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Antioxidant Evaluation of Extracts from the Peel of the Prickly Pear Cactus *Opuntia streptacantha*

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Abstract The species *Opuntia streptacantha*, commonly known as the cardon cactus, belongs to the Cactaceae family of plants. The fruit is known as tuna, and its pulp provides carbohydrates, proteins, dietary fiber, and minerals. The peel is a rich source of antioxidant compounds, including phenolics, flavonoids, and betalains, which have been demonstrated to possess beneficial properties for human health. The objective of this study was to extract compounds from the peel of *Opuntia streptacantha*, or prickly pear cactus, and evaluate their antioxidant capacity. Two extractions were conducted, one with water and the other with 60% water-ethanol, resulting in total solids yields of 4.0% and 5.3%, respectively. The ethanol extraction resulted in a higher concentration of phenolic compounds and antioxidant capacity, as evidenced by the total phenol yield of 19.98 mg gallic acid equivalents (GAE/g), flavonoid yield of 14.72 mg catechin equivalents (CE/g), and betalain yields of 0.77 mg betanin/g and 0.58 mg indicaxanthin/g. The antioxidant capacity was determined by the mean maximum effective concentration (EC_{50} µg/mL), with the following results: H_2O_2 = 1,141.0 ± 28.21 µg/mL, ROO- = no reaction, NO- = 87.0 ± 9.5 µg/mL, OH- = 1,017.0 ± 135.1 µg/mL, O_2 - = 26.88 ± 2.49 µg/mL, HCIO = 1,731 ± 51.2 µg/mL. The prickly pear peel extract was found to contain a number of compounds, including phenolic acids such as caffeic acid (5.53 mg/100g) and 3,4-dihydroxybenzoic acid (1.81 mg/100g), as well as flavonoids such as isorhamnetin 3-rutinoside (94). Additionally, the extract contains 0.07 mg/100 g of isorhamnetin, 47.69 mg/100 g of rutin, and 0.94 mg/100 g of quercetin. It also comprises betalains, including betanin (12.30 mg/100 g) and isobetanin (16.34 mg/100 g).

Keywords: *Prickly pear cactus, Opuntia streptacantha, peel, antioxidant capacity*

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1. Introduction

Beneficial properties of antioxidant compounds present in plants and vegetables, such as phenolic compounds, have been reported [1]. There are several ways to classify phenolic compounds, and one of them is to divide them into flavonoid and non-flavonoid types. Flavonoids include flavonols, flavones, isoflavones, flavanones, dihydroflavonols, anthocyanidins, and chalcones. The non-flavonoids include phenolic acids such as gallic, gentisic, caffeic, chlorogenic, sinapic, and ferulic acids.

Phenolic compounds are considered beneficial to the health of the population because they neutralize free radicals, such as reactive oxygen and nitrogen species (ROS and RNS), which are formed as a product of metabolism [2] and can cause harmful effects by disrupting vital functions at the cellular level in humans, leading to diseases such as cancer, diabetes, cardiovascular disease, hypertension, asthma, and aging [3].

The increase in ROS and RNS leads to oxidative stress and disease. Free radicals react with biomolecules such as lipids, proteins, and DNA, causing damage at the cellular level [4]. Free radicals include hydroxyl radical (OH^-), superoxide (O_2^-), nitric oxide (NO^-), nitrogen dioxide (NO_2^-), peroxyl (ROO^-), and lipid peroxyl (LOO^-) [5].

Antioxidant compounds have the ability to inhibit free radicals. They can be extracted from the edible part of fruits and vegetables, as well as from the peel, which is considered an organic waste that pollutes the environment [6]. Studies carried out on vegetable peels show interesting results, indicating that they are an important source of carbohydrates, proteins, fiber and phenolic compounds with high antioxidant capacity [7]. The peels of fruits such as the Hass avocado have a high content of pigments and phenolic compounds, in contrast to the pulp and seeds [8].

