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Bioactivity, hydrophilic, lipophilic and volatile compounds in pulps and skins of *Opuntia macrorhiza* and *Opuntia microdasys* fruits

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ABSTRACT

Opuntia genus includes several species able to grow in arid regions and known for producing delicate fruits, which are far from being thoroughly characterized. Herein, fruits from *O. macrorhiza* and *O. microdasys* were divided in skins and pulps (without including seeds) and studied for chemical composition, individual phytochemicals and bioactivity. The major volatiles were camphor and ethyl acetate, while citric acid was the main organic acid. The fatty acids detected in highest percentages were linoleic acid (skins) and lauric acid (pulps); α-tocopherol was the major isoform of vitamin E. Quercetin-3-*O*-rutinoside and quercetin-*O*-(deoxyhexoside-rutinoside) were the main phenolics in fruit skins of *O. macrorhiza* and *O. microdasys* (respectively), similarly to piscidic acid in *O. macrorhiza* pulp (*O. microdasys* pulp showed no quantifiable compounds). Betanin and isobetanin were the major betalains. All samples were antioxidant (particularly *O. macrorhiza*), but antimicrobial activity was only detected in skins. Cytotoxicity was low in all cases. Overall, these fruits proved to be potential new ingredients for food or pharmaceutical related applications, adding value to these natural species able to grow in arid environments.

1. Introduction

Opuntia genus aggregates nearly 1500 species of cactus, which are mainly distributed in Africa, Mediterranean countries, Southwestern United States, and Northern Mexico (Matthäus & Özcan, 2011). These species have the advantage of being able to grow wildly in arid regions and their fruit (specifically the mesocarp) is usually considered a delicacy (Ramadan & Mörsel, 2003), being consumed fresh or used in the form of candies, jellies or beverages (Jimenez-Aguilar, Mujica-Paz, & Welti-Chanes, 2014). It contains soluble sugars, dietary fiber, ascorbic acid, carotenoids, flavonoids, phenolic acids, betacyanins, coumarins, terpenes, alkaloids, and steroids, having some effective uses in folk medicine, *e.g.*, to treat asthma, stomach ulceration or indigestion (Chahdoura et al., 2015a; Chahdoura et al., 2015b; Chougui et al., 2013; Morales, Ramírez-Moreno, Sanchez-Mata, Carvalho, & Ferreira, 2012; Phillips et al., 2010; Pímienta-Barríos, Méndez-Mor-acan, Ramírez-Hernández, García de Alba-García, & Domínguez-Arias, 2008; Samah, Ventura-Zapata, & Valadez-Moctezuma, 2015; Schaffer, Schmitt-Schillig, Müller, & Eckert, 2005). The characterization of phenolics compounds, owing to their high antioxidant (Chougui et al., 2013), neuroprotective, anti-inflammatory, cardioprotective, hepatoprotective and antidiabetic activities (Kaur, Kaur, & Sharma, 2012) is of special interest. However, *Opuntia* spp. also contain high levels of volatiles (with a high predominance of camphor) (Chahdoura et al., 2016), which also represent a relevant field of study, owing to their progressive use by food and pharmaceutical industries (Mekni et al., 2013).

Accordingly, and taking into consideration the increasing dissemination of *Opuntia* spp. fruits, this study was designed to chemically characterize sugars, organic acids, tocopherols, fatty acids, volatiles, phenolic acids, flavonoids and betacyanins, as also to evaluate the antimicrobial activity, antioxidant activity and cytotoxicity in two

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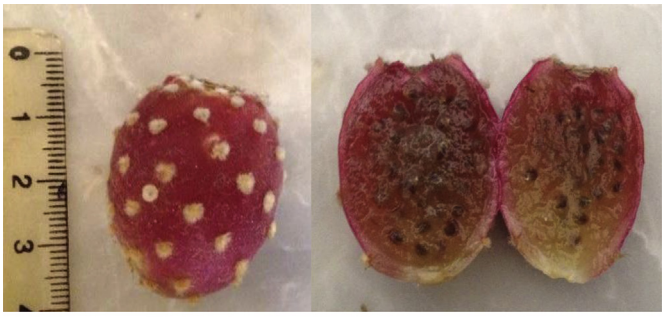

(A)

(B)

Fig. 1. Morphological appearance of *Opuntia microdasys* (A) and *Opuntia macrorhiza* (B) fruits. Inner and outer color, as well as skin to pulp ratio, can be visualized in the cut fruits. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

botanical components (pulp and skin) of *O. microdasys* and *O. macrorhiza*.

It is expected that the obtained results might contribute to promote the use of these fruits as sources of functional ingredients for food industry or other related applications.

