Pereskia aculeata leaves: properties and potentialities for the development of new products

Fabiane Grecco da Silva Porto, Angela Diniz Campos, Neftalí Lenin Villarreal Carreño and Irene Teresinha Santos Garcia

ABSTRACT
Pereskia aculeata Mill., known as Ora-Pro-Nobis or Barbados gooseberry, arouse the interest of food and pharmaceutical industries due to its bioactive compounds and mucilage. We conducted a peer-reviewed survey using Web of Science, Scopus, Scielo, Science Direct, and Scifinder platforms, as well as patent bases for new products. We selected articles which highlighted composition of leaves and applications in the development of new products. Mucilage shows great potential in the development of complexes or microparticles to transport active molecules. Reports on anti-inflammatory and anticancer properties of P. aculeata leaves open a research field to obtain pharmaceutical products. Emulsifying properties of mucilage have been explored in food processing. Another potential use is the development of films for functional and/or edible packaging. The polysaccharide chain, the main mucilage constituent, can interact with other biopolymers to be explored in colloidal chemistry for the production of biomaterials in the next years.

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1. Introduction
Pereskia aculeata Mill. (Figure S1) known as Barbados gooseberry plant or Ora-Pro-Nobis, belongs to the family Cactacea, subfamily Pereskioideae, genus Pereskia. Species belonging to this genus are native to the Americas and do not resemble...
common cacti, as they have well-developed leaves and thin stems (Souza et al. 2016a; Rosa et al. 2020). Three of the 17 known species of the genus *Pereskia*, *Pereskia bleo* (Kunth), *Pereskia grandifolia* (Haw), and *Pereskia aculeata* (Mill) are used for consumption or disease treatment in popular medicine (Sharif et al. 2013).

Sharif et al. (2013) highlighted the pharmacological relevance of plants of genus *Pereskia* and Pinto and Scio (2014) reviewed information on the biological activity and chemical composition of plants belonging to this genus.

Maciel et al. (2019) published a review on *P. aculeata* and *P. grandifolia*, focusing on agronomic studies and detailing their chemical composition in terms of protein amount, essential and non-essential amino acids and minerals. It is also important to explore their potential applications in the development of new products and update the studies with respect to their applications in nutrition and use of their bioactive compounds.

The first studies on *P. aculeata* were reported in the last century (Figures S2 and S3) and basically aimed to evaluate its chemical composition (Dayrell and Vieira 1977a, 1977b; Sierakowski et al. 1987, 1990). Investigations on planting, propagation and post-harvest conservation of *P. aculeata* have increased in recent years, mainly due to the growing interest in properties of this species in the food and pharmaceutical industries (Souza et al. 2016a; Vega et al. 2020; Monteiro et al. 2021). This species has a high protein content, it is rich in fibers and is a good source of minerals thus important for nutrition (Takeiti et al. 2009).

This plant also has important phytochemicals, such as terpenes, phenolic, and carotenoid compounds which may be associated to anti-inflammatory, antimicrobial, and antioxidant effects, due to their biological activity (Souza et al. 2016b; Garcia et al. 2019). Leaves of *P. aculeata* are also used in folk medicine to heal skin wounds or in treatment of diseases associated to inflammatory processes and cancer (Pinto et al. 2015b; Souza et al. 2016b).

*Pereskia aculeata* also has a stabilizing, emulsifying and/or thickening action, due to its high mucilage content, a natural hydrocolloid. Plant-based hydrocolloids have been studied to replace artificial emulsifiers in the food and pharmaceutical industries (Martin et al. 2017; Amaral et al. 2019; Mazon et al. 2020; Ziegler et al. 2020; Lise et al. 2021). Oliveira et al. (2019) used mucilage from leaves to develop edible packaging. The development of materials from substances of plant sources has been extensively explored, as they allow obtaining materials with non-toxic, biocompatible, biodegradable, and sustainable characteristics (Neves et al. 2020a).

In this review, we focus on *P. aculeata* leaves addressing their constituents and their potential use to develop new materials. We also investigated the nutritional aspects and their bioactive compounds.

