Biotecnia
Journal of biological and health sciences
http://biotecnia.unison.mx
Universidad de Sonora
ISSN: 1665-1456

Original Article

Phytochemical profile of *Parmentiera aculeata* (Kunth) fruit: a rich source of nutraceutical and nutrimental compounds

Perfil fitoquímico del fruto de *Parmentiera aculeata* (Kunth) Seem: una fuente rica de compuestos nutracéuticos y nutrimentales

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Abstract

The fruit of *Parmentiera aculeata* has been used locally for its medicinal properties, but its bioactive compounds had not been characterized. Thus, to identify and quantify its nutraceutical and nutritional compounds, we used UPLC-Q-TOF-MS and untargeted metabolomics, detecting 725 metabolites, including anticancer compounds (genkwanin, hespiridin, and phenylketaldoxime) and antidiabetics (metformin). We also used HPLC-DAD to quantify the phenolics cyanidin, catechin, and *p*-coumaric, 4-hydroxybenzoic, gallic and chlorogenic acids (50, 70, 80, 210, 220 and 340 μg·g^{-1} respectively) and GC-MS to analyze the vitamin profile (*β*-carotene content < 2.7 ng·mg^{-1}). Fructose, glucose and sucrose were quantified by HPLC-ELSD at 3.06, 2.77 and 78.0 mg·100 g^{-1}, respectively. Atomic emission spectra showed the content of K, Ca, P, Mg, Na, Fe, Zn to be 13 261.37, 1 114.77, 1 048.27, 932.06, 300.53, 22.03, 27.27 mg kg^{-1}, respectively. Palmitic, heptadecanoic, stearic, oleic, linoleic, arachidic, linolenic, heneicosanoic, tricosanoic were identified and quantified as the primary fatty acids using a mixture of 37 standards. These findings corroborate the medicinal, nutraceutical and nutritional contributions of this versatile fruit that show their potential as a high value-added edible crop.

Keywords: Fruit quality, kat cucumber, phytochemistry, traditional medicine, untargeted metabolomic profile.

RESUMEN

El fruto de *Parmentiera aculeata* se ha utilizado localmente por sus propiedades medicinales, pero no se han caracterizado sus compuestos bioactivos. Así, para identificar y cuantificar sus compuestos nutracéuticos y nutricionales, utilizamos UPLC-Q-TOF-MS y metabolómica no dirigida, detectando 725 metabolitos, incluidos compuestos anticancerígenos (genkwanina, hespiridina y fenilcetaldoxima) y antidiabéticos (metformina). También utilizamos HPLC-DAD para cuantificar los compuestos fenólicos cianidina, catequina y ácidos *p*-cumárico, 4-hidroxibenzoico, gálico y clorogénico (50, 70, 80, 210, 220 y 340 μg·g^{-1} respectivamente) la GC-MS se utilizó para analizar el perfil vitamínico (contenido de *β*-caroteno < 2 ng·mg^{-1}). La fructosa, la glucosa y la sacarosa se cuantificaron mediante HPLC-ELSD con 3.06, 2.77 y 78.0 mg·100 g^{-1}, respectivamente. Los espectros de emisión atómica mostraron que los contenidos de K, Ca, P, Mg, Na, Fe, Zn fueron 13 261.37, 1 114.77, 1 048.27, 932.06, 300.53, 22.03 y 27.27 mg·kg^{-1}, respectivamente. Se identificaron y cuantificaron, palmítico, heptadecanoico, esteárico, oleico, linoleico, araquídico, linolénico, heneicosanoico y tricosanoico como los principales ácidos grasos utilizando una mezcla de 37 estándares. Estos hallazgos corroboran los aportes medicinales, nutracéuticos y nutricionales de esta versátil fruta que muestra su potencial como cultivo comestible de alto valor agregado.

Palabras clave: calidad de fruta, fitoquímica, medicina tradicional, pepino kat, perfil metabolómico no dirigido.

INTRODUCTION

The tree *Parmentiera aculeata* is widely distributed throughout Mexico and Central America. Its fruit is known in Spanish as *pepino de árbol* or *pepino de ardilla*. In Mexico, it is primarily found in the central and southern regions, where it is commonly referred to as *cuajilote* (Santiago-Ruiz *et al*., 2021). In the Yucatán Peninsula, its Mayan name is *pepino kat*. The sweet and juicy fruit is highly versatile and can be consumed in both sweet and savory dishes, either as fresh fruit, in salads, or in traditional local recipes (Domínguez-Orta and Herrera-Martínez, 2018). Beyond its culinary uses, the *P. aculeata* fruit is important in traditional mexican medicine (Lim, 2012) to treat diverse disorders (e.g., inflammation, kidney pain, flu, cough, fever, diabetes and breast cancer (Pérez-Gutiérrez *et al*., 2000). In one of the few studies on medicinal compounds in *P. aculeata*, Pérez-Gutiérrez *et al*. (2000) reported that lactucin-8-*O*-methylacrylate reduced blood sugar levels in diabetic mice. Morales-Sánchez *et al*. (2015) confirmed the antiurolithic effect of the fruit in Wistar rats, and Estanislao-Gómez *et al*. (2016) demonstrated specific cytotoxic activity of a hexane extract of fruit and induction of apoptosis in breast cancer cells. Most recently, Santiago Ruiz *et al*. (2021) reported antioxidant and antimicrobial ac-

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Received: January 13, 2025
Accepted: April 15, 2025
Published: May 21, 2025

DOI: 10.18633/biotecnia.v27.2556

tivities and the polyphenol content in the pulp and seeds of *P. aculeata* and high fiber content in the fruit. Although these studies indicate that the fruit is a good source of important metabolites to treat certain diseases, our exhaustive literature search for metabolites in the fruit of *P. aculeata* showed that a complete chemical profile of bioactive metabolites had never been reported. Thus, here we identified the global metabolite profile in the fruit of *P. aculeata* and quantified the bioactive compounds that may provide nutraceutical activities, nutritional contributions and possible medicinal effects for improving consumer health.

MATERIALS AND METHODS

Source and pretreatment of fruit

Fruits of *P. aculeata* were collected in August 2021 from trees in a family plot in the municipality of Mocochá, Yucatán, Mexico (21°05′45″ N, 89° 28′ 20″ W, average altitude of 20 masl). Healthy fruits were used, without phytosanitary damage, at an optimum stage of maturity for consumption. The fruits were disinfested for 10 min in 1 % v/v hypochlorite solution and rinsed with distilled water. The fruits were cut transversally, the peel was removed, and the whole fruits were frozen in liquid nitrogen, then lyophilized (Frezer dryer, Labconco, 2.5 L-50 EE. UU) and stored in an amber container at – 20 °C) until further analysis.