2. Materials and methods

2.1. Sampling

Samples from both species (*Opuntia macrorhiza* and *Opuntia microdasys*) fruits were manually harvested (27 fruits for each species) from the cliff of Monastir in 2016. The harvesting time was defined according to fruit size (at least 3 cm long for *O. microdasys* and 5 cm long for *O. macrorhiza*) and skin color (rose-orange for *O. microdasys* and purple for *O. macrorhiza*), firmness (mature fruits are firm to touch) and after the glochids fell off (Fig. 1). Fruits free of external injuries were selected, washed and manually peeled and the resulting skin and pulp were lyophilized (LabConco, FreeZone, −105 °C, 4.5 L Cascade Benchtop Freeze Dry System, Kansas, MO, USA) and stored in a cool and dry place until use.

2.2. Phytochemical composition

In order to obtain the complete chemical characterization of the skins and pulps of *O. microdasys* and *O. macrorhiza*, their profiles in volatile, and selected hydrophilic and lipophilic compounds were analyzed.

2.2.1. Volatiles

All stages of volatile compounds analysis were detailed in a previous report (Chahdoura et al., 2016). The same solid phase micro-extraction (SPME) sampling and desorption conditions were applied for all samples. A set of blanks was measured before the first extractions and repeated at random intervals.

The GC-Electron Impact Mass Spectrometry (EIMS) analyses were performed in a Varian CP3800 (Palo Alto, CA) gas chromatograph. A DB-5 capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA, USA) and a Varian Saturn 2000 ion trap mass detector were used.

For identification purposes, the retention times and linear retention indexes were considered and compared with commercial (NIST 2014 and ADAMS) and laboratory-developed mass spectra libraries, besides checking MS data reported in literature (Adams, 2007; Davies, 1990; Jennings & Shibamoto, 1980, p. 480).

2.2.2. Hydrophilic compounds

2.2.2.1. Soluble sugars. The analyses were conducted in a high performance liquid chromatograph (HPLC) using a refraction index (RI) detector (Knauer, Smartline system 1000, Berlin, Germany) (Barreira, Pereira, Oliveira, & Ferreira, 2010). The separation of compounds was done in a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) maintained inside a thermostatted (30 °C) compartment (7971 R Grace Oven). Quantification (g/100 g of dry weight) was based in the internal standard (melezitose) method.

2.2.2.2. Organic acids. In this case, the selected equipment was an ultra-fast liquid chromatograph assembled with a photodiode array detector (UFLC-PDA; Shimadzu Corporation, Kyoto, Japan). Organic acids were separated through a Sphere Clone (Phenomenex, Torrance, CA, USA) reverse phase C_{18} column (5 μm, 250 mm × 4.6 mm i.d.), which was also maintained at constant temperature (35 °C) (Barros, Pereira, & Ferreira, 2013a). For quantification (g/100 g of dry weight) purposes, the intensities obtained at 215 and 245 nm were compared with commercial standards.

2.2.2.3. Phenolic compounds. Lyophilized samples were macerated twice (1 h + 1 h) under the same conditions (solid to liquid ratio: 1:25, 25 °C, 150 rpm) in ethanol:water (80:20 v/v). The extracts obtained after each hour were combined, filtered (Whatman no. 4 paper), dried (evaporation under reduced pressure followed by lyophilization) and further dissolved (5 mg/mL) in the extracting solvent.

The chromatographic conditions (LC-DAD-ESI/MSn) applied to obtain the phenolic profiles were previously optimized (Bessada, Barreira, Barros, Ferreira, & Oliveira, 2016). The negative mode was selected to work in the mass spectrometer.

The identification of compounds was based in chromatographic and spectral data and the quantification (mg/g of dry extract) was made from the UV–Vis absorption; the following standards (Extrasynthèse, Genay, France) were used: (A) ferulic acid; (B) *p*-coumaric acid; *p*-hydroxybenzoic acid; (D) quercetin-3-*O*-glucoside.

2.2.2.4. Betalains. The extracts described in the previous section were also characterized for their betalains profile using the same LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) system, in this case equipped with a Waters Spherisorb S3 ODS-2 C18 (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column (Roriz, Barros, Prieto, Morales, & Ferreira, 2017). The DAD intensities obtained at 480 nm (betaxanthins) and 530 nm (betacyanins) and the MS data (positive mode) were considered for compounds identification. Quantification (mg/g of extract) was done using the calibration curve (gomphrenin III: $y = 14{,}670x - 19{,}725$, $R^2 = 0.9997$).

2.2.3. Lipophilic compounds

2.2.3.1. Fatty acids. Fatty acids profiles were obtained by gas-liquid chromatography with flame ionization detection (GC-FID, 260 °C; DANI model GC 1000, Contone, Switzerland), applying previously optimized chromatographic conditions (Barros et al., 2013b). The identification

Table 1
Volatile compounds (relative percentage) detected in the skins and pulps of *Opuntia microdasys* (Lehm.) and *Opuntia macrorhiza* (Engelm.).