Research on bioactive compounds from the peel of nopal and prickly pear has brought economic benefits to the country at the agroindustrial level and to the health of the population [9]. The cactus is a cactus that easily adapts to hot and desert climates and is known to have been a source of food for pre-Columbian peoples in the Americas [10]. It is of great economic importance and its edible fruits, such as the prickly pear, are used in the diet in juices and jams due to their content of carbohydrates, lipids, fiber, and minerals, as well as fodder for livestock and vegetables for human consumption [11,12].

Prickly pear and cactus species of the genus Opuntia are rich in phenolic compounds, as in the case of the species *Opuntia streptacantha*, also known as Cardón cactus [13]. This species also contains pigments such as betalains, which in turn are subdivided into betacyanins and betaxanthins, and are used in the agrifood industry as natural colorants in culinary dishes, pastries, confectionery, sauces, yogurt, ice cream, and commercial wheat flour [14].

Betaxanthins have antioxidant activity [15] and, together with phenolic compounds, scavenge radicals that trigger degenerative diseases at both the cardiovascular and gastrointestinal levels in humans [16]. Tri-amine-betaxanthin, alanine-betaxanthin, histamine-betaxanthin, and 3-methoxytyramine-betaxanthin have been shown to have greater antioxidant, anti-inflammatory, and detoxifying capacities compared to vitamin C and flavonoids such as rutin and catechin [17,18].

Peel of the prickly pear cactus *Opuntia streptacantha* contains a large amount of phenolic compounds and is suitable for extraction [19]. Various techniques and methods are used to obtain plant extracts rich in antioxidant compounds [20]. A conventional extraction method that is still used is maceration, in which the plant tissue is crushed and mixed with solvents such as water and alcohol and left to rest for a certain period [21,22].

Few investigations have been carried out on the extraction of pigments and antioxidant compounds from the peel of the prickly pear of the genus Opuntia, so the objective of the present study was to evaluate the content of phenolic compounds and betalains of two different extracts of the peel of *Opuntia streptacantha* prickly pear and to measure its antioxidant capacity.

2. Materials and Methods

2.1. Plant Collection

The collection of *O. streptacantha* cactus was carried out in the municipality of Amacueca, in the state of Jalisco, Mexico, during the month of July 2020. Botanical identification of the species was carried out by the Herbarium of the Faculty of Biology of the University of Michoacana of San Nicolás de Hidalgo. The prickly pears were washed and disinfected with a solution of sodium hypochlorite for 30 minutes, rinsed and the thorns were removed manually. Subsequently the peel was cut into 2x2 cm squares and dried in an oven (ECOSHEL® 9053 A) at 50 °C for 24 h. The dehydrated shells were ground in a blender and sieved through a #40 mesh (400 µ). The powder sample obtained was stored in sealed amber glass bottles at a temperature of -10°C, taking care not to expose it to any light source.

2.2. Extracts

The antioxidant compounds present in the dehydrated samples of the peel of the prickly pear *O. streptacantha* were extracted by maceration according to the methodology of Zourgui *et al*. [6] with some modifications. Two extracts were prepared with different solvents, water and the 60:40% ethanol-water mixture, respectively. Each extraction was carried out in triplicate. 10 g of the dehydrated sample were added to each of the conical flasks containing distilled water and the 60:40% ethanol-water mixture. These were covered with aluminum foil and left at room temperature on a stirring plate (Cscientific cvp-2000p) for 24 to 48 h. They were then filtered with filter paper (Whatman® No. 42). The filtered solution was evaporated using a vacuum rotary evaporator (Vante RE 100-Pro). The extracts obtained were stored in amber glass bottles protected from light and at a temperature of -10°C until further analysis.

2.3. Phenolic and Betalain Content

Total phenolics

The determination of total phenols was carried out according to Hernández-Carranza *et al*. [23], by comparison with a calibration curve using gallic acid as a standard. In test tubes, a mixture was prepared separately with 2 mL of Na_2CO_3, 2.5 mL of 1N Folin-Ciocalteu reagent and 500 µL of each extract from the peel of the prickly pear *O. streptacantha*. The mixture was placed in a water bath at 50°C for 10 min and the absorbance was measured at 750 nm using a spectrophotometer (UV/Vis Spectrophotometer, VE-5600UV). The results were performed in triplicate and expressed as mg of gallic acid per gram of extract (mg GAE/g).