Chemicals and reagents

All chemicals were purchased from AccesoLab (AccesoLab S.A. de C.V., Mexico, Mexico) unless noted next. Formic acid, methanol, and acetonitrile were MS grade from Sigma-Aldrich (Merck S.A. de. CV., Mexico, Mexico). Reference standards (purity ≥ 99 %) including gallic acid, catechin and epicatechin were purchased from Sigma Aldrich (St Louis, MO, EE, UU). Ultrapure water generated using a Milli-Q Progard TS2 system (France) was used in all experiments.

Metabolite extraction

For metabolite extraction, the method previously published by Llorach *et al*. (2008) was employed, with some modifications. Compounds were extracted from 500 mg of lyophilized sample in 2 mL of 80 % v/v methanol. The mixture was vortexed (vortex Ika, MS 3, Germany) for 1 min, then centrifuged (Sorvall, model RC 6 plus, USA) (15 min at 1000 × *g*) at 4 °C. The supernatant was recovered, filtered (0.22 µm, PTFE, Agilent Technologies, Santa Clara, USA), and the solvent evaporated using a miVac vacuum centrifuge concentrator (Genevac, Ipswich, UK). The concentrated sample was reconstituted with a 90:10 acetonitrile/water Mili-Q solution and stored in an amber vial at – 20 °C until analysis.

Global analysis of metabolites using UPLC-MS-QTOF

The global metabolite analysis was based on the recommendations of Cervantes-Hernández *et al*. (2022), with some modifications. Samples were injected in a randomized order into a UPLC (Acquity class I, Waters, Milford, CA, USA) coupled with an orthogonal QTOF mass spectrometer (SYNAPT G1 HDMS; Waters) and separated chromatographically on a reverse-phase CSH C18 column (2.1 mm × 150 nm, 1.7 mm; Waters) at 35 °C. Mobile phase A (ultrapure water and 1 % v/v formic acid) and mobile phase B (100 % v/v acetonitrile) were applied in a gradient with a flow rate of 0.3 mL·min^{-1}: 90 – 20 % A for 18 min, 0.90 % A for 18.10 to 25 min, 0 to 18 min, 10 – 80 % B; 18.18 to 25 min, 100 – 10 % B.

The mass spectrometer range was set from 50 to 1500 Da. Both ionization modes were used separately. For positive electrospray ionization (ESI^+) mode, the conditions were capillary voltage 3 kV, cone voltage 40 V, source temperature 120 °C, desolvation temperature 350 °C, desolvation gas flow 500 L·h^{-1}.

For the negative mode (ESI^-), capillary voltage was 2.5 kV, cone voltage 40 V, source temperature 120 °C, desolvation temperature 300 °C, and desolvation gas flow was 500 L·h^{-1}. Leucine–enkephalin (2 ng·mL^{-1}) was infused as the LockSpray reference internal mass calibrant at 5 mL·min^{-1}, and its signal was monitored every 10 s. Data were collected in the continuum mode with an MS scan time of 1.5 s. For both ionization modes, Ar was used as the collision gas with collision energy in the trap region of 10 eV (Function 1, low energy) and ranging from 20 – 50 eV (Function 2, high voltage).

Metabolite profile analysis

Positive and negative electrospray ionization data were independently analyzed using Progenesis QI for small molecules software (Non-Linear Dynamics, Waters, Milford, MA, United States). Alignment, normalization, and deconvolution were set at standard parameters. Chemspider Databases (https://www.chemspider.com/), PlantCyc (https://www.plantcyc.org/), KEGG (https://www.genome.jp/kegg/), HMDB (https://www.hmdb.ca/), and ChEBI (https://www.ebi.ac.uk/chebi/init.do) and an in-house database were used for preliminary identification of compounds with a minimum match of 80% for precursor ions; MS/MS data, retention times values, and isotope distribution were included for increasing match score values.

Extraction of phenolic compounds and HPLC-DAD analysis

Polyphenols were extracted as described by Molina-Quijada *et al*. (2010). The material was subjected to two extractions. The extract was injected (60 µL) in duplicate into a C18 HPLC column (5 µm, 25 cm × 4 mm, Supelcosil™ LC-18, SUPELCO, Bellefonte, PA, USA) in an Agilent 1260 Infinity HPLC with a photodiode array detector and ChemStation software (Agilent Technologies). The mobile phase consisted of methanol (A) and acidified water (5% v/v formic acid) (B) and with 1 mL min^{-1} flow. The samples were eluted using the following gradient: 2% A and 98% B as initial conditions, 32% A and 68 B for 30 min, 40% A and 60% B for 40 min, 95% A and 5% B for 50 min and finally 95% A and 5% B for 55 min.

The reference standards included gallic acid (270 nm), dihydroxybenzoylic acid (330 nm), epicatechin (280 nm), syringic acid (270 nm), catechin (280 nm), myrecithin (370

nm), cyanidin (280 nm), ferulic (320 nm), vanillic (260 nm), caffeine (270 nm), sinapic acid (320 nm), 4-hydroxybenzoyl acid (260 nm), hydrated rutin (260 nm), caffeic acid (320 nm), *p*-coumaric (320 nm), resveratrol (320 nm).

Quantification of fat-soluble vitamins by HPLC-DAD

The extraction was done in triplicate using 50 mg of lyophilized sample and 500 µL of dichloromethane. The quantification of fat-soluble vitamins was carried out according to Mattila *et al*. (1995) and Mahmoodani *et al*. (2017). The mixture was sonicated (Fisher Scientific, model FS30, USA) for 5 min, homogenized with a vortex shaker (brand and model as mentioned above) for 30 s, and then centrifuged (Hermle labnet, model Z206A, USA) for 5 min at 13 500 × *g*. The supernatant was transferred to a new tube with 500 µL of Mili-Q water and centrifuged again for 5 min at 5000 × *g*; 100 µL of the organic extract was combined with 500 µL of methanol, and 10 µL was injected into a liquid chromatograph (Agilent Technologies, 1200 series, diode array detector, USA) (HPLC-DAD), with an Eclipse Plus XDB, C18 column (4.6 × 250 mm, 5 µm). The mobile phase was 100% methanol, temperature 30 °C, with 1 mL·min^{-1} flow rate for 15 min. Wavelengths used were 265 nm for cholecalciferol and ergocalciferol, and 380 nm for β-carotene. Calibration curves were generated for each standard using 0–70 µg·mL^{-1}. The retention times were compared to those of reference standards of ergocalciferol, cholecalciferol and *β*-carotene and to the UV-Vis spectra to identify the vitamins.