2. Methodology

We conducted a peer-review of scientific articles on Web of Science, Scielo, Scopus, Science Direct and Scifinder platforms, which contained the words Ora-Pro-Nobis or *Pereskia aculeata* or Barbados gooseberry. Figure S2 shows the distribution of articles per year in each base surveyed in a total of 349. After a preliminary analysis, we
excluded the repeated articles and discarded those that dealt only with the phenotypic, genetic characterization of the species and those about control, when *P. aculeata* was considered exotic plant. Figure S3 shows the distribution of articles evaluated in this review, resulting in 145 articles from the search during the entire consultation period (until Abril 2021). The content of these articles was analyzed for the development of this work, focusing on the properties and potential applications of *P. aculeata* leaves. Patents, which explored the development of products based on *Pereskia aculeata*, were also searched, with the same keywords, on the platforms Scifinder, United States Patent and Trademark Office (USPTO), Derwent Innovations index, Spacenet and Patentscope – WIPO. The results are shown in Table S1.

### 3. Nutritional aspects

Takeiti et al. (2009) carried out a study detailing the nutritional components present in *Pereskia aculeata* leaves. Maciel et al. (2019) reported that the amount of minerals in ora-pro-nobis are superior to those of spinach and sweet potato leaves. The leaves of this plant have the highest protein contents among vegetables with an average of 28 g/100 g dry weight of total proteins (Amaral et al. 2019). Its proteins have a high content of essential and non-essential amino acids with good digestibility (around 85%), providing a high-quality nutritional ingredient (Maciel et al. 2019; Morais et al. 2019). Those characteristics have led to the exploration of this plant as an alternative source for industrial production of protein concentrates. Morais et al. (2019) studied the recovery and purification of *P. aculeata* leaf proteins by combining isoelectric precipitation to the addition of salts. The best result recovered 69% of protein with ammonium sulfate at concentrations from 0.8 to 1 mol·L\(^{-1}\), at 85°C in pH 3.0. Neves et al. (2020b) developed a process capable of recovering 94.45% of proteins using polyacrylamide cryogel amino acid functionalized. Protein purified by this method presented a secondary structure composed mainly of \(\beta\) leaf and \(\alpha\) helix, indicating structures with an ordered conformation. Not only the protein content, but also the high content of mineral salts, are important for nutrition and have drawn interest in the use of *P. aculeata* in the food industry. The *P. aculeata* leaves contain Fe (14.2 mg/100 g), P (156 mg/100 g), Mg (1,900 mg/100 g), Mn (46.4 mg/100 g), Ca (3,420 mg/100 g), K (1,632 mg/100 g), Zn (26.7 mg/100 g), B (25.55 mg/100 g), and Cu (1.4 mg/100 g), which are important in several metabolic processes (Takeiti et al. 2009).

Several works have evaluated the addition of *P. aculeata* leaf flour to processed foods, such as meat for the production of hamburgers, pre-commercial mixes of cakes, pasta, ice cream, dairy drinks, and sausages, aiming to increase the nutritional value of these foods (Sato et al. 2019; Mazon et al. 2020; Rosa et al. 2020; Ziegler et al. 2020). Further studies are needed to optimize the incorporation of *P. aculeata* nutrients into processed foods, mainly into processing techniques. In addition to the nutritional benefits, studies have shown biological effects of adding *P. aculeata* flour to diets. Studies on the use of *P. aculeata* in the diet of rats found an improvement in their intestinal mobility, as well as a reduction in visceral fat and lipid profile, when submitted to a diet containing 30% (w/w) of leaf flour, during a week. The effect observed on intestinal mobility was attributed to the high fiber content in *P. aculeata* flour (around 32%
Another study was also conducted with flour of leaves added in cookies to the diet of overweight men. In the study, the authors observed that the addition of this plant to the diet increased the gastrointestinal health of the subjects, compared to the control group that did not consume flour from the leaves of the plant (Vieira et al. 2019). Vieira et al. (2020) conducted a similar study adding *P. aculeata* flour to a drink offered to women in a prospective, double blind, randomized clinical trial. The authors observed that the drink increased satiety, reducing weight and body fat. According to the authors, this effect may be related to the dietary fiber in *P. aculeata* flour. Fibers can delay gastric emptying and decrease the rate of glucose absorption in the small intestine, contributing to a reduction in feeding and satiety regulation, interfering with the general energy balance, promoting an improvement in body composition. Furthermore, the same authors suggest that a kinetic effect may occur due to the presence of phytochemical compounds, such as polyphenols, which also contribute to the reduction of body fat, either by enzyme inhibition or thermogenesis. Therefore, besides adding nutritional characteristics, *P. aculeata* leaf flour promotes beneficial effects to the body, a functional ingredient to be added to foods.

