Research, Society and Development, v. 11, n. 6, e55211629315, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i6.29315

Ora-pro-nobis - chemical characterization and sourcing of crude extract through different extraction methods: a review

Ora pro nóbis – caracterização química e obtenção do extrato bruto através de diferentes métodos de extração: uma revisão

Ora pro nobis – caracterización química y obtención del extracto bruto através de diferentes métodos de extracción: una revisión

Received: 04/18/2022 | Reviewed: 04/26/2022 | Accept: 05/06/2022 | Published: 05/10/2022

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Abstract
Ora-pro-nobis has its center of origin in tropical America. It is found in Brazil, from the Northeast to Rio Grande do Sul. The plant belongs to the Cactaceae family, Pereskia genus. Its leaves and fruits contain bioactive compounds that may have expectorant properties and can be used to alleviate inflammatory processes in traditional medicine. Despite its richness, the plant is still little used by the general population and the food and pharmaceutical industries. Some extraction methods are used to obtain bioactive compounds from crude plant extracts, such as maceration, supercritical fluid, and ultrasound-assisted extraction, which have stood out for meeting the principles of green chemistry. The chemical characterization of the extracts obtained through chromatographic analysis by GC-MS/MS and LC-MS/MS proves to be extremely effective, as it allows quantifying the various chemical species present in the extract, making it possible to evaluate and compare the results generated in each chosen extraction condition. The objective of this research is to conduct a review study about the *Pereskia aculeata* Miller plant. To this end, an exploratory study was carried out, by means of theoretical surveys through Science direct, Capes, Scielo and Pubmed databases. Extracting from plants using different methods is an important step for the industry, ensuring improvement in the quality of products, especially in the food and pharmaceutical sectors. In this sense, no comparative study was found in the literature employing different extraction methods for ora-pro-nobis in the sense of identifying the best method, one with better yield and greater amount of bioactive compounds. The search for different extraction strategies integrated with processes and products that can extract intelligently, minimizing waste, shows an innovative potential in future research.
Keywords: Chromatographic analysis; Bioactive compounds; Extraction; *Pereskia aculeata* Miller.

Resumo
A ora-pro-nóbis tem o seu centro de origem a América tropical. Encontra-se no Brasil desde o Nordeste até o Rio grande do Sul. A planta pertence à família Cactaceae, ao gênero Pereskia. Suas folhas e frutos contem compostos bioativos que podem apresentar propriedades expectorantes e podem ser utilizados no abrandamento de processos inflamatórios na medicina tradicional. Apesar de sua riqueza, a planta ainda é pouco utilizada pela população geral e indústrias alimentícia e farmacêutica. Alguns métodos de extração são utilizados para obter compostos bioatvos do extrato bruto de plantas, tais como, maceração, fluído supercrítico e extração assistida por ultrassom que têm se destacado por atenderem aos princípios da química verde. A caracterização química dos extratos obtidos através da análise cromatográfica por CG-MS/MS e LC-MS/MS prova-se extremamente eficaz, pois, possibilita a quantificação das variadas espécies químicas presente no extrato, permitindo avaliar e comparar os resultados gerados em cada condição de extração escolhida. O objetivo deste trabalho é realizar um estudo de revisão acerca da planta *Pereskia aculeata* Miller. Para isto, foi realizado um estudo exploratório, por meio de levantamento teóricos em base de dados Science direct, Capes, Scielo, Pubmed. Realizar a extração de plantas por diferentes métodos, é um passo importante para a indústria, assegurando melhoria na qualidade de produtos, principalmente no setor alimentício e farmacêutico. Neste sentido, não foi encontrado na literatura estudo comparativo de diferentes métodos de extração da ora-pro-nóbis, identificando melhor método, com melhor rendimento e maior quantidade de compostos biotivos. A busca de estratégia de extrações diferentes, que estejam integradas a processos e produtos que consigam extrair de forma inteligente, minimizando desperdícios, demonstra um potencial inovador em pesquisas futuras.
Palavras-chave: Análise cromatográfica; Compostos bioativos; Extração; *Pereskia aculeata* Miller.