Carbohydrate identification

For the identification of carbohydrates, the method reported by Chen *et al*. (2021) was followed. The lyophilized sample (50 mg) was combined with 2 mL of Milli-Q water, sonicated for 5 min, homogenized on a vortex shaker (brand and model as mentioned above) for 30 s, and centrifuged at 5000 × *g* for 10 min. The supernatant was filtered through a 0.22 µm filter, diluted 1:150 in Mili-Q water, then 10 µL was injected in triplicate into an Agilent 1200-Altech3300 evaporative light scattering detector liquid chromatograph (HPLC-ELSD) with a Prevail carbohydrate ES column (Alltech associates inc) (4.5 × 250 mm, 5 µm). The column temperature was set at 30 °C, flow rate 1 mL·min^{-1}, evaporation temperature 38.2 °C, drying gas flow rate 1.4 L·min^{-1}, and detector flow gain 16.

For quantifying fructose, glucose and sucrose, standard curves were generated using reference standards (0 to 150 mg·L^{-1}) (fructose: R^2 = 0.9994, glucose: R^2 = 0.9988, sucrose: R^2 = 0.9983).

Quantification of minerals

K, Ca, Mg, Na, P, Fe and Zn were identified in triplicate samples, using 100 mg of lyophilized sample in 10 mL of HNO_3 (JT Baker Instra 69 – 70 %) in PTFE beakers, to carry out the digestion program using a microwave (MARS 5 CEM brand). The digestion program consisted of a 15 min ramp until reaching 175 °C and maintaining this temperature for 10 min. Temperature was controlled using an RTP 300 PLUS fiber optic sensor, and power was set to 400 W. The digested sample was brought to 25 mL in a glass flask and analyzed using the inductively coupled plasma emission spectrometer iCAP 6500 DUO (Thermo Electron Corp., Altrincham, UK) with an RF power of 1150 W, auxiliary gas flow of 0.5 L·min^{-1}, and nebulizer gas flow of 0.60 L·min^{-1} (U.S. EPA. 2014. "Method 6010D (SW-846).

Determination of fatty acids

The method reported by Cavonius *et al*. (2014) was followed. The lyophilized sample (50 mg) was combined with 400 µL of BF_3 (10 % v/v in methanol) and 600 µL of methanol, heated at 60 °C for 90 min, then 500 µL of hexane were added, followed by 4 mL of Milli-Q water. The sample was vortexed (brand and model as mentioned above), then centrifuged at 1000 x *g* for 15 min. The organic phase was used for analysis and injected in triplicate.

The GC-MS was pre-calibrated with perfluorurotributylamine, and calibration scale for masses (<5 ppm) and relative intensities of ions (*m/z* 30 – 400) were adjusted. Samples were injected in triplicate onto an Agilent J & W HP-88 column (100 m × 0.25 mm) × 0. 2 µm in splitless mode, with injection temperature 250 °C, carrier gas flow (He) 1 mL·min^{-1}, temperature ramp: 120 °C (1 min), 175 °C (10 min), 210 °C (4 min), 212 ºC (12 min), 230 °C (3 min), 240 °C (1 min), transfer line temperature 230 °C, ionization source temperature 250 °C, quadrupole temperature 200 °C, electron ionization energy 70 eV, mass range 30–400 *m/z*, solvent delay 8.5 min. Fatty acids were identified based on their retention times compared with those of the standards and comparing their mass spectra with the NIST 11 library and were quantified using an external calibration method and a mixture of 37 fatty acid methyl ester standards (Supelco 37 component FAME Mix CRM-47885); standard curves were prepared for each compound at 0 to 200 µg·mL^{-1}, and 37 calibration curves were prepared for each analyte, using the quantifier ions 74, 55, 67 and 79 *m/z* for saturated, monounsaturated, two unsaturated and polyunsaturated fatty acids (three or more unsaturations), respectively. Compounds were quantified by extrapolating the signal intensities of the samples with respect to the equations for the standard curve obtained for each standard.

RESULTS AND DISCUSSION

Global metabolite profile by UPLC-QTOF-MS

Here, we developed a method using UPLC-Q-TOF-MS that separated the chemical constituents in the fruits of *P. aculeata*, and using untargeted metabolomics, we identified 725 chemical species after peak selection from the masses obtained in positive (ESI^+) and negative (ESI^-) electrospray ionization mode. In ESI^+ mode, 375 metabolites were identified; 245 were pre-identified, and 44 were accepted as present (Table 1). In ESI^- mode, 350 metabolites were detected; 275 were pre-identified, and 60 were accepted (Table 2).

The fruit of *P. aculeata* contained phytochemical compounds, including flavonoids, piperazines, coumarins, ses-

quiterpenes, carbohydrates, fatty acids and vitamins among other metabolites, these compounds exhibit diverse bioactivities with potential health benefits (Keservani *et al*., 2020; Ullah *et al*., 2020), suggesting that *P. aculeata* can be considered a functional food. Additionally, most of the compounds found are probably marker metabolites of *P. aculeata*, since they are not present in frequently consumed foods such as citrus and other fruits or vegetables, according to a review by Rafiq *et al*. (2021).

An important major group of biomolecules identified in the fruit were flavonoids, the largest group of natural phenolic compounds. Seven flavonoids were identified in the fruit: 5-hydroxy-4′,7,8-trimethoxyflavone, 4′,5-dihydroxy-7-methoxy-6-methylflavone, epicatechin 3-glucoside, genkwanin and hesperidin. Genkwanin and hesperidin are known to diminish symptoms of some neurodegenerative diseases such as Parkinson, Alzheimer's, diabetes and cancer (Xiong *et al*., 2019; Menyiy *et al*., 2023). Genkwanin, a methoxyflavone found in several plant species, has remarkable antioxidant and anti-inflammatory activities, through the activation of glucokinase. In addition, it has antihyperglycemic activity, cardioprotective and neuroprotective properties, antitumor activity, and antibacterial, antiviral and dermaprotective effects (Menyiy *et al*., 2023). Hesperidin regulates lipid metabolism and helps maintain optimal glucose and antioxidant levels and prevent apoptosis and inflammation, provides multiple benefits to skin functions and is used to treat obesity (Xiong *et al*., 2019). Piperazines are also used to treat a wide range of diseases (Faizan *et al*., 2024). Some piperazine derivatives were initially used as anthelmintics, but also have anticancer, antioxidant, cognition enhancing, antimicrobial, antibacterial, antiviral, antidiabetic and antidepressant properties among others (Keservani *et al*., 2020).

Coumarins also possess a wide range of biological activities, including antibacterial, antimicrobial, antioxidant, anti-inflammatory, antiarrhythmic, spasmolytic and antiviral activities (Ostrowska, 2020).

Among sesquiterpene compounds, zaluzanin C has been isolated from other natural products and demonstrates antioxidant, antimicrobial, anti-inflammatory, antiulcer, antiviral, antimalarial and anticancer activities. In addition, it is proposed as an effective hepatoprotectant (Lee *et al*., 2023).