4. Bioactive compounds and methods to obtain

Souza et al. (2014) studied the essential oils of *P. aculeata* leaves obtained by hydro distillation. The authors identified 30 compounds, with about 29.4% corresponding to oxygenated diterpenes. The essential oils were evaluated in vitro against gram-positive bacteria showing a weak inhibitory activity of these microorganisms. In a subsequent study, the authors detected 24 compounds in essential oils of this plant, of which 44.92% of the constituents were oxygenated sesquiterpenes. The authors attributed to the environmental conditions for plant growth, as well as age or even the method of harvesting or processing the leaves as possible causes for the differences in the composition of essential oils (Souza et al. 2016b).

Pinto et al. (2016) reported that dry leaves of *P. aculeata* were extracted to exhaustion with methanol and the resulting extract was fractionated in hexane. After removing the solvent, this fraction was tested in edema caused in ears of rats. The results demonstrated intense anti-inflammatory activity without clinical signs of topical toxicity. Pharmacological tests suggested a mechanism of action similar to glucocorticoids and were related to sterols in the hexanoic fraction, such as taraxerol, taraxasterol, and phytol.

The authors also showed that the hydromethanolic fraction of the plant methanolic extract, after removing the solvent, has antinociceptive activity at the peripheral and central levels. The authors conducted tests on rats and suggested that the presence of alkaloids and quercetin in this extract fraction is responsible for the effect observed (Pinto et al. 2015a).

Souza et al. (2016b) evaluated antimicrobial properties in petroleum ether, chloroform, and methanol extracts obtained from *P. aculeata* leaves. The solvents of the extracts were evaporated under reduced pressure and the authors found antibacterial and antifungal activity in these products and associated the antioxidant activity to the presence of phenolic compounds. The methanolic extract, with a higher concentration...
of polyphenols, also showed greater antioxidant activity. Garcia et al. (2019) evaluated the phenolic profile of *P. aculeata* leaves after hydroalcoholic extraction. Caffeic acid (\((2R,3R)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3-hydroxybutanedioic\) was responsible for practically half of the concentration of total phenols. Moreover, another phenolic acid was detected and other eight flavonoids derived from caffeic acid. The authors demonstrated its antioxidant and antimicrobial activity against gram-positive and gram-negative bacteria, suggesting a broad spectrum of antibiotic activity.

Pinto et al. (2012) evaluated the cytotoxic effect of extracts from leaves against human promyelocytic leukemia cells and human breast adenocarcinoma cells (HL60 and MCF-7 cell lines, respectively). The leaves were dried and extracted firstly with methanol, followed by extraction with hexane, dichloromethane, ethyl acetate and butanol. The authors performed a phytochemical characterization of the extracts and detected the presence of phenols, flavonoids, and alkaloids. All extracts presented a cytotoxic behavior against the tumor cells studied and showed no cytotoxicity against non-tumor cells. Authors also found that methanolic extract and its fractions did not show cytotoxicity against macrophages with \(IC_{50} > 100 \mu g/mL\), demonstrating good selectivity. Souza et al. (2016b) evaluated the cytotoxic effect of extract of *P. aculeata* leaves on human neuroblastoma cells SH-SY5Y. However, further studies on this topic are important to answer questions about safe dose and possible cytotoxic active components.

