*, the chemical composition of *P. grandifolia* is still poorly understood. Thus far, the presence of alkaloids, carotenoids, phytosterol, and fatty acid esters have been linked. The fruits were found to contain an oleoanolic acid saponin, and the essential oil extracted from the dried leaves is rich in manool oxide and phytol.

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* (23°46'11.8" S; 53°16'42.6" W) and *P. grandifolia* (23°46'09.2" S; 53°16'42.6" 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 Paranavai, 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. 40°C), 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* (PGF) 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. 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:

\[
\text{Radical Scavenging Ability} \% = \left[ 1 - \frac{A_s}{A_o} \right] \times 100 \quad \text{Eq. (1)}
\]

where \(A_s\) and \(A_o\) 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. 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, PFG, PGL) at different concentrations (62.5, 125, 250, 500, 1000, 2000 μg/μl) 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 (EC50) was expressed as μg/ml.

**Ferric reducing power antioxidant (FRAP) assay**

The reducing power of PAF, PAL, PFG, and PGL extracts was determined by FRAP assay with minor modifications.6 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 50ºC 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:

\[
\text{Inhibition} \% = \left[ 1 - \frac{(A_{\text{sample}} - A_{\text{control}})}{(A_{\text{control}} - A_{\text{control}})} \right] \times 100
\]

Eq. (2)

where \( A_0 \) and \( A_2 \) 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, PFG, PGL) was assessed by means of a bioautographic assay23 as previously described.24 The acetylcholinesterase enzyme (AChE; Sigma-Aldrich®) was dissolved in a triis-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 F364 plates (10 x 10 cm, 0.2 mm thickness; Merck®). To monitor possible active compounds21, 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) guidelines26 with minor changes.27 A total of 10 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 (LC50) 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 LC50.

**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, PFG, 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. 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 (LC50). Finally, the extracts were classified as active (LC50 ≤ 1000) or inactive (LC50 > 1000).

**Evaluation of in vitro antiproliferative activity**

Following the NCI-60 protocol with minor modifications, 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 CO2, 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, GI50 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.

### 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 |
|-------------------|------|------|------|------------------|
|                   | EC50 (μg/ml) | EC50 (μg/ml) | μM TE/100 g DW | EC50 (μg/ml) |
| PAF               | 1612.9 ± 50.2a | 1209.8 ± 61.1a | 5.9 ± 1.1b     | 2516.8 ± 101.9a |
| PAL               | 3351.5 ± 109.1c | 2851.7 ± 101.4c | 17.7 ± 1.7b   | 3523.4 ± 189.8c |
| PGF               | 4132.3 ± 138.4d | 3305.6 ± 123.7d | 9.8 ± 1.4c    | 5330.6 ± 217.4d |
| PGL               | 4950.2 ± 150.5e | 3712.5 ± 135.4e | 24.3 ± 1.6b   | 2701.0 ± 105.7e |
| Trolox            | 101.2 ± 10.1a  | 74.5 ± 5.5a    | -              | 188.7 ± 3.5a    |

Values are means ± standard deviation. **The same letters within the same column were not significantly different.**

**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.* 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 EC50 values: highly active (EC50 < 50 μg/ml); moderately active (EC50 = 50-100 μg/ml); weakly active (EC50 = 100-200 μg/ml) or inactive (EC50 > 200 μg/ml). Our results (Table 1) reveal that active compounds are present in each extract, although
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*2 and *P. bleo*.12

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.39 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.*24 have recently reported that *Opuntia ficus-indica* extracts had substantial neuroprotective activity against AlCl₃-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 mollusccidal 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 WHO24, 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 (LC₉₀ = 31.2 µg/ml and LC₅₀ = 19.2 µg/ml) can be classified as a moderate mollusccidal agent and the leaf

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**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 | Rₜ spot | 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.
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) | 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_{50} (μg/ml)        | > 400.0 | > 400.0 | 90.4 | 31.2 | < 5.0       |
| LC_{90} (μg/ml)        | > 400.0 | > 400.0 | 66.6 | 19.2 | < 5.0       |
| LC_{95} (μg/ml)        | > 400.0 | > 400.0 | 49.0 | 11.8 | < 5.0       |

*Niclosamide: LC_{50} ≤ 0.5 μg/ml; nt: no tested.

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. Among all extracts evaluated, only the PGF extract showed an antiproliferative activity (mean log GI_{50} = 1.5) with the ability to moderately inhibit leukemia 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. 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) | 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  | 96.7 ± 0.5  | 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         | 30.0 ± 1.4  | 3.3 ± 0.5   | 0.0         |
| 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_{2}Cr_{2}O_{7} (LC_{50} = 20.1 ± 1.0 μg/ml)
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. 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). Furthermore, the methanol extract of P. bleo leaves significantly reduced the viability of human nasopharyngeal epidermoid cells (KB; IC_{50} = 6.5 μg/ml) and breast carcinoma cells (T-47D cell line; IC_{50} = 2.0 μg/ml). In T-47D cells, the methanol extract of P. bleo leaves induced apoptosis by promoting activation of caspase-3 and c-myc genes. According to Siew et al., 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. 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. 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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