Although the fruit of *P. aculeata* has been used in traditional medicine to treat diabetes mellitus (Pérez-Gutiérrez *et al*., 2000), the therapy has been empirical. However, its efficacy has been attributed to lactucin-8-*O*-methacrylate (Pérez-Gutiérrez *et al*., 2000). Our UPLC-QTOF-MS analysis also revealed the possible presence of metformin in the fruit of *P. aculeata*, which is an antidiabetic derived from a natural active product called galegine, a guanidine derived from the plant *Galega officinalis* (French lilac) that was used in medieval times to relieve excessive urination in diabetics (Andrade-Cetto and Heinrich, 2005). Genkwanin and hesperidin also exhibit antidiabetic effects. Along with 8-*O*-methacrylate these compounds likely contribute to hyperglycemic effects when the fruit is consumed. These results indicate that *P. aculeata* is a promising source of antidiabetic compounds. However, *in vivo* or *in vitro* studies are needed to determine the mechanism of the antidiabetic action of this fruit.

Another interesting compound found in the fruit is dycthioquinazole C, which has also been reported to alleviate symptoms caused by neurodegenerative diseases (Xiao-Fei *et al*., 2018). Dictyoquinazoles A–C are natural alkaloids in the edible mushroom (*Dictyophora indusiata*) used in Chinese food and medicine (Zhang *et al*., 2023). They have a unique quinazoline moiety, which is rarely found in natural products. Such alkaloids protect neurons from neurotoxicity induced by glutamate, *N*-methyl-*D*-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (Oh and Song, 2007).

Cynaratriol, a sesquiterpenic lactone with a typical tricyclic guaianolide skeleton, was also identified in *P. aculeata* and has been isolated from the plant *Centaurea musimomum* (Milošević *et al*., 2010). It is also used in traditional medicine because of its various biological activities such as antimicrobial activity.

Estanislao-Gómez *et al*. (2016) conducted studies to evaluate the *in vitro* cytotoxic and pro–apoptotic potential of hexane extract from *P. edulis* on the MDA-MB-231 breast cancer cell line. However, the specific metabolites responsible for these effects were not identified. We also identified glucosinolate phenylketaldoxime, which has been reported to have biological properties and potential uses as a cancer preventive and therapeutic agent (Wittstock and Halkier, 2000; Ashani and Silman, 2008). It likely contributes to the anticancer activity of the fruit. However, further investigation of the biological activities of the compounds in *P. aculeata* fruit and extracts is needed.

Polyphenol profile from HPLC–DAD analysis

Of the six polyphenols identified and quantified, chlorogenic acid (340 ± 0.00 μg·g^{-1}) was most abundant, followed by gallic acid (220 ± 0.15 μg·g^{-1}), 4-hydroxybenzoic acid (210 ± 0.00 μg·g^{-1}), *p*-coumaric acid (80 ± 0.00 μg·g^{-1}), catechin (70 ± 0.00 μgg^{-1}) and cyanidin (50 ± 0.00 μg·g^{-1}). Given the importance of these compounds, the fruit is an important nutraceutical source with health benefits. These types of compounds represent a diverse, bioactive, and widely distributed class of secondary plant metabolites. They form an essential part of the human diet due to beneficial properties that could help decrease the incidence of chronic degenerative diseases, cardiovascular diseases, cancer, liver disorders, obesity and diabetes (Rasouli *et al*., 2017). They also possess broad health-promoting properties related to the treatment of metabolic syndrome and are anti-inflammatory, antioxidant, antilipidemic, antihypertensive and antineurodegenerative (Santana-Gálvez *et al*., 2017). Chlorogenic acid also has liver and kidney protective activities (Wang *et al*., 2022).

The occurrence, composition and content of phenolic compounds in fruits can vary considerably. For example, *p*-coumaric acid is present in oranges and currants; gallic acid in bananas, pitayas and avocados; catechin in red grapes and sweet cherries; and cyanidin in raspberries, pomegranates

Table 1. Compounds extracted from the fruit of *Parmentiera aculeata* and determined using positive ionization mode.
Tabla 1. Compuestos extraídos del fruto de *Parmentiera aculeata* y la determinación usando el modo de ionización positivo.

Metabolite	Formula	Class
6-Amino-4-[3-[[4-(4-fluorophenyl)-1-piperazinyl]methyl]-4-methoxyphenyl]-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile	$C_{26}H_{27}FN_6O_2$	Piperazine
Indoleacrylic acid	$C_{11}H_9NO_2$	Indol
3-(4-Hydroxyphenyl)-2-oxiranecarboxylic acid	$C_9H_8O_4$	Phenol
Zaluzanin C	$C_{15}H_{18}O_3$	Sesquiterpenoid
2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol	$C_{13}H_{22}O_{13}$	Oxolane
Dyctioquinazol C	$C_{18}H_{18}N_2O_5$	Quinazoline
2-(2-Methylpropyl)-thiazole	$C_7H_{11}NS$	Thiazole
3-(4-Hydroxyphenyl)-2-oxiranecarbaldehyde	$C_9H_8O_3$	Phenol
5-Methylthio-2-oxopentanoic acid	$C_6H_{10}O_3S$	Fatty acid
Ahaleboside	$C_{15}H_{16}O_8$	Coumarin glycoside
N(2)-(2-carboxyethyl)-L-arginine	$C_9H_{18}N4O_4$	L-α-amino acid (guanidine)
L-2-Amino-5-hydroxypentanoic acid	$C_5H_{11}NO_3$	α-Amino acid
Phenylacetaldoxime	C_8H_9NO	Glucosinolate
4-Acetanisole (also called 4′-metoxiacetofenona)	$C_9H_{10}O_2$	Alkyl-phenylketone
5-Hydroxy-4′,7,8-trimethoxyflavone	$C_{18}H_{16}O_6$	Flavonoid
4-(Fluoromethyl)-4-hydroxy-2-oxanone	$C_6H_9FO_3$	δ-Lactone
(2*E*)-4-hydroxy-5-methyl-2-propylidene-3(2*H*)-furanone	$C_8H_{10}O_3$	Furanone
2,5,5,8α-Tetramethyl-3,5,6,8a-tetrahydro-2*H*-chromen-3-ol	$C_{13}H_{20}O_2$	Megastigmane glycoside
Glaucarubolone 15-*O*-*β*-D-glucopyranoside	$C_{26}H_{36}O_{13}$	Quassinoid
Lactarofulvene	$C_{15}H_{16}$	Sesquiterpenoid
Metformin (1+)	$C_4H_{12}N_5$	Guanidine
Isopentenyl phosphate	$C_5H_{11}O_4P$	Isoprenoid phosphate
3,6-dioxocyclohexa-1,4-dien-1-olate	$C_6H_3O_3$-	Benzoquinone
Cethexonium	$C_{24}H_{50}NO^+$	Cyclohexanol
Obtusafuran	$C_{16}H_{16}O_3$	Benzofuran
Pyrethrolone	$C_{11}H_{14}O_2$	Alcohol
(±)-3-(Methylthio)heptanal	$C_8H_{16}O_S$	α-Hydrogen aldehyde
1-Hydroxyepiacorone	$C_{15}H_{24}O_3$	Acyloin
(R)-Pterosin *β*	$C_{14}H_{18}O_2$	Indanone
(5*α*,8*β*,9*β*)-5,9-Epoxy-3,6-megastigmadien-8-ol	$C_{13}H_{20}O_2$	Benzopyran
(α ±)-Mandelic acid	$C_8H_8O_3$	Aromatic α-hydroxy acid
Cynaratriol	$C_{15}H_{22}O_5$	Guaianolide
Dehydrovomifoliol	$C_{13}H_{18}O_3$	Enone
2,2,6,7-Tetramethylbicyclo[4.3.0]nona-1(9),4-diene-7,8-diol	$C_{13}H_{20}O_2$	Tertiary alcohol
6′-*O*-cinnamoylmussaenosidic acid, (rel)-	$C_{25}H_{30}O_{11}$	Terpene glycoside
5-*O*-(trans-feruloyl)- L-arabinofuranose	$C_{15}H_{18}O_8$	*O*-Acyl carbohydrate
Cis,*trans*-hepta-2,4,6-trienoic acid	$C_7H_8O_2$	Monocarboxylic acid
2-Hydroxy-*p*-mentha-1,8-dien-6-one	$C_{10}H_{14}O_2$	Menthane monoterpenoid
7-Amido-7-deazaguanosine	$C_{12}H_{15}N_5O_6$	Nucleoside
Helioside A	$C_{39}H_{52}O_{23}$	Carbohydrate
Genkwanin	$C_{16}H_{12}O_5$	Flavonoid
3-(Methylthio)butyraldehyde	$C_5H_{10}OS$	Aldehyde
Furylacetone	$C_7H_8O_2$	Ketone
8-Methylquinoline	$C_{10}H_9N$	Quinoline
γ-L-glutamylbutirosin B	$C_{26}H_{48}N_6O_{15}$	Enzyme