Total Flavonoids

Total flavonoids were evaluated by comparison with a standard calibration curve using catechin as the standard element and according to Zhishen *et al*. [24]. Briefly, 2 mL of the extract and a 2% $AlCl_3$ solution were added to a test tube, the tube was vortexed for a few minutes, the sample was incubated for 1 h at 20 °C, and the reading was taken at an absorbance of 490 nm in a spectrophotometer (UV/Vis spectrophotometer, VE-5600UV). Quantification was performed in triplicate and the result was expressed as mg catechin/g (mg CE/g).

Betalaíns

Betalain content was determined by the method described by Stintzing *et al*. [25]. The diluted extracts were prepared with McLvaine buffer (pH 6.5) and read at an absorbance of 535 and 483 nm. Results were expressed as betacyanins and indicaxanthin equivalents.

2.4. Evaluation of Antioxidant Capacity

The antioxidant capacity of *O. streptacantha* cactus rind extract was evaluated using spectrophotometric methods, which assessed the ability to scavenge oxidant

species of biological relevance, such as superoxide anion ($-O_2^-$), peroxyl (ROO-), hydroxyl (OH), hypochlorous acid (HClO), hydrogen peroxide (H_2O_2), and nitric oxide (NO).

Peroxyl (ROO^-)

The peroxyl radical (ROO^-) scavenging capacity was determined according to the method described by López & Lissi [26]. 10 mL of the extracts were prepared at different concentrations [200 to 4,000 ppm] with an ethanol/H_2O solution. A solution containing 1.5 mL of red pyragol (30 µM) was mixed with 150 µL of solutions of the extracts and Oligopin at different concentrations in test tubes, then 25 µL of 2-azobis (2-methylpropionamidine) dihydrochloride (AAPH) was added.) [600 mM], were placed in a water bath at 37°C for 2 hours, and the absorbance was measured at 540 nm in a spectrophotometer (UV/Vis Spectrophotometer, VE-5600UV).

Superoxide anion($\bullet O_2^-$)

The ability to scavenge the superoxide anion ($-O_2^-$) was evaluated by a non-enzymatic method described by Nishikimi *et al*. [27]. 20 mL of the extract were prepared at different concentrations [1,500 ppm] and the standard quercetin [200 ppm]. 1 mL nitro blue tetrazolium (NTB), 1 mL NADH and 1 mL extract were mixed. The reaction was started with 150 µL phenazine methosulfate (PMS) and transferred to a water bath for 15 min. The absorbance of the solutions was read using a spectrophotometer (UV/Vis Spectrophotometer, VE-5600UV) at a wavelength of 560 nm.

Nitric oxide (NO^-)

Nitric oxide (NO^-) radical uptake was determined by the method described by Sreejayan [28]. The extracts were prepared with sodium nitroprusside [10 mM] in phosphate buffer (0.5 M, pH 7.4) with 0.5 mL of samples containing the extracts [500 ppm] and the curcuma standard [250 ppm] at different concentrations and then incubated at 37°C for 2. Then 0.5 mL of Griess reagent was added and allowed to stand at room temperature for 1 h. The absorbance was measured at 548 nm in a spectrophotometer (UV/Vis Spectrophotometer, VE-5600UV).

Hydrogen Peroxide (H_2O_2)

For the ability to eliminate hydrogen peroxide (H_2O_2), what was done by Ruch *et al*. [29]. 20 mL of the extract with different concentrations [1,500 ppm] and the gallic acid standard [500 ppm] were prepared. To 1.7 mL of the extract and standard solution (gallic acid), 600 µL of H_2O_2 solution [40 mM] were added and allowed to stand for 3 min at room temperature. The absorbance of the solutions was measured in a spectrophotometer (UV/Vis Spectrophotometer, VE-5600UV) at a wavelength of 230 nm.

Hypochlorous acid (HClO)

The method of Aruoma & Halliwell [30] was used with slight modifications to calculate the ability to inhibit hypochlorous acid (HClO). In a test tube, 1 mL of the extract at different concentrations [2,500] and the vitamin C standard [150 ppm] was added to 1 mL of HClO [18 mM] in phosphate buffered saline (PBS) and allowed to stand for 15 min in a water bath at 37°C, 1 mL of catalase [1 mg/mL] was added for 15 min at 37°C. Reading was performed at a wavelength of 245 nm.