Constituents	LRI	*O. microdasys*		*O. macrorhiza*		*t*-Student test	
		skin	pulp	skin	pulp	skins	pulps
Ethyl acetate	616	–	–	–	54 ± 1	–	–
Isoamyl alcohol	736	–	–	–	0.60 ± 0.01	–	–
1-pentanol	771	–	–	–	0.31 ± 0.03	–	–
Hexanal	803	0.61 ± 0.04	–	1.12 ± 0.05	–	< 0.001	–
Butyl acetate	813	–	–	–	0.22 ± 0.02	–	–
(E)-3-hexen-1-ol	853	–	–	–	0.29 ± 0.04	–	–
1-hexanol	870	1.0 ± 0.1	1.9 ± 0.01	–	1.8 ± 0.1	–	0.030
Isopentyl acetate	878	–	–	–	2.6 ± 0.1	–	–
Heptanal	902	0.8 ± 0.1	–	1.5 ± 0.1	–	< 0.001	–
1-pentyl acetate	918	–	–	–	3.9 ± 0.1	–	–
1-heptanol	971	–	1.2 ± 0.0	–	0.31 ± 0.04	–	< 0.001
3-octanone	986	–	–	–	0.26 ± 0.03	–	–
-6-methyl-5-hepten-2-one	897	0.59 ± 0.03	1.51 ± 0.05	1.03 ± 0.05	–	< 0.001	–
2–octanone	992	–	0.98 ± 0.05	–	–	–	–
2-pentyl furan	993	–	–	–	1.9 ± 0.1	–	–
Ethyl hexanoate	998	0.40 ± 0.04	–	–	–	–	–
n-decane	1000	1.5 ± 0.1	–	–	–	–	–
Octanal	1003	1.8 ± 0.1	–	0.67 ± 0.05	–	< 0.001	–
(Z)-3-hexenyl acetate	1008	–	–	–	3.6 ± 0.2	–	–
1-hexyl acetate	1010	–	–	–	15.3 ± 0.4	–	–
α-terpinene	1020	–	–	1.8 ± 0.1	–	–	–
p-cymene	1028	–	–	1.0 ± 0.1	–	–	–
Limonene	1032	–	–	4.2 ± 0.2	–	–	–
Benzyl alcohol	1034	2.2 ± 0.1	1.3 ± 0.1	2.1 ± 0.1	–	0.001	–
1,8-cineole	1035	–	2.1 ± 0.1	–	–	–	–
Isooctanol	1058	–	–	1.4 ± 0.1	–	–	–
(E)-2-octenal	1063	1.9 ± 0.1	–	–	–	–	–
1-octanol	1072	3.1 ± 0.2	8.9 ± 0.4	–	0.61 ± 0.04	< 0.001	< 0.001
cis-linalool oxide	1076	–	–	6.5 ± 0.2	–	–	–
trans-linalool oxide	1090	0.60 ± 0.04	–	6.3 ± 0.2	–	< 0.001	–
2-nonanone	1093	–	–	–	0.30 ± 0.04	–	–
n-undecane	1100	0.38 ± 0.04	–	–	–	–	–
Linalool	1101	–	5.1 ± 0.4	3.9 ± 0.2	0.58 ± 0.04	–	< 0.001
Nonanal	1104	16.4 ± 0.4	6.6 ± 0.2	6.9 ± 0.2	–	< 0.001	–
Phenylethyl alcohol	1111	–	–	6.5 ± 0.2	1.9 ± 0.1	–	–
Methyl octanoate	1128	0.43 ± 0.04	–	–	–	–	–
Camphor	1146	49 ± 1	26 ± 1	39 ± 1	1.2 ± 0.1	< 0.001	< 0.001
(E)-2-nonenal	1164	1.1 ± 0.1	–	0.87 ± 0.05	–	0.001	–
1-nonanol	1172	0.98 ± 0.05	22 ± 1	–	0.44 ± 0.05	–	< 0.001
α-terpineol	1191	–	–	0.81 ± 0.05	–	–	–
cis-Dihydrocarvone	1194	–	–	0.69 ± 0.05	–	–	–
Ethyl octanoate	1197	0.81 ± 0.05	–	–	–	–	–
n-dodecane	1200	–	–	0.89 ± 0.05	–	–	–
Decanal	1206	0.79 ± 0.05	2.2 ± 0.1	0.89 ± 0.05	–	0.015	–
1-octyl acetate	1213	0.34 ± 0.04	–	–	3.0 ± 0.1	–	–
Carvone	1244	–	1.8 ± 0.1	–	–	–	–
2-phenylethyl acetate	1258	–	–	3.3 ± 0.1	0.52 ± 0.04	–	
(E)-2-decenal	1263	0.51 ± 0.04	–	–	–	–	–
1-decanol	1273	–	1.8 ± 0.1	–	–	–	–
(E)-anethole	1285	–	3.3 ± 0.1	–	–	–	–
2-undecanone	1293	–	0.9 ± 0.1	–	1.8 ± 0.1	–	< 0.001
Undecanal	1307	0.79 ± 0.05	–	–	–	–	–
1-nonyl acetate	1313	–	–	–	2.6 ± 0.1	–	–
α-terpinyl acetate	1352	–	1.7 ± 0.1	–	–	–	–
Cyclosativene	1370	0.30 ± 0.03	–	–	–	–	–
α-copaene	1377	3.9 ± 0.2	–	1.3 ± 0.1	–	< 0.001	–
Ethyl decanoate	1385	0.72 ± 0.05	–	–	–	–	–
n-tetradecane	1400	–	–	–	0.19 ± 0.02	–	–
1-decyl acetate	1412	–	–	1.4 ± 0.1	–	–	–
cis-threo-davanafuran	1418	3.2 ± 0.1	1.3 ± 0.1	–	–	–	–
β-caryophyllene	1419	–	2.5 ± 0.1	–	–	–	–
(E)-Geranylacetone	1454	0.41 ± 0.01	–	–	–	–	–
Caryophyllene oxide	1582	–	1.5 ± 0.1	–	–	–	–
2-Ethylhexyl salicylate	1808	2.2 ± 0.1	1.4 ± 0.1	4.5 ± 0.4	–	< 0.001	–
Percentage of identified compounds		96.57	98.20	96.55	98.26	–	