Table 2. Compounds extracted from the fruit of *Parmentiera aculeata* and determined using negative ionization mode.
Cuadro 2. Compuestos extraídos del fruto de *Parmentiera aculeata* y la determinación usando el modo de ionización negativo.

Metabolite	Formula	Class
α-D-Glucopyranoside	$C_6H_{12}O_6$	Hexose
Sucrose	$C_{12}H_{22}O_{11}$	*O*-Glycosyl
3,4,5-Trihydroxy-6-[4-(1-hydroxy-2-oxo-2-phenylethyl)phenoxy]oxane-2-carboxylic acid	$C_{20}H_{20}O_9$	Stilbene glycoside
Dihydroxyfumitremorgin C	$C_{22}H_{25}N_3O_5$	β-carboline
Lusitanicoside	$C_{21}H_{30}O_{10}$	Phenolic glycoside
Maltulose	$C_{12}H_{22}O_{11}$	Fatty acyl glycoside of mono and disaccharide
Citbismine C	$C_{37}H_{36}N_2O_{11}$	Acridone
D-Glucaro-1,4-lactone	$C_6H_8O_7$	γ- butyrolactone
Khelmarin D	$C_{28}H_{24}O_8$	Angular pyranocoumarin
Pelargonidin-3-rhamnoside-5-glucoside	$C_{27}H_{31}O_{14}+$	Anthocyanidin-5-*O*-glycoside
3,4,5-trihydroxy-6-{[5-hydroxy-8-(hydroxymethyl)-8-methyl-4-oxo-2-phenyl-4*H*,8*H*-pyrano[2,3-f]chromen-3-yl]oxy}oxane-2-carboxylic acid	$C_{26}H_{24}O_{12}$	Coumarine
L-Sorbose	$C_6H_{12}O_6$	Monosacharide
Salicylic acid *β-D*-glucoside	$C_{13}H_{16}O_8$	Phenolic glycoside
Isolariciresinol-9′-*O*-*α*-*L*-arabinofuranoside	$C_{25}H_{32}O_{10}$	Lignan glycoside
D-Vacciniin	$C_{13}H_{16}O_7$	Benzoic acid ester
Chrysaloin	$C_{21}H_{22}O_8$	Anthracene
5-Methylthioribose	$C_6H_{12}O_4S$	Pentose
Caffeoyl C1-glucuronide	$C_{15}H_{16}O_{10}$	Hydroxycinnamic acid glycoside
2-Oxo-3-(phosphonooxy)propyl decanoate	$C_{13}H_{25}O_7P$	Ester
Xanthoxylin	$C_{10}H_{12}O_4$	Alkyl-phenylketone
Riboflavin	$C_{17}H_{20}N_4O_6$	Flavin (vitamina B2)
Salicylic acid *β-D*-glucoside	$C_{13}H_{16}O_8$	Phenolic glycoside
Ethylvanillin glucoside	$C_{15}H_{20}O_8$	Phenolic glycoside
Myricanene B 5-[arabinosyl-(1-6)-glucoside]	$C_{32}H_{42}O_{13}$	Meta,meta-bridged biphenyl
Citbismine B	$C_{36}H_{34}N_2O_{11}$	Acridone
Dictyoquinazol A	$C_{17}H_{16}N_2O_4$	Quinazoline
Quinic acid	$C_7H_{12}O_6$	Quinic acid
5*Z*-Caffeoylquinic acid	$C_{16}H_{18}O_9$	Quinic acid and derivates
3,4,5-trihydroxy-6-{[3-(6-hydroxy-7-methoxy-2*H*-1,3-benzodioxol-5-yl)propanoyl]oxy}oxane-2-carboxylic acid	$C_{17}H_{20}O_{12}$	Flavonoid
6-*O*-*p*-Coumaroyl-*D*-glucose	$C_{15}H_{18}O_8$	Coumaric acid ester
(*S*)-Isosclerone	$C_{10}H_{10}O_3$	Tetralin
5-(3′,5′-Dihydroxyphenyl)-γ-valerolactone-*O*-glucuronide-*O*-methyl	$C_{18}H_{22}O_{10}$	Phenolic glycoside
Citbismine A	$C_{35}H_{32}N_2O_{10}$	Acridon
Lacto-*N*-tetraose	$C_{26}H_{45}NO_{21}$	Oligosaccharid
trans-3-Hydroxycotinine glucuronide	$C_{16}H_20N_2O_8$	O-glucuronid
3,4,5-trihydroxy-6-[3-(2-methyl-3-oxopropyl)phenoxy]oxane-2-carboxylic acid	$C_{16}H_{20}O_8$	Phenolic glycosid
2-*O*-Benzoyl-*D*-glucose	$C_{13}H_{16}O_7$	Hexose
β-D-fructosyl-α-*D*-(6-*O*-(*E*))-feruloylglucoside	$C_{21}H_{28}O_{12}$	Cinnamic acid ester
4′,5-Dihydroxy-7-methoxy-6-methylflavone	$C_{17}H_{14}O_5$	7-O-methylated flavonoid
Epicatechin 3-glucoside	$C_{21}H_{24}O_{11}$	Flavonoid-3-O-glycoside
Hesperidin	$C_{28}H_{34}O_{15}$	Flavonoid-7-O-glycoside
trans-Δ-Viniferin 3″-glucoside	$C_{34}H_{32}O_{11}$	2-arylbenzofuran flavonoid
3′-Ketolactose	$C_{12}H_{20}O_{11}$	Disaccharide
trans-*δ*-Viniferin 3′′-glucoside	$C_{34}H_{32}O_{11}$	2-arylbenzofuranflavonoid
Ascladiol	$C_7H_8O_4$	Butenolide
2′′-Methoxy-(*S*)-oleuropein	$C_{26}H_{34}O_{14}$	Terpene glycoside
Tavulin	$C_{15}H_{20}O_4$	Germacranolide and derivates
Naematolone	$C_{17}H_{22}O_5$	Sesquiterpenoid
Citbismine F	$C_{36}H_{34}N_2O_{10}$	Acridon
β-D-Glucose	$C_6H_{12}O_6$	Hexose
Galactinol	$C_{12}H_{22}O_{11}$	O-glycosyl compound
Isolimonic acid	$C_{26}H_{34}O_{10}$	Naphtofuran
Sesamolinol 4′-*O*-*β*-*D*-glucosyl (1-6)-*O*-*β*-*D*-glucoside	$C_{32}H_{40}O_{17}$	Phenolic glycoside
2-Fucosyllactose	$C_{18}H_{32}O_{15}$	Oligosaccharide
Uzarigenin-3-[xylosyl-(1-2)-rhamnoside]	$C_{34}H_{52}O_{12}$	Cardenolide glycosid and derivatives
Corchorifatty acid F	$C_{18}H_{32}O_5$	Linoleic acid and derivatives
LysoPA (0:0/18:1(9*Z*))	$C_{21}H_{41}O_7P$	2-acylglycerol-3-phosphate
Phenethyl decanoate	$C_{18}H_{28}O_2$	Fatty acid ester
24-Methylenepollinasterol	$C_{29}H_{48}O$	Ergosterol and derivatives