Hydroxyl (OH^-)

To calculate the scavenging capacity of the hydroxyl radical (OH^-), it was determined according to the method formulated by Smirnoff & Cumbes [31]. The extract solutions were prepared at different concentrations [1,500 ppm] and the gallic acid standard [500 ppm]. 1 ml of the extract solution, 300 µL $FeSO_4$ [8 mM], 250 µL H_2O_2 [20 mM] were added to test tubes. To start the reaction, 1 ml of salicylic acid solution [3 mM] was added and then incubated at 37°C for 30 min, then distilled water was added up to 450 µl, centrifuged at 3500 rpm for 15 min, and the supernatant was collected, and the absorbance (A1) was measured at a wavelength of 510 nm using a spectrophotometer (UV/Vis spectrophotometer, VE-5600UV).

2.5. Characterization of Compounds by HPLC Chromatography

The ethanolic extract was evaluated by chromatography because it showed the best values for total phenolic compounds, total flavonoids, and antioxidant capacity. A high-performance liquid chromatograph (HPLC, Agilent 1260 Infinity Series) equipped with an autosampler, column oven, diode array detector (DAD), fluorescence detector (FLD) and Discovery C18, 250 × 4.6 analytical column was used., 5 µm, according to the methodology reported by Schieber *et al*. [32] and Albano et al. [33].

2.6. Statistical Analysis

All assays were performed in triplicate using GraphPad Prism 8 software. Results were expressed as mean ± standard deviation (SD). The yield of the extract and the amount of phytochemicals such as total phenolics, flavonoids, betacyanins, and betaxanthins were determined using a Student's t-test ($p < 0.05$). Analysis of variance (ANOVA) and Tukey test ($p<0.05$) were used for antioxidant capacity. Spearman correlation tests ($p<0.05$) were used to evaluate the correlation between phytochemicals and antioxidant capacity of the extract.

3. Results

3.1. Phenolic Compounds and Betalains

Table 1 shows the yield and phytochemical contents of the extracts from the peel of prickly pear *O. streptacantha*, where significantly ($p<0.05$) higher values of yield, total phenolic compounds and total flavonoids were observed in the ethanol extract. The values of yield, total phenolic compounds and total flavonoids were 5.30±0.60%, 19.98±0.42 mg GAE/g and 14.72±0.48 mg CE/g, respectively, in the ethanolic extract of *O. streptacantha* compared to the extract in water where the yield was 4.00±0.80%, the values of total phenolic compounds were 14.88±0.26 mg GAE/g and the values of total flavonoids were 12.07±0.12 mg CE/g. The contents of total phenolic compounds obtained in the present investigation were similar to those reported by Zourgui *et al*. [6] in prickly pear peel of *O. streptacantha*, these authors reported a higher content of total phenolic compounds and total

flavonoid compounds being 24.65 mg GAE/g and 14.08 mg CE/g, respectively, in ethanol-water extracts, compared to 12.78 mg GAE/g of phenolic compounds and total flavonoid compounds, with 8.95 mg CE/g obtained in aqueous extracts.

On the other hand, the extraction of betacyanins and betaxanthins was favored in water, where there were significantly higher concentrations ($p<0.05$) of betacyanins, 1.97±0.11 mg BET/g, and of betaxanthins, 1.35±0.33 mg IN/g, in relation to those obtained in the ethanol-water extract, which were 0.77±0.03 mg BET/g and 0.58±0.04 mg IN/g of betacyanins and betaxanthins, respectively.