No signs of toxicity have been reported in studies conducted with *P. aculeata* leaves for topical use in rats (Pinto et al. 2015a; 2016). In the same way, the ethanolic extract of *Pereskia aculeata* leaves (after removal of the solvent), did not show oral toxicity in rats when administered a single dose of up to 5000 mg per kilogram of body weight (Silva et al. 2017).

Table S2 summarizes the biological activities for the different extracts obtained from *Pereskia aculeata* leaves. These findings reinforce its potential for the development of pharmaceutical products. Therefore, the literature lacks definitions as to the quality assurance control of these extracts. For both the literature lacks definitions and the quality assurance control of these extracts and even quality control should be discussed, as well as what markers are used for it, biological markers directly related to the observed activities, have not been identified yet.

5. Mucilage of *Pereskia aculeata* leaves
Chemical composition, as well as physical-chemical aspects of mucilage, depends on the extraction methods, addressed in this Chapter.

5.1. Chemical composition and extraction methods
Mucilage of *P. aculeata* leaves is composed mainly of complex, highly branched polysaccharides, and arabinogalactans (Martin et al. 2017; Amaral et al. 2019).

Martin et al. (2017) carried out the physical-chemical characterization of this mucilage obtained by aqueous extraction at room temperature. After extraction, the mucilage was precipitated with ethanol and dried at 25 °C, containing 48% (w/w) of total carbohydrates, 19% (w/w) of proteins and minerals containing Na, Mg, P, Ca, Zn, Fe,
Mn, and P. The authors reported that the main mucilage component was type I arabinogalactans, which has a chain of β-D-galactopyranose linked (1/4) replaced by arabinose and galactose units, along with fucose units and partially esterified galacturonic acid. Another important aspect highlighted is the behavior of polyelectrolyte, emulsifier, and stabilizer presented by *P. aculeata* mucilage, attributed to its heterogeneous macromolecular profile and its interfacial adsorption properties.

Lima Junior et al. (2013) developed a process to extract mucilage from *P. aculeata* leaves at low cost and environmentally friendly and viable at industrial scale. Mucilage was extracted by hot water extraction and the authors optimized parameters, such as temperature and raw material/solvent ratio, which showed the best results when 2.46 to 3.70 L of water per kilo of leaves and temperatures between 54.6 and 80°C were used. After extraction, activated carbon was filtered to remove pigments, then the mucilage precipitated with the addition of ethanol and it was dried at 40°C. The mucilage obtained by this process showed 46.88% (w/w) of total carbohydrates, 10.47% (w/w) of minerals such as Ca, K, P, Mg, S, and Fe and 0.44% of uronic acid (w/w). Uronic acid is present in the arabinogalactan structure. In hot extraction, the polysaccharide groups can undergo self-hydrolysis. Martin et al. (2017) showed that the uronic acid content in mucilage was 26% (w/w) for extraction in cold water, which preserves the original polysaccharide structure.

### 5.2. Physical-chemical properties

We characterized thermal stability and microstructure of mucilage obtained from *P. aculeata* leaves. Mucilage loses water at approximately 102.1°C and decomposes at approximately 218.8°C. This material presents a highly porous structure with small particles bonded to the larger ones. Mucilage has a spongy appearance, which is characteristic of materials with hygroscopic behavior and the presence of minerals confirms its polyelectrolyte characteristics (Conceiçao et al. 2014).

Pseudoplastic suspensions were obtained with mucilage dispersed in water at concentrations between 1% and 5% (w/w) and temperatures between 2 and 42°C. The highest mucilage concentration produced the greatest deformability and the highest structural strength. The consistency index increased with increasing mucilage concentration and decreased with increasing temperature. The rheological analyses with frequency scanning allowed the characterization as concentrated solutions up to 3% (w/w) and as a gel, concentration was above 5% (w/w) (Junqueira et al. 2019b).