and acerolas (Haminiuk and Maciel, 2012). Chlorogenic acid has been identified in thistle, potato, chrysanthemum, strawberry, mango, blueberry and mulberry leaves (Šilarová *et al*., 2019; Wang *et al*., 2022) and is the main phenolic compound in eggplants (Colak *et al*., 2022). However, the contents of different polyphenols in fruits vary depending on abiotic and biotic factors such as light and UV exposure, temperature, and heavy metal accumulation (Zagoskina *et al*., 2023). It is important to clarify that consuming fruits with high levels of polyphenols, does not generally correlate with higher concentrations of active metabolites because of differences in the bioaccessibility and bioavailability of the compounds. Bioavailability of a compound depends on its bioaccessibility from the food matrix after digestion (Haminiuk *et al*., 2012) and is closely related to the metabolic capacity of the intestinal microbiota in the consuming organism (Gonthier *et al*., 2003).

Quantification of fat-soluble vitamins

For the HPLC-DAD analysis of *β*-carotene (provitamin A), ergocalciferol (vitamin D2) and cholecalciferol (D3), the calculated limits of detections and quantification are given in Table 3. Calibration curves were generated from 0 to 70 μg·mL^{-1}, and three blanks were injected to calculate the limits of detection and quantification.

Table 3. Calibration equation, limits of quantification and detection of fat-soluble vitamins.
Tabla 3. Ecuación de calibración, límites de cuantificación y detección de las vitaminas liposolubles.

Compound	Regression equation	R^2	LOQ (μg·mL^{-1})	LOD (μg·mL^{-1})
β-Carotene	Abs = 1.94*xC* + 1.21	0.9977	0.015	0.045
Ergocalciferol	Abs = 0.394*xC* + 0.799	0.9997	0.017	0.051
Cholecalciferol	Abs = 2.66*xC* – 1.99	0.9976	0.009	0.028

In Figure 1a, a signal is seen that coincides with the retention time of *β*-carotene, but its intensity was below the limit of quantification (LOQ = 0.045 μg·mL^{-1}), which would be equivalent to analyzing a sample with at least 2.7 ng of *β*-carotene per milligram of sample, the minimum amount to quantify an analyte with the method used. In Figure 1b, note that there are no signals for cholecalciferol or ergocalciferol.

Carbohydrate identification

Sugars and other carbohydrates are essential for fruit quality because they impact flavor and texture. They are synthesized during fruit growth and ripening, and sucrose, fructose and glucose predominate (Julius *et al*., 2017), as observed in *P. aculeata* fruit. For fructose, the retention time was 6.57 min, glucose 7.99 min and sucrose 10.21 min (Figure 2). Sucrose was most abundant (78.0 mg·100 mg^{-1}) followed by fructose (3.06 mg·100 mg^{-1}) and glucose (2.77 mg·100 mg^{-1}). These results are important because carbohydrates are fundamental for human nutrition, energy when broken down, for structural and functional roles. From a nutritional point of view, glucose and fructose, which is broken down into glucose, are among the most important because the brain depends on a continuous supply of glucose that can be completely oxidized to CO_2 and water (Plaza-Díaz *et al*., 2013).

Quantification of macro- and micronutrients

Minerals are key elements of a healthy diet because they have both direct and indirect effects on human health, although specific daily requirements vary from person to person depending on several factors such as age, sex, mass, activity level and general health status (Gush *et al*., 2021). Of the macronutrients in the fruit of *P. aculeata*, K was most abundant, followed by Ca, P, Mg and Na, which according to Chinaza *et al*. (2020), are very important minerals for humans. Among the micronutrients, zinc and iron were present at relatively similar levels (Figure 3).