A recent study showed that the peels of wild prickly pears of different Opuntia species cultivated in Mexico, such as *O. macrocentra, O. phaeacantha, O. engelmannii and O. ficus-indica*, are important sources of phenolic compounds and betalains, these authors reported for the different Opuntia species of total phenolics ranging from 3. 70-7.11 mg GAE/g, flavonoids from 3.18-5.01 mg CE/g, betacyanins from 0.09-1.16 mg/g and betaxanthins from 0.06-0.08 mg/g in methanolic extracts, these values were lower than those shown in the present investigation for the peel of *O. streptacantha* species. It is important to mention that the variations in the content of phenolic compounds and betalains in the extracts are related to various factors, including the Opuntia species, fruit maturity, climate, extraction method and solvent, as well as phytochemical quantification methods [34,35]. However, the results show that the shells of *O. streptacantha* species may be a better source of bioactive compounds than the shells of other Opuntia species.

In the extraction of phytochemical compounds, one of the most important factors is the polarity of the solvent, as it has a great impact on the type of compounds to be extracted and their performance [36]. Previous research has shown that solvents such as water, methanol, ethanol or their combinations are ideal for the efficient extraction of both phenolic compounds and betalains [6,23]. According to the data obtained, total phenolic compounds were less polar than betalains, as phenolic compounds were better extracted with ethanol-water and betalains were more efficiently extracted in water. The extraction yield of total phenolic compounds and total flavonoid compounds was higher in ethanol-water than in water, reaching a value of 34.2% and 21.9%, respectively. On the other hand, the yield of betacyanins and betaxanthins was higher in water than in ethanol-water by 155.8% and 132.7%, respectively.

3.2. Antioxidant Activity

Table 2 shows the antioxidant activity against the radicals NO^-, H_2O_2, HClO, O_2^-, OH^-, ROO^- of the aqueous and ethanolic extracts of *O. streptacantha*, where it is observed that the ethanol-water extract presented significantly ($p<0.05$) greater antioxidant activity against these radicals, compared to the water extract. The results indicated that the bioactive compounds extracted with ethanol-water showed greater activity against free radicals (ROS and RNS) in the following order $O_2^- > NO^- > OH^- > H_2O_2 > HClO > ROO^-$.

An evaluation was carried out with different standards used to estimate the antioxidant activity against the respective radicals (Table 2). The values of quercetin and the ethanol-water extract of *O. streptacantha* did not show a statistically significant difference ($p<0.05$) against the inhibition of the O_2^- radical, obtaining EC50 values of 26.88±2.49 µg/mL and 20.25±0.77 µg/mL. mL, respectively. On the other hand, the ethanol-water extract of *O. streptacantha* peel showed an EC_{50} of 87.0±9.5 µg/mL and turmeric showed an EC_{50} of 119.5±3.3 µg/mL against the NO^- radical.

These results indicated that the hydroalcoholic extract of *O. streptacantha* peel was more effective than turmeric in scavenging NO^- radicals. However, the hydroalcoholic extract of *O. streptacantha* showed lower activity against H_2O_2 radicals (1,141.0±28.21 µg/mL) and HClO (1,731±51.2 µg/mL) compared to vitamin C (48.31±7.36 and 108. 9±2.9 µg/mL), and lower activity against OH^- radicals (1,017.0±135.1 µg/mL) compared to gallic acid (90.80±4.05 µg/mL), and no effect against ROO radicals (>4,000 µg/mL).

In a study by Avila-Nava *et al.* [37], who investigated the antioxidant mechanisms of *O. ficus-indica* cladode extracts, they found EC_{50} values for OH^- radicals of 5600 µg/mL and O_2^- of 1874 µg/mL, which were higher than those previously reported. However, in the present study, these authors reported lower values for the radicals HClO (180 µg/mL) and H_2O_2 (6.9 µg/mL) than those we reported in the *O. streptacantha* prickly pear peel extracts (Table 2). These differences suggest that the antioxidant mechanism is specifically influenced by the type of active molecule present in each extract.

Table 1. Composition of phytochemicals extracted from the peel of prickly pear cactus *O. streptacantha*

	Extraction	
	Water	Ethanol-Water
Yield (%)	4.00±0.80[b]	5.30±0.60[a]
Total phenolics (mg GAE/g)	14.88±0.26[b]	19.98±0.42[a]
Total flavonoids (mg CE/g)	12.07±0.12[b]	14.72±0.48[a]
Betacyanins (mg BET/g	1.97±0.11[a]	0.77±0.03[b]
Betaxanthins (mg IN/g)	1.35 ±0.33[a]	0.58±0.04 [b]

GAE, gallic acid equivalents; CE, catechin; BET, betacyanins; IN, indicaxanthin. Values are expressed as mean ± standard deviation. Different letters indicate statistical differences with a significance of $p<0.05$. Student's t test.