LRI: linear retention indices on DB-5 column.

step was achieved by comparing the retention times of fatty acid methyl esters prepared from fatty acids present in *Opuntia* samples with a commercial standard mixture. For quantification purposes, peak areas were converted to relative percentages using CSW 1.7 software (DataApex 1.7, Prague, Czech Republic).

2.2.3.2. Tocopherols. Tocopherols composition was obtained in an HPLC (Knauer, Smartline system 1000, Berlin, Germany) system assembled with a fluorescence detector (FP-2020; Jasco, Easton, MD, USA) and a Polyamide II (5 μm, 250 × 4.6 mm) normal-phase column (YMC Waters America, Inc., Allentown, PA, USA). Compounds quantification (mg/100 g of dried weight) was done using the internal standard (tocol) method (Barros et al., 2013b).

2.3. Bioactive properties evaluation

2.3.1. Antioxidant activity

Each hydroethanolic extract prepared for phenolic compounds analysis was successively diluted (50–0.156 mg/mL). The obtained set of solutions was screened for DPPH radical-scavenging, reducing power, inhibition of β-carotene bleaching and TBARS formation inhibition assays (Barros et al., 2013b) and the EC_{50} value (mg/mL extract) of each assay was calculated by interpolation.

2.3.2. Cytotoxic activity

Different portions of the lyophilized extracts prepared for phenolic compounds analysis, were dissolved in water (8 mg/mL) and successively diluted to prepare a working set of concentrations (400–12.5 μg/mL). The cytotoxic activity of these solutions was tested in four human tumor cell lines (using a cellular density of approximately 1.0×10^4 cells/well): MCF-7 (breast adenocarcinoma), HCT15 (colon carcinoma), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma). Cells lines were incubated for 48 h (Abreu et al., 2011). In addition, a primary cell culture (PLP2) obtained from fresh porcine liver was used to evaluate the hepatotoxicity in non-tumor cells. The sulforhodamine B assay was applied in all cases (Abreu et al., 2011) and ellipticine (Sigma-Aldrich, St. Louis, MO, USA) was tested as positive control. Results were expressed as GI_{50} (the concentration of extract that inhibits the cell growth at a 50% rate) values (μg/mL extract).

2.3.3. Antibacterial activity

Other portions of the same extracts described used to determine the antioxidant activity and cytotoxicity were additional screened for antibacterial activity. The selected bacterial species included Gram positive (*Staphylococcus aureus* - ATCC 6538, *Bacillus cereus* - clinical isolate, *Micrococcus flavus* - ATCC10240, and *Listeria monocytogenes* - NCTC7973) and Gram negative (*Enterobacter cloacae* - ATCC 35030, *Escherichia coli* - ATCC 35210, *Pseudomonas aeruginosa* - ATCC 27853, and *Salmonella typhimurium* - ATCC 13311) strains. For a better characterization, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were both obtained (Dias et al., 2016). Streptomycin and ampicillin were used as positive controls, while a simple DMSO solution (5%) served as negative control.