K is the most abundant mineral in fruits and vegetables, but Ca has significant impacts food quality. A diet rich in K contributes to lower blood pressure, regulate the heartbeat, it aids in muscle contraction and is essential for sending nerve impulses to release energy from fats, carbohydrates and proteins. On the other hand, Ca is essential for the formation o f bones and teeth. There is also evidence linking hypertension with Ca deficiency (Vincente *et al*., 2014). Magnesium (Mg) is also important for bones and teeth and participates

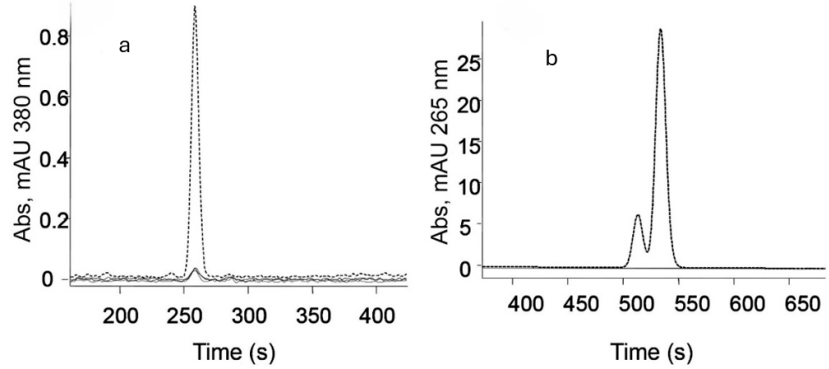

Figure 1. Overlay chromatograms of (a) β-carotene standard (--) and *P. aculeata* sample (−), (b) cholecalciferol or ergocalciferol standard (--) and *P. aculeata* sample (–).
Figura 1. Cromatogramas superpuestos de (a) estándar de β-caroteno (--) y muestra de *P. aculeata* (–), (b) estándar de colecalciferol o ergocalciferol (--) y extracto de *P. aculeata* (–).

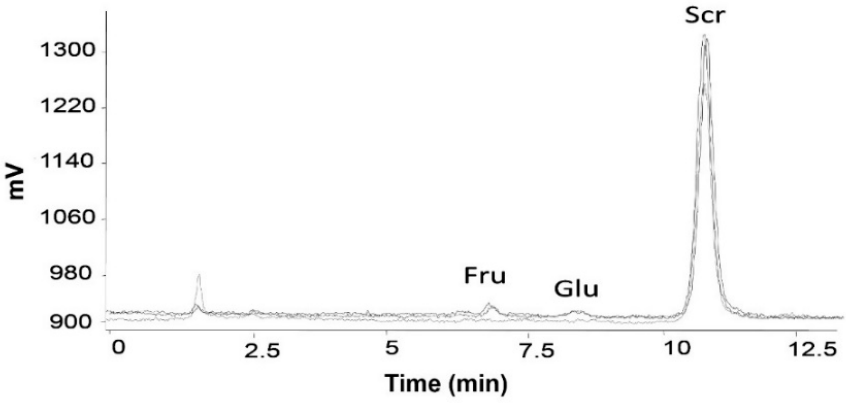

Figure 2. Typical chromatogram of *P. aculeata* aqueous extracts for quantifying the carbohydrates fructose (Fru), glucose (Glu), and sucrose (Scr).
Figura 2. Cromatograma típico de extractos acuosos de *P. aculeata* para la cuantificación de los carbohidratos fructosa (Fru), glucosa (Glu) y sacarosa (Scr).

in cell metabolism and the activity of 300 enzyme systems. Similarly, P is essential for important enzymes that store and release energy necessary for body functions. Among the micronutrients, zinc is essential for the activity of more than 300 enzymes, plays a vital role in host defense against pathogens (Lozano-Muñoz and Díaz, 2020), and iron is required by many enzymes and proteins, notably in hemoglobin to prevent anemia (Chinaza *et al*., 2020).

Based on data from the National Library of Medicine in the United States, the fruit of *P. aculeta* satisfies the recommended daily intake of K, Ca, Mg, P, Na, Fe and Zn (respectively, 3800–4700, 1000–1200, 130–420, 500–700, 1200–1500, 8–18 and 5–11 mg·g^{-1}) for children, women and men.

Determination of fatty acids

Figure 4 shows the typical chromatogram for total ions in the fruit of *P. aculeata* for the identified fatty acids (FAs) enlisted in Table 4. The FAs with the highest abundance in the fruit were in decreasing order oleic acid (C18:1n9c), palmitic acid (C16:0), linolenic acid (C18:3n3), linoleic acid (C18:2n6c, omega 6). One FA was also detected but could not be quantified because it was not included in the mixture of commercial standards used. However, with the help of mass spectrometry analysis, we identified it with a probability of 68.6% as heptadienoic acid (C7:2) by comparing its spectrum against the NIST library. Figure 5 shows the mass spectrum for the derivative (methyl ester).

Long-chain polyunsaturated FAs can regulate a broad set of homeostatic and inflammatory processes linked to numerous diseases, directly or through conversion into locally acting bioactive metabolites (Buczynski *et al*., 2019). However, endogenous synthesis of these molecules is much lower than expected, and as a consequence, these compounds must be included in the human diet to maintain health (Zárate *et al*., 2017).

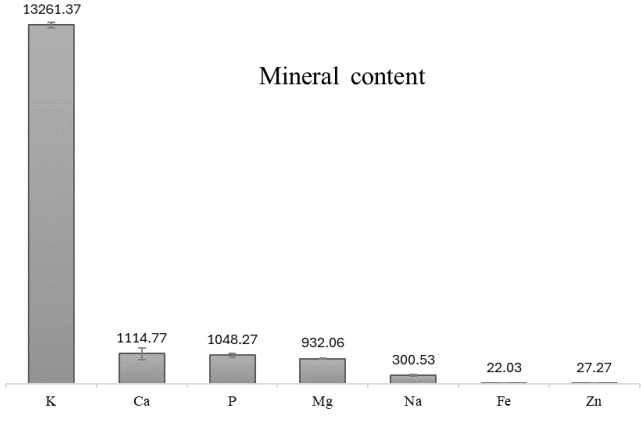

Figure 3. Mineral content of *P. aculeata* fruit (mg·kg^{-1}).
Figura 3. Contenido de minerales del fruto de *P. aculeata* (mg·kg^{-1}).

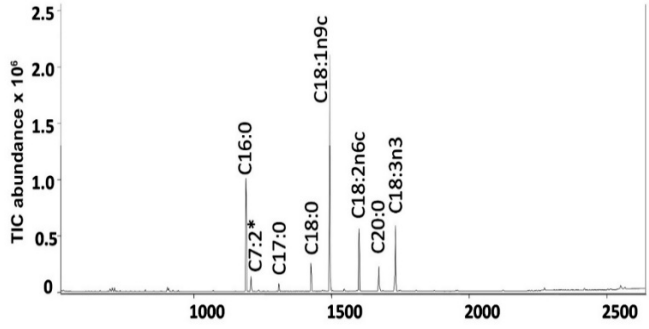

Figure 4. Total ion chromatogram (TIC) of *P. aculeata* fruit analyzed by GC-MS. *Compound identified with a probability of 68.6% (heptadienoic acid, C7:2) in a comparison of its spectrum with the NIST library, palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), arachidonic acid (C20:0), arachidonic acid (C20:0), linolenic acid (C18:3n3).
Figura 4. Cromatograma de iones totales (CIT) del fruto de *P. aculeata* analizado por GC-MS. Compuesto identificado con una probabilidad de 68.6 % (ácido heptadienoico C7:2) en comparación con los espectros de la librería NIST. Ácido palmítico (C16:0), ácido heptadecanoico (C17:0), ácido esteárico (C18:0), ácido oleico (C18:1n9c), ácido linoleico (C18:2n6c), ácido araquidónico (C20:0), ácido linolénico (C18:3n3).