Table 2. Antioxidant activity of *O. streptacantha* cactus peel extracts

Sample type	NO• EC_{50} (µg/mL)	H_2O_2 EC_{50} (µg/mL)	HClO EC_{50} (µg/mL)	$O_2^{\bullet-}$ EC_{50} (µg/mL)	OH• EC_{50} (µg/mL)	ROO• EC_{50} (µg/mL)
Water Extract	148.7±14.46[a]	1,291.0±86.85[a]	>2000	41.48±9.68 [a]	1,335.0±142.0 c [a]	>4,000
Extract Ethanol-Water	87.0±9.5[c]	1,141.0±28.21 c [b]	1,731±51.2[a]	26.88±2.49 [b]	1,017.0±135.1 c [b]	>4,000
Curcum	119.5±3.3[b]					
Vitamin C		48.31±7.36 [c]	108.9±2.9 [b]			
Quercetin				20.25±0.77 [b]		
Gállic Acid					90.80±4.05 [c]	
Oligopine						701.3±13.05

Values are expressed as mean ± standard deviation. Different letters indicate statistical differences with a significance of p<0.05. (ANOVA, Tukey test).

Table 3. Correlation (Spearman) between the antioxidant activity and the content of total phenolics, flavonoids, betacyanins and betaxanthins of water and ethanol-water extracts of *O. streptacantha*

	$NO^{\bullet}$	H_2O_2	HClO	$O_2^{\bullet}$	$OH^{\bullet}$
Total phenolics	r = -0.94* p = 0.017	r = -1.0* p = 0.003	r = -0.50 p = 0.990	r = -0.900* p = 0.016	r = - 0.50 p = 0.990
Total flavonoids	r = -0.88* p = 0.033	r = -0.75 p = 0.115	r = -0.50 p = 0.990	r = -0.657* p = 0.175	r = - 0.50 p = 0.990
Betacyanins	r = -0.89* p = 0.028	r = 0.81* p = 0.033	r = -0.86 p = 0.667	r = -0.98* p = 0.006	r = - 0.866 p = 0.667
Betaxanthins	r = -0.88* p = 0.03	r = 0.88* p = 0.055	r = -0.50 p = 0.990	r = -0.88* p = 0.033	r = - 0.50 p = 0.990

* Correlation is significant (p<0.050)

The antioxidant mechanisms of phenolic acids and flavonoids are due to their molecular structure composed of phenolic rings in resonance with one or more hydroxyl groups (OH^-), therefore they can eliminate chemical species that initiate peroxidation, preventing the formation of hydroxyl radicals. coming from the Fenton reaction and deactivation of the superoxide anion [15]. Betacyanins have also been shown to be good donors of electrons and hydrogen atoms [38].

Table 3 presents a Spearman correlation analysis, where the association between the different bioactive compounds and their ability to scavenge different types of free radicals is observed. The data showed that the phenolic compounds present in *O. streptacantha* extracts have the ability to inhibit NO and H_2O_2 radicals (r= -0.94, p= 0.017 and r= -1.0, p= 0.003). Flavonoids showed a high and significant correlation (r= -0.88, p= 0.033) to eliminate NO^- radicals. On the other hand, betacyanins had the ability to neutralize NO^- and O_2^- radicals (r= -0.89, p= 0.028 and r=-0.98, p= 0.006, respectively) and betaxanthins had a high and significant effect against NO^- and O_2^- radicals (r= -0.88, p= 0.03 and r= -0.88, p= 0.033, respectively).

It was shown that the antioxidant capacity presented by the different bioactive compounds in the cactus pepper peel extracts of *O. streptacantha* is mainly related to the scavenging of nitric oxide (NO^-), followed by superoxide anion (O_2^-) and finally nitric peroxide hydrogen (H_2O_2). This is the first study to specifically demonstrate the ability of different bioactive compounds present in *O. streptacantha* peel extracts to inhibit radicals of biological interest, such as ROS and RNS.