Resumen
La ora-pro-nóbis tiene su origen en la América tropical. Se encuentra en Brasil desde el noreste hasta Rio Grande do Sul. La planta pertenece a la familia Cactaceae, al género Pereskia. Sus hojas y frutos contienen compuestos bioactivos que pueden tener propiedades expectorantes y pueden usarse para mitigar procesos inflamatorios en la medicina tradicional. A pesar de su riqueza, la planta todavía es poco utilizada por la población en general y las industrias alimentaria y farmacéutica. Se utilizan algunos métodos de extracción para obtener compuestos bioactivos a partir del extracto crudo de plantas, como la maceración, el fluido supercrítico y la extracción asistida por ultrasonidos que se han destacado por cumplir con los principios de la química verde. La caracterización química de los extractos obtenidos mediante análisis cromatográfico por CG-MS / MS y LC-MS / MS resulta sumamente eficaz, ya que permite la cuantificación de las distintas especies químicas presentes en el extracto, permitiendo la evaluación y comparación de los resultados generados en cada condición de extracción elegida. El objetivo de este trabajo es realizar un estudio de revisión sobre la planta *Pereskia aculeata* Miller. Para ello, se realizó un estudio exploratorio, a través de un relevamiento teórico en la base de datos Science direct, Capes, Scielo, Pubmed. Se concluye que la extracción de plantas por diferentes métodos es un paso importante en las industrias, asegurando la mejora en la calidad de los productos, así como no existe un estudio comparativo de diferentes métodos de extracción de ora-pro-nóbis, identificando un mejor método con óptimas Producir. La búsqueda de diferentes estrategias de extracción, que se integren con procesos y productos que puedan extraer de manera inteligente, minimizando los residuos, demuestra un potencial innovador en futuras investigaciones.
Palabras clave: Análisis cromatográfico; Compuestos bioactivos; Extracción; *Pereskia aculeata* Miller.