2.3.4. Antifungal activity

Extracts evaluated for antibacterial activity were also assessed pertaining their antifungal capacity against eight reference species: *Aspergillus fumigatus* (ATCC1022), *Aspergillus ochraceus* (ATCC12066), *Aspergillus versicolor* (ATCC11730), *Aspergillus niger* (ATCC6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC9112), *Penicillium verrucosum* var. *cyclopium* (food isolate), and *Trichoderma viride* (IAM 5061) (Soković & Van Griensven, 2006). In line with the approach followed in the antibacterial activity, the MIC (lowest concentration without visible growth at the binocular microscope) and the minimum fungicidal concentration (MFC) were obtained for each extract. Bifonazole and ketoconazole were, in this case, used as positive controls.

2.4. Statistical analysis

The analytical assays described previously were conducted using three independent samples (n = 3); likewise, each of these samples was analyzed in triplicate. Except for antimicrobial assays, the results were expressed as mean values ± standard deviation (SD). The *t*-Student test was consecutively performed to compare the results from skin extracts and from pulp extracts. A 5% significance level was considered in the tests conducted in IBM SPSS Statistics for Windows, version 22.0. (IBM Corp., USA).

3. Results and discussion

3.1. Aroma volatiles

The volatile compounds identified in the skins and pulps of *O. microdasys* and *O. macrorhiza* are listed in Table 1. In total, 64 compounds were detected, but camphor was the single one common to both skins and pulps. From a quantitative point of view, the percentage of identified compounds varied between 96.55% (*O. macrorhiza* skins) and 98.26% (*O. macrorhiza* pulps). However, the individual volatiles profiles were quite dissimilar, either when comparing different botanical parts of the same species, or the same botanical part of different species. *O. microdasys* fruit skins presented 28 different compounds, while only 21 were possible to be identified in pulps; likewise, skins and pulps of *O. macrorhiza* fruits had only 4 common compounds. Camphor was the most abundant volatile in *O. mycrodasis* skins (49%) and pulps (26%), followed by nonanal (16.4%) and 1-nonanol (22%), respectively. Camphor was also the major compound in the fruit skins of *O. macrorhiza* (39%), which also showed significant percentages of nonanal (6.9%), *cis*-linalool oxide (6.5%), phenylethyl alcohol (6.5%), and *trans*-linalool oxide (6.3%). The predominance of camphor is in agreement with the volatile profiles that were previously characterized in different *O. microdasys* flowering stages (Chahdoura et al., 2016). This compound was previously reported for its biological properties and industrial applications, particularly in medical products (Santos & Cabot, 2015). However, the main compound detected in the pulps of *O. macrorhiza* fruits was ethyl acetate (52%), followed by 1-hexyl acetate, which was also present in high percentage (15.3%).

The dissimilarity among the profiles observed in each pulp suggests that these species might be used in different applications, since each volatile compound has different odor thresholds (ranging from a few ppb to several ppm), thereby being expected to produce different aromas (Žulj, Maslov, Tomaz, & Jeromel, 2015).

The differences in volatile profiles were also observed among other species such *O. ficus-indica*, which, despite presenting nonanal as its main volatile, had highly dissimilar profile when compared to the species studied herein (Farag, Maamoun, Ehrlich, Fahmy, & Wesjohann, 2017).

3.2. Chemical composition (hydrophilic compounds)

3.2.1. Sugars

The detected soluble sugars were fructose, glucose and sucrose (Table 2), all, as easily expectable, presenting higher concentrations in pulps than in skins. However, it was somehow surprising to verify that sucrose, the most abundant sugar in *O. microdasys* pulp (50 g/100 g), could not be detected in the pulp of *O. macrorhiza*. In fact, all sugars presented significant ($p < 0.050$) differences among both species, either comparing skins or pulps. Nevertheless, the fruits from both species should be similarly sweet, since the lower sugar quantity in *O. macrorhiza* (76 g/100 g dw *vs*. 82 g/100 g in *O. microdasys*) might be easily compensated by having only fructose and glucose (both having higher sweeter power than sucrose). In fact, the high fructose content in *O.*

Table 2
Chemical composition (hydrophilic compounds) of skins and pulps of *Opuntia microdasys* and *Opuntia macrorhiza*.

Parameter	*O. microdasys*		*O. macrorhiza*		*t*-Student test	
	skin	pulp	skin	pulp	skins	pulps
Sugars (g/100 g dw)						
Fructose	5.5 ± 0.2	14.9 ± 0.3	10.3 ± 0.5	41 ± 1	< 0.001	< 0.001
Glucose	6.4 ± 0.4	16.9 ± 0.4	4.6 ± 0.2	35 ± 1	< 0.001	< 0.001
Sucrose	3.6 ± 0.2	50 ± 1	1.0 ± 0.1	–	< 0.001	–
Total sugars	15.5 ± 0.4	82 ± 1	16.0 ± 0.5	76 ± 2	0.099	< 0.001
Organic acids (g/100 g dw)						
Oxalic acid	0.95 ± 0.04	0.012 ± 0.002	0.83 ± 0.03	0.056 ± 0.003	< 0.001	< 0.001
Quinic acid	0.44 ± 0.05	0.011 ± 0.001	0.27 ± 0.03	0.021 ± 0.002	< 0.001	< 0.001
Malic acid	1.26 ± 0.05	0.045 ± 0.004	0.87 ± 0.03	0.043 ± 0.003	< 0.001	0.358
Citric acid	3.9 ± 0.1	2.6 ± 0.2	11.7 ± 0.3	1.23 ± 0.03	< 0.001	< 0.001
Ascorbic acid	0.076 ± 0.004	0.015 ± 0.001	0.027 ± 0.002	0.98 ± 0.05	< 0.001	< 0.001
Organic acids	6.6 ± 0.3	2.7 ± 0.2	13.7 ± 0.3	2.3 ± 0.1	< 0.001	< 0.001