Kang et al [39] showed that *O. humifusa* cladode extracts can have anti-inflammatory effects by inhibiting NO production in mouse cell lines and identified the isorhamnetin isomers, which are flavonoids, as responsible for this effect. A recent study showed that *O. streptacantha* fruit extracts protect against liver damage in a rat model by reducing mitochondrial O_2^- production and thus oxidative stress, an effect attributed to flavonoids, phenolic compounds and betalains [40]. It is crucial to understand the mechanism of action of the different molecules, since their potential application in the treatment of various diseases depends on it.

The ethanol-water extract of *O. streptacantha* peels was the one that showed the greatest activity in eliminating ROS and RNS, so it was selected to carry out the identification and quantification of its phenolic compounds and betalains specifically.

3.3. Identification and Quantification of Phenolic Compounds and Betalains by HPLC

Table 4 shows the concentration and retention time of the phenolic compounds and betalains that were identified in the ethanol-water extract of the prickly pear peels of *O. streptacantha* and in Figure 1 the representative chromatograms in the identification of these compounds bioactives, where their retention time and type of molecule are indicated. Among the phenolic acids detected, caffeic acid was found at a concentration of 5.53 mg/100 g and 3,4-dihydroxybenzoic acid at 1.81 mg/100 g. On the other hand, a greater quantity and variety of flavonoids were found, such as isorhamnetin-3-rutinoside (94.07 mg/100 g), isorhamnetin (47.69 mg/100 g), rutin (3.07 mg/100 g) and quercetin (0.94 mg/100 g). 100g). And some betalains were identified and quantified, such as betanin (12.30 mg/100 g) and isobetanin (16.34 mg/100 g).

The evaluation of the concentration and type of secondary metabolites present in the prickly pear peel extract of *O. streptacantha* is essential to know their potential effects on the body, as well as their technological

application. López-Palacios and Peña-Valdivia [41] reported on the phytochemical profile of the cladodes of *O. streptacantha*, where the presence of caffeic acid was detected at 0. 513 mg/100 g, which was a lower concentration than that determined in the present study (Table 4); however, these authors also identified chlorogenic acid, p-coumaric acid, ferulic acid, vanillic acid and syringic acid in the cladodes, which were not detected in the peels of prickly pear fruits of *O. streptacantha* in our study. On the other hand, the flavonoids found in the cladodes of *O. streptacantha* included isorhamnetin (0.951 mg/100 g), rutin (0.357 mg/100 g), and epigenin (0.024 mg/100 g), and in the hydroalcoholic extract of the peels of the fruits of *O. streptacantha* in the present study, the presence of isorhamnetin-3-rutinoside, isorhamnetin and rutin was determined in higher concentrations than those reported by López-Palacios and Peña-Valdivia [41], and also confirmed the presence of quercetin, as shown in Table 4. It is important to mention that in both the cladodes and the peel of the fruit of *O. streptacantha*, the predominant type of flavonoid was isorhamnetin (and its glycosides in the peel of the fruit).

There are many factors that can influence the biosynthesis and accumulation of secondary metabolites, including genetic, morphogenetic, and environmental factors, among others [42]. However, it has been shown that isorhamnetin and its glycosides are the flavonoids found in the highest concentration in extracts of the genus Opuntia, mainly in the peel of its fruits, and their presence has been described as a biochemical fingerprint [43]. Previous research has shown that the flavonoids present in Opuntia, particularly isorhamnetin and its glycosides, reduce the inflammatory process by reducing lipid peroxidation and restoring antioxidant systems such as superoxide dismutase, catalase and glutathione, which counteract the effects of oxidative stress in animal models. [43,44,45].