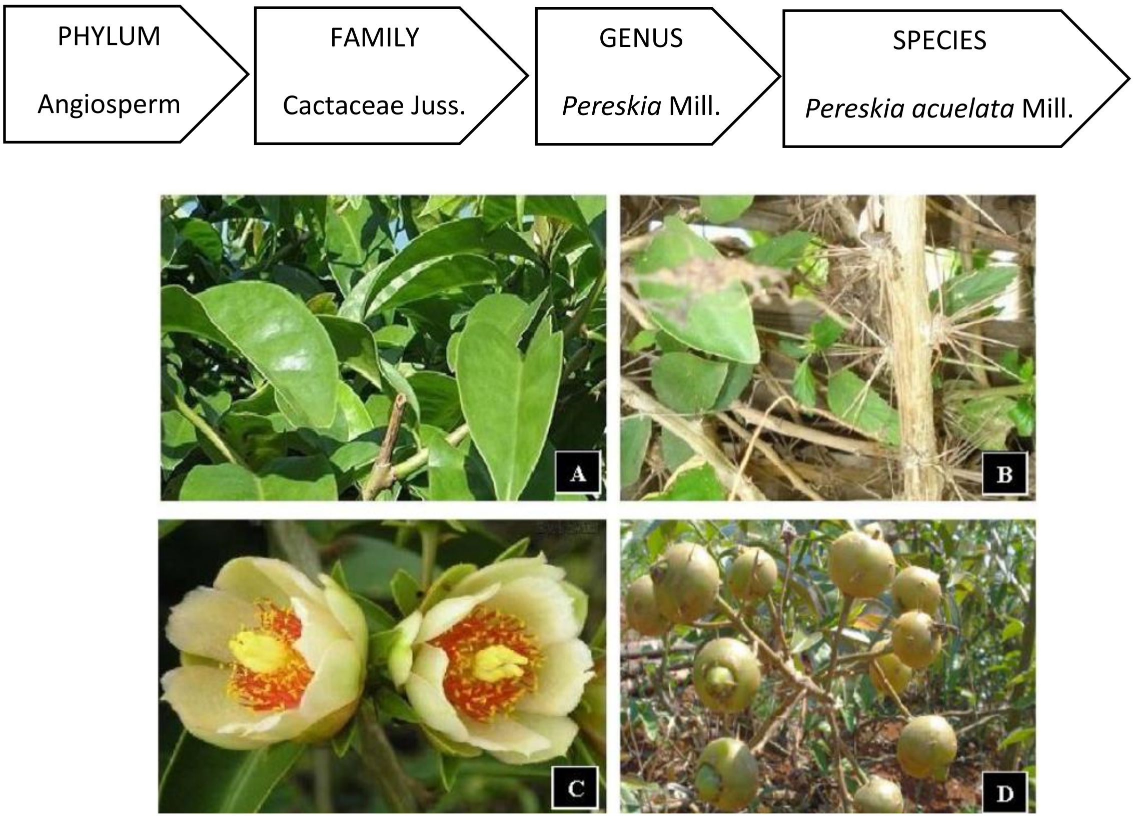
1. Introduction

Pereskia aculeata Miller, known in Brazil as ora-pro-nóbis (OPN) and other medicinal plants, have been widely used in traditional medicine (Hao, 2019). Due to their chemical composition with high protein and vitamin content, as well as mineral salts, they are linked to health maintenance (Manaf, *et al*., 2016). Non-conventional food plants, called PANCs, are among them; they are not produced on a commercial scale and are still little explored in Brazil, despite the country's intense biodiversity (Moraes *et al*., 2021; Moraes *et al*., 2020; Santos *et al*., 2015; Miranda *et al*., 2009; Souza *et al*., 2009). PANCs include *Pereskia aculeata* Miller, popularly known in Brazil as *ora-pro-nobis*. (Brasil, 2010; Trentin *et al*., 2020).

The term PANC (*Planta Alimentícia Não Convencional*, Non-Conventional Food Plant) was created in 2008 by the biologist, Professor and Doctor Valdely Ferreira Kinupp and is link to a food variation potential, with a reduction in environmental impacts and enhancement of natural resources. PANCs are characterized by fruits, leaves, flowers, rhizomes, and other parts of plants of native or exotic species, that can be consumed by humans in the Brazilian territory, be they found in nature or domesticated and grown commercially (Kinupp & Lorenzi, 2014). Some of these plant species have cultural, ecological and economic importance (Kinupp& Barros, 2008). PANCs also play the role of functional foods, as they are composed of essential vitamins, antioxidants, minerals, in addition to fiber, that is, all important nutrients for good health maintenance (Kelen *et al*., 2015). Among the PANCs, *Pereskia aculeata* Miller can be mentioned.

Pereskia aculeata Miller (Figure 1) is composed of leaves, flowers, stem and fruits and has the following botanical classification: the plant belongs to the Plantae Kingdom, Magnoliopsida Class, Caryophyllales order, Cactaceae Family, *Pereskia* genus (Kinupp, 2008).

Figure 1. Taxonomic hierarchy of the *Pereskia aculeata* Miller species. Ora-pro-nobis: A – Leaves; B – Lignified stem with needle-shaped thorns; C – Flowers and D – Unripe fruits.



Source: Adapted from Zappi *et al.*, (2020); Santos *et al.*, (2015).

Plants belonging to the Cactaceae family usually have a desert and semi-desert natural habitat, which provides them with peculiar adaptations that are important for survival, due to the scarcity of water and nutrients, allowing them to be perennial even when adhered to these Xerophytic environments (Altesor & Ezcurra, 2003). Some of their defense mechanisms are associated with phytochemical compounds present in their structures, such as alkaloids, flavonoids, terpenes and tannins, all substances associated with biological activities (Harlev *et al*., 2012).

Ora-pro-nobis contains 20% of nutrients in its leaf mass, depending on its cultivation. Large amounts of amino acids, such as lysine and tryptophan, are found in it (Kazama *et al*., 2012). It is rich in soluble fiber, important for the digestive and intestinal process. It contains vitamins A, B and C, important for the immune process, eyes and skin, in addition to minerals, such as iron, calcium and phosphorus (Vicente *et al*., 2020; Francelin *et al*., 2021).

Studies have evidenced the characterization of several chemical compounds from the crude extract of *Pereskia aculeata* Miller. Bioactive compounds, such as tannins, flavonoids, phenolics and trypsin inhibitors, have been identified (Cruz *et al*., 2021; Ngo *et al*., 2017; Almeida *et al*., 2014), including α-carotene, β-carotene, lutein, stigmasterol, α-cryptoxanthin, β-cryptoxanthin and Sitosterol (Cruz *et al*., 2021; Agostini-Costa *et al*., 2014).