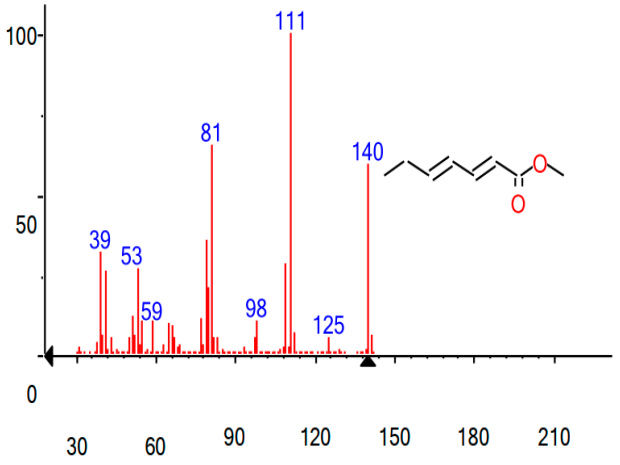

Figure 5. Mass spectrum of the derivatized compound (fatty acid methyl ester) found in *P. aculeata* at retention time 20.13 min (68.6% probability of being heptadienoic acid).
Figura 5. Espectro de masas del compuesto derivatizado (éster metílico de ácido graso) encontrado en *P. aculeata* en un tiempo de retención de 20.13 min. (68.6% de probabilidad de ser ácido heptadienoico).

The long-chain FAs in *P. aculeata* fruit, such as oleic acid (OA), could be considered as a potential source of beneficial lipids, considered to be the main component responsible for the human health benefits of the Mediterranean diet rich in fruits, vegetables and especially olive oil. OA-inclusive diets are associated with a decreased risk of coronary heart disease, cardiometabolic risk, obesity, type 2 diabetes and hypertension. Studies have also suggested that OA may have a protective effect against cardiovascular disease, age-related cognitive decline and Alzheimer's disease. Additionally, insulin sensitivity is relatively impaired with diets low in OA or rich in palmitic acid (Arsic *et al*., 2019). Possible protective effects of OA against the promotion and progression of some human cancers, mainly breast, colorectal and prostate cancers, have also been suggested (Binukumar, 2005).

Palmitic acid has fundamental biological functions at the cellular and tissue levels as an essential component of membrane, secretory and transport lipids with an important role in protein palmitoylation and signaling molecules (Agostoni *et al*., 2016). Studies highlight that α-linolenic acid, an omega-3 polyunsaturated FA, is an essential fatty acid found in walnuts, chia, flaxseed, some green leafy vegetables and some fatty fish. In recent years, -linolenic acid has been associated with benefits similar to those of eicosapentaenoic acid and docosahexaenoic acid, which have important roles in brain development, the brain network, and the immune system. the neural network and cardiovascular disease (Pandohee, 2022). The fruit of *P. aculeata* has been of great interest as a source of plant derived omega-3 fatty acids among nonmeat-eaters. The linoleic acid in the fruit of *P. aculeata* could also be very beneficial because it is important for various cellular activities in mammals. It is also a major component of adipose tissue in the brain, and it participates in developing and maintaining the central nervous system (Olatunya and Adesina, 2024).

The fruit of *P. aculeata* could serve as a suitable alternative source of FAs and could even replace marine sources in the case of linolenic acid.

Table 4. Mean (±SD) of fatty acids content in fruit of *P. aculeata*.
Tabla 4. Medias (± D.E) del contenido de ácidos grasos en el fruto de *P. aculeata*.

Fatty acid	Quantifier ion	Retention time (min)	Content (μg·g^{-1})
Palmitic acid (C16:0)	74 *m/z*	19.83	38.8 ± 0.71
Heptadecanoic acid (C17:0)	74 *m/z*	21.82	2.33 ± 0.08
Stearic acid (C18:0)	74 *m/z*	23.77	6.91 ± 0.12
Oleic acid (C18:1n9c)	55 *m/z*	24.91	87.1 ± 1.36
Linoleic acid (C18:2n6c)	67 *m/z*	26.69	21.9 ± 1.81
Arachidonic acid (C20:0)	74 *m/z*	27.91	2.60 ± 0.10
Linolenic acid (C18:3n3)	67 *m/z*	28.88	29.5 ± 0.27
Heneicosanoic acid (C21:0)	74 *m/z*	30.17	0.23 ± 0.02
Tridecanoic acid (C23:0)	74 *m/z*	35.42	0.35 ± 0.01

In general, our global metabolite profile of the fruit of *P. aculeata* and quantification of bioactive beneficial compounds supports and underscore the fruit´s traditional medicinal uses of the fruit and highlights the importance of further study of the biological and chemical properties.

CONCLUSIONS

Using untargeted metabolomics based on UPLC-QTOF-MS, we identified 725 metabolites that are probable markers of *P. aculeata* fruit and included flavonoids, piperazines, coumarins and sesquiterpenes and anticancer molecules such as genkwanin, hesperidin, and phenylketaldoxime and antidiabetic molecules such as metformin. The high content of beneficial polyphenols and essential long chain polyunsaturated fatty acids make the fruit an important nutraceutical source. Among carbohydrates, sucrose predominated, followed by fructose and glucose. The mineral content in the fruit (K, Ca, P, Mg, Na, Zn and Fe) meets the recommended daily intake based on data from the National Library of Medicine in the United States. Our findings provide new and interesting data and a scientific basis for the high value of the metabolites in *P. aculeata* that have diverse biological activities and benefits for human health. The fruit is a good functional and nutritional food and thus should be more widely promoted for consumption. However, further *in vitro* or *in vivo* studies of these promising metabolites are recommended to provide strong evidence to support the numerous potential medicinal uses of *P. aculeata*.

ACKNOWLEDGMENTS

The authors express their gratitude to Julio Alejandro Rivera Haro for his invaluable support and for providing access to the facilities at the Chemical Analysis Laboratory at CIMAV Monterrey, which were crucial for the determination of minerals. We also thank Daniel Alberto León Soqui for his assistance in the determination of phenolic compounds. Their contributions were essential to the success of this research.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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