macrorhiza pulp might be important for its commercial exploitation.

On the other hand, the skins of both *Opuntia* species showed similar ($p = 0.099$) quantities of total sugars (15.5 g 100/g dw in *O. microdasys*; 16.0 g/100 g dw in *O. macrorhiza*).

3.2.2. Organic acids

In line with the results of sugar composition, organic acids profiles were also different for each *Opuntia* species, as they were among fruit pulps and skins within the same species (Table 2). Actually, and except for malic acid in pulps ($p = 0.358$), all detected compounds presented significant differences ($p < 0.050$). Organic acids were detected in higher quantities in fruit skins (6.6 g/100 g in *O. microdasys*, 13.7 g/100 g dw in *O. macrorhiza*) than in pulps (2.7 g/100 g dw in *O. microdasys*, 2.3 g/100 g in *O. macrorhiza*), mainly due to the high levels of citric acid. The second and third most abundant organic acids in *Opuntia* skins were malic and oxalic acid, both detected in quantities around 1 g/100 g dw. In comparison to other botanical parts, fruit skins contain higher levels of organic acids than cladodes (Chahdoura et al., 2014a) and flowers (Chahdoura et al., 2016), which, nonetheless, have higher quantities than seeds (Chahdoura et al., 2015a). As far as we know, there are no reports describing the organic acids composition in the pulps and skins of the *Opuntia* species studied herein; however, some previous works reporting the chemical characterization of *O. joconostle*, *O. matudae* (Morales et al., 2012), *O. ficus-indica*, *O. lindheimeri*, *O. streptacantha* and *O. stricta* var. *stricta*, presented lower ascorbic acid contents (Kuti, 2004).

3.2.3. Phenolic compounds

Some chromatographic data were considered in the identification of phenolic compounds, including retention time, λ_{max}, pseudomolecular ion, and main fragment ions in MS^2 (Table 3).

Overall, twelve different phenolic compounds were identified, three phenolic acids (peaks 1–3, Table 3) and nine flavonoids (isorhamnetin, quercetin and kaempferol derivatives: peaks 4 to 12). All compounds were previously described in *Opuntia* spp. (Chahdoura et al., 2014a,b; Melgar et al., 2018; Morales, Barros, Ramírez-Moreno, Santos-Buelga, & Ferreira, 2014, 2015), and the assumptions therein were also taken into account. *O. macrorhiza* skins showed higher concentrations of phenolic compounds than the same botanical part in *O. microdasys*. In addition, the pulps of this last species did not reveal the identified compounds in quantifiable concentrations. In turn, *O. macrorhiza* skins revealed quercetin-3-*O*-rutinoside (1.13 ± 0.01 mg/g of extract) as the main phenolic compound, while the same parts of *O. microdasys* presented quercetin-*O*-(deoxyhexoside-rutinoside) as the major component (0.17 ± 0.01 mg/g of extract). Otherwise, *O. macrorhiza* pulps presented piscidic acid as the main molecule (0.38 ± 0.04 mg/g of extract).

3.2.4. Betalains

The nine identified betalains (peaks 13–21, Table 3) were also previously identified in *Opuntia* spp. (Castellanos-Santiago & Yahia, 2008; Melgar et al., 2018; Morales et al., 2014, 2015). Betalain contents were significantly higher in *O. macrorhiza* when compared to *O. microdasys*. As expectable, these components were more concentrated in skins than in the respective pulps, and the main compounds were betanin and isobetanin in both parts and species.

3.3. Chemical composition (lipophilic compounds)

3.3.1. Fatty acids

The main fatty acids detected in *Opuntia* samples are listed in Table 4. Besides those, caprylic acid (C8:0), capric acid (C10:0), eicosatrienoic acid (C20:3n3), heneicosanoic acid (C21:0), gondoic acid (C22:1n9) and nervonic acid (C24:1n9) were detected in trace amounts (< 0.2%).