Some methods used to obtain the crude extract of plants and separate bioactive compounds are: supercritical fluid, maceration, hydrodistillation and ultrasound-assisted technique (Sarvin *et al*., 2018). Each method has different specifications regarding the extractive process, but all methods have characteristics that meet the principles of Green Chemistry, due to the use of solvents with lower environmental impact and/or in reduced volumes (Oliveira *et al*., 2016; Knez *et al*., 2014; Brasil, 2010).

Chromatographic analysis is extremely important for the chemical characterization of the crude extract sourced from the plants, as it separates, identifies and allows quantifying the various chemical species contained in the extract, making it possible to evaluate and compare the results generated in each chosen extraction technique and condition (Naczk and Shahidi, 2004; Collins *et al*., 2006; Garmus *et al*., 2015; Song *et al*., 2019).

Therefore, the objective of this research was to conduct a review study on the *Pereskia aculeata* Miller plant, describing its botanical characteristics, chemical composition, extraction methods used to obtain its crude extract, in addition to performing a chemical characterization from the extract in order to specify the main compounds obtained in each extractive process.

2. Methodology

To carry out this review study, original full texts of articles, theses or dissertations, written in English, Portuguese and/or Spanish, with free and/or paid access, were selected using the methodology Preferred Report Items for Systematic Reviews and Meta-analyses. The consultations for setting up the database for this article were done on these platforms: Science Direct, Scielo, Pubmed, Redaly and *Portal de Periódicos Capes* (https://www.periodicos.capes.gov.br). A search for more recent works was established.

The following descriptors were used to search for data: *Pereskia aculeata* Miller, *ora-pro-nobis,* extraction methods, Maceration, supercritical fluid, ultrasound extraction, liquid chromatography, gas chromatography, chemical characterization, solvents and biodegradable solvents. The criteria chosen for exclusion were: articles published before 2010, undergraduate course papers, papers without defined language. After research, the articles were tabulated in order of author, which methods were used in their research, how the chemical characterization of the compounds was carried out, and which were the main compounds found in each work.

3. Results and Discussion

After researching the scientific literature, the articles were tabulated in order of author, which methods were used in their research, how the chemical characterization of the compounds was carried out, and which were the main compounds found in each work. Thus, 10 articles were found, evidencing different approaches regarding the extraction and chemical characterization of *Pereskia Aculeata* Miller in obtaining bioactive compounds, proteins and carbohydrates, as shown in Table 1.

The *Pereskia* genus is considered the least advanced of the Cactaceae family; it has 17 species divided into two sub-groups, distributed mainly in the regions between Brazil and Mexico (Sharif *et al*., 2013). These plants contain woody stems, juicy leaves and flowers distributed in terminal summits (Souza *et al*., 2009; Kinupp, 2007). *Pereskia aculeata* Miller is considered a scrambling shrub; its leaves (Figure 1A) are green, have a symmetrical, elliptical shape, and a leathery texture (Rosa & Souza, 2003). Its stem (Figure 1B) have needle-shaped thorns, which, in the older branches, grow in clusters (Brasil, 2010). Its flowers (Figure 1C) are small and white and have fruits (Figure 1D) with seeds immersed in a gelatinous mass, with a color that varies between yellow and red when ripe, and green when unripe (Rosa & Souza, 2003).

Table 1. Comparison between different extraction methods, chemical characterization and main compounds obtained from *ora-pro-nobis*, by different authors.