The use of *P. aculeata* mucilage in the food industry, whether as an emulsifier, thickener, or nutritional value aggregator, is strongly affected by its interaction with the dispersion medium, since its viscoelastic behavior and characteristics in solution can be affected by several factors, such as nature and concentration of other species, temperature, pH, among others. Therefore, the analysis of these effects is important for modeling processes and the proper use of mucilage (Amaral et al. 2018; 2019). Junqueira et al. (2019a) used this approach and studied the behavior of this mucilage in a 20% soy oil emulsion in water (w/w) in the presence and absence of sucrose and/or sodium chloride at pH values 4 and 7. Powdered mucilage was added at a concentration of 2.5 g/100 g and sucrose and NaCl concentrations were 15 and 1 g/100 g, respectively. After 24 hours of preparation, NaCl destabilized the emulsions and
sucrose increased the emulsion viscosity in the continuous phase, which hindered mobility and agglutination of oil droplets. At pH 2, there was a tendency toward destabilization of the emulsions, which corresponded to the isoelectric point of the proteins in the mucilage. Although mucilage was effective in the formation of emulsions with desirable characteristics for application in food, such as high zeta potential, pseudoplastic rheological behavior, and mean diameter smaller than $6.32 \pm 0.16 \mu m$, the presence of sodium chloride or acidic pH depreciates these characteristics, requiring the addition of another stabilizing agent.

Amaral et al. (2019) used the same approach to evaluate how sucrose, NaCl, and calcium chloride influenced thermal stability, rheological behavior, and molecular structure of mucilage. The authors used different model solutions with concentrations ranging from 0% to 5% of mucilage and 0%, 10%, and 20% of sucrose, 0%, 2%, and 4% of sodium chloride, 0%, 0.5%, and 1% of calcium chloride and pH values 3, 7, and 11. The authors observed that the addition of salts and sucrose affected the structure of the exposed mucilage surface. The addition of sucrose or salts did not change the rheological behavior of systems; however, the addition of sodium chloride reduced viscosity. The authors observed that at 20°C, at concentrations up to 1.25%, mucilage presented a Newtonian behavior that became viscoelastic within the concentration range from 2.5% to 5%. The thermal behavior was influenced by mucilage concentration and addition of NaCl or sucrose. The addition of NaCl and sucrose to the system with *P. aculeata* mucilage decreased roughness, indicating that NaCl and sucrose increased the electrostatic repulsive forces between the mucilage molecules. Macromolecules underwent changes in ionization of the structural groups involved in inter and intramolecular interactions induced by these additives.

Junqueira et al. (2018) evaluated the influence of the drying method to obtain mucilage on rheological characteristics and on stability of emulsions. Two processes were studied: drying by freeze drying and heating at 60°C in a vacuum oven. They analyzed emulsions with 10 g of soybean oil and 40 g of reconstituted mucilage with concentrations between 0.5 and 3.0 g/100 g H2O. Emulsions obtained from lyophilized mucilage showed higher viscosities and the drying process did not affect stability of emulsions.

Lago et al. (2019) studied stability of soybean oil and water nanoemulsions with *P. aculeata* mucilage prepared with ultrasound. The process parameters were optimized for 5 min of sonication time with an ultrasound power amplitude of 90%. Concentrations below 5% (w/w) soybean oil and between 1% and 1.5% (w/w) mucilage showed the best results for the formation of nanoemulsions. The emulsion containing 1% (w/w) soybean oil and 1.5% (w/w) mucilage showed the best stability, remaining stable for 30 days of storage. In their study, all colloidal systems presented the Newtonian behavior.

Studies on *P. aculeata* mucilage demonstrate its capacity as an emulsifier, strengthening and encouraging the perspective of its use in formulations of food products.

### 6. Development of new products

Despite the growing interest and research on *P. aculeata* in recent years, few studies have explored its constituents for applications in the development of new products, either exploring the mucilage characteristics or its bioactive compounds.
The first studies report on arabinogalactan complexes obtained from *P. aculeata* with metals, such as Co, Cu, Mn, and Ni. Merce et al. (2001b) characterize these complexes and metal ions are bonded to the polymeric network. Arabinogalactan is an edible biopolymer and the characterization of Fe ions highlights the possibility of using these complexes as carriers of metal ions, essential for the human body (Merce et al. 2001a). In addition, Maciel et al. (2020) developed colloidal microparticles based on chitosan and pectin, using aqueous extract of *P. aculeata* leaves as Fe source. Microparticles can be an innovative strategy to fortify food, since nutritional Fe deficiency is a frequent health problem worldwide.