The similarity among the profiles of the skins of both *Opuntia* fruits is obvious, with C18:2n6 as the major compound in both cases (50.6% in *O. microdasys*; 53.0% in *O. macrorhiza*), followed by C16:0 (17.1% in *O. microdasys*; 19.3% in *O. macrorhiza*), C18:1n9 (12.9% in *O. microdasys*; 8.6% in *O. macrorhiza*) and C18:3n3 (4.0% in *O. microdasys*; 5.5% in *O. macrorhiza*). Fruit pulps, on the other hand, presented great differences in their fatty acids profiles. Lauric acid (C12:0) was the major (54%) fatty acid in *O. macrorhiza*, which also presented significant percentages of C20:1 (8.5%), C18:2n6 (8.3%) and C16:0 (5.3%). The fruit pulps of *O. microdasys*, in turn, presented three major fatty acids, C12:0 (20.1%), C16:0 (18.5%) and C18:2n6 (16.7%), in addition to their relevant percentages of C18:3n3 (6.8%) and C16:1 (6.7%). In either case, the percentages of fatty acids presented significant differences among all samples, except in the case of C24:0 among skins ($p = 0.053$).

In what concerns grouped fatty acids, the skins of *O. microdasys* and *O. macrorhiza* fruits presented a high percentage (54.8% and 58.7%, respectively) of polyunsaturated fatty acids (PUFA), which could be advantageous for their application in dietetic formulations. Fatty acids quantified in pulps, on the other hand, were mainly saturated (63.5% in *O. microdasys*, 76.7% in *O. macrorhiza*), which can assure a good oxidative stability when using their juice in food applications. The percentages of saturated, monounsaturated and polyunsaturated fatty acids were also significantly different among species, for both botanical parts.

Table 3
Retention time (Rt), wavelengths of maximum absorption in visible region (λ_{max}), mass spectral data, identification and quantification of phenolic compounds and betacyanins detected in *Opuntia microdasys* (OMi) and *Opuntia macrorhiza* (OMa) skin and pulp.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion (*m/z*)[a]	MS^2 (*m/z*)	Tentative identification	Quantification (mg/g)				*t*-Student test	
						Skins		Pulps			
						OMi	*OMa*	*OMi*	*OMa*	skins	pulps
Phenolic compounds											
1	4.06	297	255	193(100), 179(4), 165(4), 149(5)	Piscidic acid	nd	nd	nd	0.38 ± 0.04	–	–
2	8.33	326	355	193(100)	Ferulic acid hexoside	nd	0.012 ± 0.001	nd	nd	–	–
3	11.23	310	325	163(100)	*p*-Coumaric acid hexoside	nd	0.038 ± 0.002	nd	nd	–	–
4	13.90	334	755	609(35), 301(100)	Quercetin-*O*-(deoxyhexosyl-rutinoside)	0.17 ± 0.01	nd	nd	nd	–	–
5	15.80	323	739	285(100)	Kaempferol-*O*-(di-deoxyhexosyl-hexoside)	0.13 ± 0.01	nd	nd	nd	–	–
6	16.70	352	769	315(100)	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside)	0.16 ± 0.01	0.12 ± 0.01	nd	nd	< 0.001	–
7	17.36	352	609	301(100)	Quercetin-3-*O*-rutinoside	nd	1.13 ± 0.01	nd	nd	–	–
8	18.45	347	463	301(100)	Quercetin-3-*O*-glucoside	nd	0.11 ± 0.01	nd	nd	–	–
9	18.68	351	769	315(100)	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside)	0.13 ± 0.01	nd	nd	nd	–	–
10	20.60	340	593	285(100)	Kaempferol-3-*O*-rutinoside	nd	0.10 ± 0.01	nd	nd	–	–
11	21.40	354	623	315(100)	Isorhamnetin-*O*-(deoxyhexosyl-hexoside)	nd	0.88 ± 0.03	nd	0.007 ± 0.001	–	–
12	23.72	353	623	315(100)	Isorhamnetin-*O*-(deoxyhexosyl-hexoside)	nd	0.15 ± 0.01	nd	nd	–	–
Phenolics						**0.58 ± 0.01**	**1.55 ± 0.03**	**-**	**0.38 ± 0.04**	**< 0.001**	**-**
Betacyanins											
13	22.04	534	713	551(20), 389(100), 345(5), 150(5)	Betanidin-5-*O*-β-sophoroside	0.45 ± 0.02	nd	nd	nd	–	–
14	22.20	534	551	389(100), 345(5), 150(5)	Betanidin-5-*O*-β-glucoside (betanin)	1.0 ± 0.1	76 ± 3	0.13 ± 0.01	10.5 ± 0.3	< 0.001	< 0.001
15	23.22	534	713	551(20), 389(100), 345(5), 150(5)	Isobetanidin-5-*O*-β-sophoroside	0.40 ± 0.04	nd	nd	nd	–	–
16	23.70	534	551	389(100), 345(5), 150(5)	Isobetanidin-5-O-β-glucoside (Isobetanin)	1.1 ± 0.1	42 ± 1	0.007 ± 0.001	6.2 ± 0.2	< 0.001	< 0.001
17	24.65	534	551	507(7), 389(100), 345(5), 301(5)	Betanidin-6-*O*-β-glucoside (Gomphrenin I)	0.25 ± 0.03	1.0 ± 0.1	0.020 ± 0.002	0.13 ± 0.01	< 0.001	< 0.001
18	25.19	530	637	389(100)	6′—O-Malonylbetanin (Phyllocactin)	nd	0.44 ± 0.04	0.013 ± 0.001	0.082 ± 0.004	–	< 0.001
19	25.72	469	549	387(100)	14,15-Dehydrobetanin (Neo-betanin)	0.43 ± 0.05	2.4 ± 0.1	0.005 ± 0.001	0.45 ± 0.04	< 0.001	< 0.001
20	26.09	539	389	343(100), 150(60)	Betanidin	nd	9.3 ± 0.3	0.007 ± 0.001	0.70 ± 0.04	–	< 0.001
21	28.01	538	389	343(100), 150(45)	Isobetanidin	nd	0.43 ± 0.03	0.005 ± 0.001	0.28 ± 0.02	–	< 0.001
Betacyanins						**3.6 ± 0.1**	**131 ± 4**	**0.19 ± 0.01**	**18.4 ± 0.2**	< 0.001	< 0.001