AUTHOR	EXTRACTION METHOD	CHEMICAL CHARACTERIZATION	MAIN COMPOUNDS
Cruz *et al*., 2021	Soxhlet/ Lyophilization	Liquid Chromatography (HPLC/DAD/UV)	Flavonoids; Caffeic Acid; Rutin, Quercetin
Garcia *et al*., 2019	Shaker/ Lyophilization	Liquid Chromatography (LC-MS)	Caffeic acid; Quercetin-3-O-rutinoside; Isorhamnetin-O-pentoside-O-rutinoside
Hoscher *et al*.,2019	Clevenger device	Gas Chromatography (GC-MS)	Phytol, isotorquatone, 2-hexyl-(E)-cinnamaldehyde, linoleic acid
Didini, 2019	Bain-Marie	Liquid Chromatography (HPLC-DAD-MS)	Caffeic acid; Quercetin-3-O-rutinoside
Francisco, 2018	Crushing	Liquid Chromatography (LC-MS)	Aspartate; Threonine; Serine; glutamate
De Souza et *al*., 2017	Maceration/Soxhlet	Thin-Layer Chromatography (TLD)	Phenols; flavonoids; catechins; alkaloids.
Souza *et al*., 2016	Maceration	Gas Chromatography (GS-MS)	Phytol; linoleic acid; Methyl Linoleate
Martin *et al*., 2015	Soxhlet	Gas Chromatography (GC-MS) and Liquid Chromatography (HPLC)	L-Arabinose; D-Galactose; L-Rhamnose; D-Galacturonic Acid
De Souza, 2014	Lyophilization	Liquid Chromatography (LC-MS)	Chlorogenic Acid; Caffeic Acid; Ferulic Acid
Souza *et al*., 2014	Crushing	Gas Chromatography (GS-MS)	1-Tetradecene; 9-Decenylacetate; (E)-β-Ionone; n-Pentadecane
Agostini-Costa *et al*., 2014	Maceration	Liquid Chromatography (HPLC-PAD)	Lutein; α-cryptoxanthin/ Zeinoxanthin; β-cryptoxanthin; α-carotene

Source: Authors (2022).

The *Pereskia aculeata* Miller species is known by several names in Brazil, such as *groselheira-de-barbados*, *carne-de-pobre* and *lobrobó*, with *ora-pro-nobis* being the most common one (Brasil, 2015; Kinupp & Lorenzi, 2014). The plant is easily grown, as it is rustic, perennial and propagates easily. Its planting method is the use of cuttings, for providing greater efficiency, shortening its production cycle (Trentin *et al*., 2020). Rooting strength, in addition to the quality and quantity of cuttings (with the latter being healthy and vigorous matrices), varies according to species type, environmental conditions, growing method, besides the internal condition of the plant itself (Madeira *et al*., 2016).

The leaves, flowers, stem and fruits of *ora-pro-nobis* can be used in food preparation (Kinupp & Lorenzi, 2014), and for medicinal purposes, as it confers emollient power, assists in inflammatory treatments, osteoporosis, iron deficiency anemia, hemorrhoids, varicose veins, intestinal problems, skin diseases, in addition to having an antifungal and antibacterial function (Souza *et al*., 2016).

Ora-pro-nobis contains 20% of nutrients in its leaf mass, depending on its cultivation. In general, it is rich in proteins, fibers and essential amino acids such as lysine and tryptophan (Kazama *et al*., 2012), with highlight to lysine, since the content found is higher compared to that of other leafy vegetables such as lettuce, cabbage and spinach (Almeida *et al*. 2014).

In the work described by Taco (2011), 100 g of black beans contain 4.5 grams of protein, while 100 grams of crushed leaves in dry weight of *ora-pro-nobis* has 18.95 grams of protein, revealing a higher value when compared to beans (Francelin *et al*., 2021). In the study by Almeida *et al*. 2014, the authors found a content of 28.99 g/100 g of total proteins in the flour from *ora-pro-nobis* leaves. According to the WHO (World Health Organization), 15% of the daily human diet must come from proteins, which is equivalent to 40 g day; thus, with approximately 200 gof the plant, it is possible to almost meet the daily protein need.

For food to be considered rich in fibers that are important for the digestive and intestinal process, it must have 6 grams of fiber/100 grams of product (Brasil, 2010). According to Takeiti *et al*. 2009, the content of soluble fibers found in the dried leaves of *ora-pro-nobis* was 5.2 g/100 g, which is close to the indicated value.

Considering the microelements, some studies point to *ora-pro-nobis* leaves as good sources of minerals in the daily diet. The studies by Takeiti *et al*. 2009 and by Almeida *et al*. 2014 have found high iron levels, with 14.2 and 20.56 mg/100 g, which are classified as superior to the levels of spinach and other leafy vegetables. The same studies have also found 3420 mg/100g (Takeiti *et al*., 2009) and 1346.67 mg/100 g (Almeida *et al*., 2014) of calcium in dry leaves of the plant, which are higher than the values found in whole milk powder (570 mg/100 g), Minas Frescal cheese (579 mg/100 g), Parmesan 992 mg/100 g, according to the Taco (2011).