Arabinogalactan extracted from *P. aculeata* leaves was also added to reconstituted bacterial cellulose films, in a study conducted by Lucyszyn et al. (2016). The incorporation of hydrocolloid obtained films with better mechanical properties in relation to the original material also with good viability and cell adhesion.

Regarding the exploration of bioactive compounds in *P. aculeata* leaves, Pinto et al. (2016) developed a gel for pharmaceutical applications. The gel basis was composed of the methanolic extract of *P. aculeata*, as well as the fraction obtained in hexane from this extract. The gels showed anti-inflammatory properties and were efficient in accelerating wound healing in rats. In subsequent studies, the authors developed an herbal cream for clinical practice, containing hexane fraction of *P. aculeata* leaves as an active ingredient. The anti-inflammatory activity of the cream was similar to dexamethasone and some authors have suggested its use in the treatment of psoriasis (Pinto et al. 2020). All of these preliminary tests were performed on rats.

The use of mucilage extracted from *P. aculeata* leaves in blends composed with gum Arabic and guar gum has been studied in fermented milk drinks to replace fat and ensure quality during shelf life. Amaral et al. (2018) concluded that 70% of *P. aculeata* mucilage and 30% of guar gum presented better performance, with higher values of apparent viscosity.

Oliveira et al. (2019) obtained compact, flexible, smooth, dark-colored films from mucilage, with potential use in packaging for food sensitive to light. As mucilage did not present toxicity, the film could be used in edible packaging. The best characteristics found, namely lower permeability to water vapor, higher elongation at break, and higher Young’s modulus, were obtained in films prepared from a solution with 1.5% (w/w) mucilage and 20% (w/w) glycerol. Despite the promising results, further studies are needed to improve the mechanical and barrier properties of films for the development of intelligent and/or edible packaging.

Mucilage isolated of *P. aculeata* leaves was used by Neves et al. (2020a) to obtain microcapsules with whey protein. The microparticles were efficient to encapsulate alpha tocopherol. Encapsulation of bioactive compounds with low solubility in aqueous media is of great interest for the pharmaceutical and food industries.

7. Conclusion and perspectives
The nutritional aspects of *P. aculeata* leaves with their high content of proteins, fibers, and minerals are promising for the development of food products. The isolation of these compounds, as well as their processing and incorporation in food, still requires
applied research. In addition, the use of complexes or microparticles to transport minerals requires studies on the viability for industrial application.

The bioactive compounds obtained from *P. aculeata* leaves showed promising anti-inflammatory and anticancer properties without negative effects on normal cells or signs of toxicity for ingestion or topical use. This fact can stimulate the exploration of this plant to obtain pharmaceutical products in the next years. In this sense, it is necessary to develop pharmacological quality controls, such as chemical markers. The anti-inflammatory topical activity of *P. aculeata* leaves has already been evaluated in animals and clinical tests for use in humans are expected to be developed.

The heterogeneous macromolecular profile and the interfacial adsorption properties of mucilage of *P. aculeata* leaves make it a natural alternative source to synthetic emulsifiers. Blends with other natural emulsifiers emerge as alternative for use in processed foods. Research on composition of these blends, determining expiration date, and the sensory analysis are still necessary for their application.

Films obtained with mucilage of *P. aculeata* leaves as the main constituent have shown potential to obtain functional edible packaging and future studies should investigate their mechanical and barrier properties.

Type I arabinogalactan is the main component of mucilage of *P. aculeata* leaves, containing units of galacturonic acid in the side chain. This functional group can promote interactions with other biopolymers. Cold extraction preserves these functional groups in the polysaccharide chain. The addition of this mucilage to the bacterial cellulose for film formation improved its mechanical properties. Few studies have reported on the interaction of mucilage with other biopolymers, which is a topic with potential to be explored to obtain new products.

Mucilage is efficient to obtain microcapsules and encapsulation of active ingredients to protect microcapsules and improve their availability is an active field of investigation.

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