Gómez-López *et al*. [46] analyzed the betanin profile of methanolic extracts of the fruit peels of *O. stricta var*. Dilleni and found values of betanin of 83.12 mg/100 g and isobetanin of 42.78 mg/100 g, which are concentrations higher than those reported in the present study, which were 12.30 and 16.34 mg/100 g of betanin and isobetanin, respectively. It is important to mention that betalain content varies depending on Opuntia species and extraction method, among other factors [34,35]. Betalains are important pigments in the peels of Opuntia sp. Fruits, where betanins predominate and have been shown to have effects on oxidative stress in humans due to their high bioavailability, they prevent DNA damage induced by radicals, peroxynitrite ($ONOO^-$) and hydrogen peroxide (H_2O_2) [46,47], have also been associated with hepatoprotective and anti-inflammatory effects [48].

The research conducted by Gómez-López *et al*. [46] showed that the gastrointestinal bioavailability of phenolic compounds and betalains present in the peels of the fruits of *O. stricta var*. Dilleni is comparable and even greater (depending on the type of compound) than those present in the pulp of the fruits. Therefore, although the peels of fruits of the genus Opuntia have traditionally been considered organic waste, recent research, such as this one, has shown that they are a valuable source of bioactive compounds, and their antioxidant activity suggests great potential in combating oxidative stress. present in various diseases.

Table 4. Identification and quantification of phenolic compounds and betalains in the ethanol-water extract of *O. streptacantha* fruit peels by HPLC

Compound	Retention Time (min)	Concentration (mg/100 g)
Caffeic acid	20.84	5.53
3,4-dihydroxybenzoic acid	16.94	1.81
t-ferulic acid	31.72	3.55
Isorhamnetin	59.27	47.69
Isorhamnetin 3-rutinoside	39.06	94.07
Quercetin	51.10	0.94
Routine	33.84	3.07
Betanin	18.62	12.30
Isobetanine	21.06	16.34

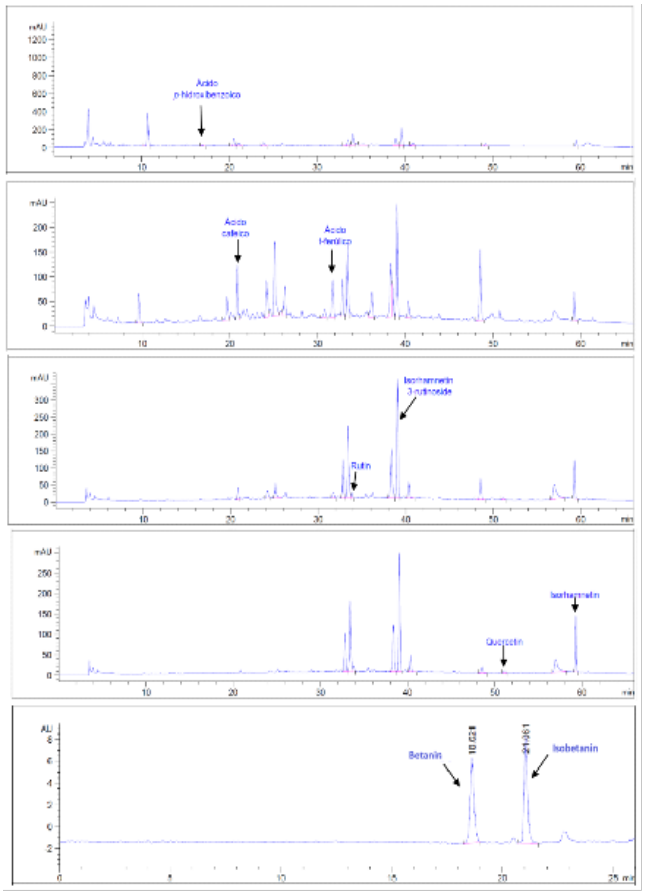

Figure 1. Representative chromatograms of the analysis of phenolic compounds and betalains in the ethanol-water extract of *O. streptacantha* peels by HPLC

4. Conclusions

A higher yield of solids, a higher concentration of total phenolic compounds and total flavonoid compounds and a higher antioxidant capacity were obtained when the extraction was performed with the 60% ethanolic solution. Therefore, this is a new alternative for the use of *Opuntia streptacantha* prickly pear peel waste as an ingredient for

the formulation of functional foods for the prevention of diseases related to oxidative stress.

Conflict of Interest

All authors declare no conflicts of interest.

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