In addition, the leaf mass of *ora-pro-nobis* has vitamins A, B and C, important for the immune process, eyes and skin (Vicente *et al*., 2020; Francelin *et al*., 2021). The absence of foliar toxicity makes it an important and useful food (Almeida-Filho and Cambraia, 1974), characterizing a complementary source in the diet of many Brazilians (Almeida *et al*., 2014; Takeiti *et al*., 2009).

Some methods used to obtain the crude extract of plants and separate bioactive compounds are: supercritical fluid, maceration, hydrodistillation and ultrasound-assisted technique (Cruz *et al.*, 2021; Ojha *et al*., 2020; FERREIRA *et al*., 2020; Sarvin *et al*., 2018; Bernardo *et al*., 2016; Boukroufa *et al*., 2015). Each method has different specifications regarding the extractive process, but all methods have characteristics that meet the principles of Green Chemistry, due to the use of solvents with lower environmental impact and/or in reduced volumes (Oliveira *et al*., 2016; Knez *et al*., 2014; Brasil, 2010).

Extraction by hydrodistillation is very common and recommended by the World Health Organization (WHO), due to its low cost and practicality. To carry out this method, Clevenger or Soxhlet devices are used; they are attached to flasks, where the plant material is inserted together with an amount of distilled water that, after boiling, produces steam containing the volatile compounds; the steam is then dragged to the condenser, and from this stage, the extract and vapor condense and separate by density (Cruz *et al.*, 2021; Venskutonis, 2003; Jantan *et al*. 2003).

Ultrasound-assisted extraction is based on the propagation of ultrasonic waves and of the resulting cavitation forces; the bubbles that are created can clash intensely and generate local pressure, causing tissue rupture, thus favoring the release and penetration of solvents into intra-cellular layers of the plant, increasing their extraction rate (Knorr *et al*., 2004; Shalmashi, 2009; Goula, 2013; Santos *et al.*, 2015). The advantage of this extraction method is the simplicity of the equipment, reduced volumes of solvents used, and/or use of biodegradable solvents, and the possibility of using different solvents for the process, in addition to low cost and extraction time (Cruz *et al*., 2021; Freitas *et al*., 2021; Brum & Arruda, 2009).

The supercritical fluid extraction (SFE) method can be defined as the solubilization of compounds from a solid or liquid matrix in a solvent under supercritical conditions. Solvents are used above their critical points to extract soluble components from a mixture. That is, this method is used in conditions of temperature and pressure above the critical point, ensuring high solvating power and diffusion coefficient, low surface tension and viscosity. Environmental benefits are added to that, as CO_2 is used as a solvent, which is considered a cleaner process for applying Green Chemistry (Freitas *et al*., 2021; Silveira *et al*., 2012; Tian *et al*., 2012; Santos *et al*., 2015; Knez *et al*., 2014). According to Da Silva *et al*. (2016), when the SFE method is performed, applied to natural products, the latter will have the following as main bioactive compounds: antioxidant compounds (41% of studies), compounds with antibacterial activity (10% of studies), and antitumor compounds (18% of studies).

The Maceration extraction technique explores the phenomenon of solvent diffusion through the plant tissue. In this procedure, the botanical material must be divided into small fragments and subjected to contact with the solvent for a determined time, which can vary from three hours to three weeks, at room temperature. It is necessary to revolve them in a determined time and, at the end, filter and press them. The process is non-selective, slow, unfeasible for extracting all of the active ingredient, but preliminary to other extraction processes such as: percolation, infusion and decoction (Zanella *et al.*, 2021, Handa, 2008; ANVISA, 2010). As for its types, maceration can be static or dynamic. In the first case, the contact of the solvent with the plant fragments is made for an established time and at rest. In the case of dynamic maceration, the mixture for extraction is agitated for a certain time, which can increase the extraction yield (ANVISA, 2010). The advantage of this technique is the low cost and ease of operation (Zanella *et al.*, 2021, Handa, 2008).

After the crude extract of *ora-pro-nobis* is obtained using the extractive methods described above, chromatographic analysis is extremely important for its chemical characterization, as it separates, identifies and allows quantifying the various chemical species present in the extract, making it possible to evaluate and compare the results generated in each chosen extraction technique and condition (Naczk & Shahidi, 2004; Collins *et al*., 2006; Garmus *et al*., 2015; SONG *et al*., 2019). It is a physical and chemical method that separates the components of a mixture, consisting of a mobile and a stationary phase (Collins *et al*., 2006). Two chromatographic analysis methods can be used for this characterization: GC-MS (Gas Chromatography coupled with the detector by Mass Spectrometry, and LC-MS (High-Performance Liquid Chromatography coupled with Mass Spectrometry).

Compared with other types of chromatography, gas chromatography has excellent power to separate or resolve the volatile and thermally stable constituents of a mixture, allowing for the analysis of dozens of substances present in the sample. It also has a high degree of sensitivity in detecting components, allowing the study of substances in concentrations of the nanogram order. As an important characteristic, the technique requires small amounts of sample for analysis, representing a great advantage in the use of this analytical method (Skoog, 2006; Collins *et al*., 2006).

Liquid chromatography is one of the most used techniques for separation, identification and quantification of phenolic compounds (Naczk &Shahidi, 2004). With technological advances, it was possible to use support (stationary phase) with very small particles (diameter < 2.0 μm) that can withstand high pressures. Due to the use of columns with high separation capacity (stationary phase) combined with the liquid mobile phase, the technique requires the use of specific equipment, resulting in analyses with better resolution, faster separations, lower consumption of mobile phase, less amount of waste generated, and lower cost with solvent, being a suitable method for separating ionic species and/or macromolecules (Peres, 2002).

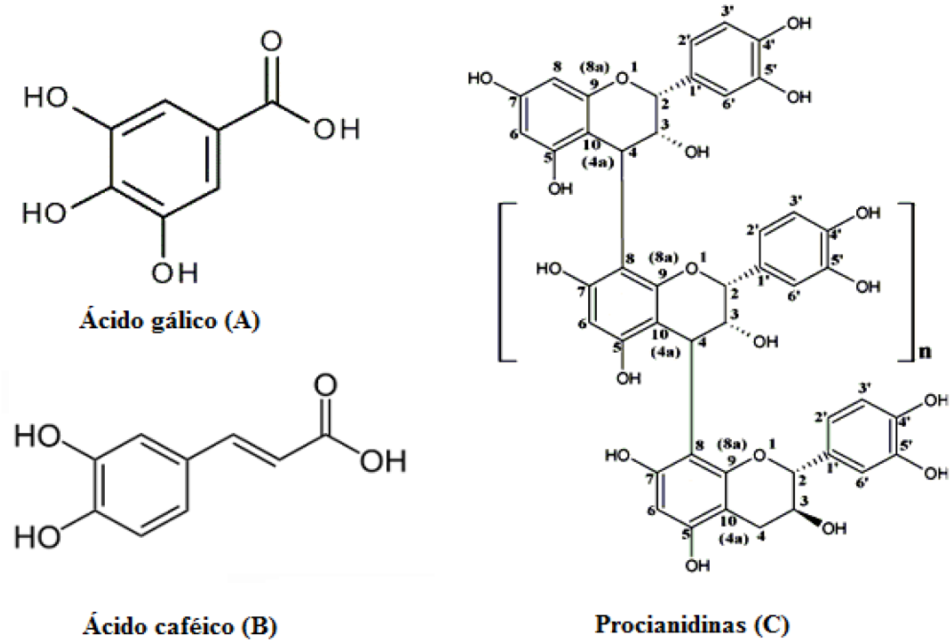
Studies show chemical compounds characterized from the crude extract of *Pereskia aculeata* Miller. Bioactive compounds, such as tannins, flavonoids, phenolics, trypsin inhibitors, have been identified (Cruz *et al.*, 2021; Almeida *et al*., 2014), including α-carotene, β-carotene, lutein, stigmasterol, α-cryptoxanthin, β-cryptoxanthin and Sitosterol (Agostini-Costa *et al*., 2014).

Food bioactive compounds (FBAs) are defined as extra nutritional compounds generally present in small amounts in foods that vary widely in chemical structure. FBAs are divided into different classes, with polyphenols, carotenoids and glucosinolates being the three main groups present in everyday human diet (Horst *et al*., 2016).

According to Sujhata et al. (2017), bioactive compounds usually have the following characteristics: they play a part in antimicrobial, anti-inflammatory, anticancer, androgenic and dermatogenic activities, are hypocholesterolemic and cholesterolic, have antioxidant and antitumor properties, and are antispasmodic, vasodilators, antidiabetic, hepatoprotective, hypoglycemic and antihepatotoxic.

Among bioactive compounds, phenolic compounds can be highlighted, which are molecules containing a phenol group (functional hydroxyl group in aromatic ring) and can have a high antioxidant content. These aromatic molecules act as defense agents to the stresses caused to vegetables and fruits, giving greater astringency, color and flavor to these foods (Arnoso *et al*., 2019). Phenolics make up a group with widely diversified structures, with more than 8000 molecules currently known, which can range from simple phenolic molecules, such as gallic acid (Fig. 2A) and caffeic acid (Fig. 2B), to highly polymerized compounds, such as procyanidins (Fig. 2C) (Cheynier, 2012).

Figure 2. Structural formulas of the phenolic compounds: A – Gallic acid; B – Caffeic acid; and C – Procyanidins.



Source: Adapted from Azmir *et al*. (2013); Cong-Cong *et al*., (2017); Gonçalves *et al*., (2019).

Garcia *et al*. (2019) performed an advanced chemical characterization of the organic leaves of *Pereskia aculeata* Miller. The authors identified a total of ten phenolic compounds in the hydroethanolic extract of the leaves: two phenolic acids (caffeic acid derivatives) and eight flavonoids (quercetin, kaempferol, and isorhamnetin glycoside derivatives). Caffeic acid was the major compound (accounting for more than 49% of the phenolic content of the extract), followed by quercetin-3-O-rutinoside (14.99%) and isorhamnetin-O-pentoside-O-rutinoside (9.56 %). This extract also presented important biological activities (antioxidant and antimicrobial potential) and had its absence of toxicity proven (Gonçalves *et al*., 2019).

In Hoscher *et al*. (2019), the authors performed the chemical characterization of *ora-pro-nobis* leaves through gas chromatography coupled with mass spectrometry to obtain essential oil. Through the analysis, they identified twenty substances, including the classes of sesquiterpenes (41.17%), diterpenes (5.88%) and phenylpropanoids (23.5%). The detailed phytochemical study revealed that the major substances were phytol, isotorquatone, 2-hexyl-(E)-cinnamaldehyde and linoleic acid. Furthermore, the authors compared different methods for drying the leaves of the plant as to the yield of essential oil obtained.

However, despite extensive research on the crude extract and essential oil of *Pereskia aculeata* Miller, there is a lack of studies comparing different extraction methods by means of different solvents, with chemical characterization by gas and/or liquid chromatography, in order to optimize the procedure to source specific bioactive compounds. Additionally, there is a lack when it comes to the use of the ultrasound-assisted and supercritical fluid extraction methods, which are very effective and important methods for the application of Green Chemistry, due to the use of solvents of lower environmental impact and/or in reduced volumes (Oliveira *et al*., 2016; Santos *et al*., 2015; Knez *et al*., 2014; Brasil, 2010).

4. Final Considerations

The review of the articles analyzed in this paper highlights *ora-pro-nobis* as a great potential in the food and pharmaceutical industrial sectors. It is important to understand which extractive method is most effective in extracting bioactive, phenolic and antioxidant compounds. The best alternative to be chosen is one that combines product extraction processes with low energy consumption, reduced unit operations, lower consumption of conventional solvents and/or use of biodegradable solvents that promote greater yield, thus developing a cleaner and more efficient extraction condition, providing the industrial sector with innovative technologies that present such characteristics. Therefore, seeking different extraction strategies, integrated with clean-technology processes, shows an innovative potential in future research.

Acknowledgments

The authors would like to thank Brazilian National Council for Scientific and Technological Development – CNPq (Process no. 308505/2018-2), as well as the Coordination for the Improvement of Higher Education Personnel - CAPES and the research support of the Cesumar Institute of Science, Technology and Innovation - ICETI and the Cesumar University – Unicesumar.

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