[a] Negative mode ($[M-H]^-$) was used for phenolic compounds identification; positive mode ($[M+H]^+$) was used for betacyanin identification.

Table 4
Chemical composition (lipophilic compounds) of skins and pulps of *Opuntia microdasys* and *Opuntia macrorhiza*.

Parameter	*O. microdasys*		*O. macrorhiza*		*t*-Student test	
	skin	pulp	skin	pulp	skins	pulps
Fatty acids (relative percentage)						
C6:0	0.12 ± 0.01	3.9 ± 0.1	nd	2.2 ± 0.1	–	< 0.001
C12:0	1.1 ± 0.1	20.1 ± 0.4	1.4 ± 0.1	54 ± 1	< 0.001	< 0.001
C14:0	0.87 ± 0.04	3.9 ± 0.1	1.4 ± 0.1	9.4 ± 0.2	< 0.001	< 0.001
C15:0	0.13 ± 0.01	5.4 ± 0.1	0.24 ± 0.03	0.41 ± 0.04	< 0.001	< 0.001
C16:0	17.1 ± 0.3	18.5 ± 0.5	19.3 ± 0.4	5.3 ± 0.2	< 0.001	< 0.001
C16:1	0.47 ± 0.05	6.7 ± 0.2	1.1 ± 0.1	2.4 ± 0.1	< 0.001	< 0.001
C17:0	0.21 ± 0.02	1.5 ± 0.1	0.29 ± 0.03	0.75 ± 0.05	< 0.001	< 0.001
C18:0	2.8 ± 0.1	5.4 ± 0.2	3.2 ± 0.1	1.4 ± 0.1	< 0.001	< 0.001
C18:1n9	12.9 ± 0.3	1.3 ± 0.1	8.6 ± 0.2	1.1 ± 0.1	< 0.001	0.002
C18:2n6	50.6 ± 0.4	16.7 ± 0.3	53.0 ± 0.5	8.3 ± 0.3	< 0.001	< 0.001
C18:3n3	4.0 ± 0.2	6.8 ± 0.2	5.5 ± 0.2	1.9 ± 0.1	< 0.001	< 0.001
C20:0	2.9 ± 0.2	1.8 ± 0.1	2.4 ± 0.1	0.65 ± 0.04	< 0.001	< 0.001
C20:1	0.7 ± 0.1	4.4 ± 0.2	0.10 ± 0.02	8.5 ± 0.3	< 0.001	< 0.001
C20:2	0.13 ± 0.01	0.66 ± 0.04	nd	1.0 ± 0.1	-	< 0.001
C22:0	3.6 ± 0.1	0.69 ± 0.05	1.7 ± 0.2	0.48 ± 0.04	< 0.001	< 0.001
C23:0	0.19 ± 0.02	0.85 ± 0.05	0.10 ± 0.01	1.0 ± 0.1	< 0.001	< 0.001
C24:0	1.6 ± 0.1	1.5 ± 0.1	1.4 ± 0.2	0.60 ± 0.04	0.053	< 0.001
SFA	30.7 ± 0.3	63.5 ± 0.5	31.5 ± 0.4	76.7 ± 0.5	< 0.001	< 0.001
MUFA	14.5 ± 0.3	12.4 ± 0.3	9.9 ± 0.2	12.1 ± 0.4	< 0.001	0.043
PUFA	54.8 ± 0.4	24.1 ± 0.4	58.7 ± 0.5	11.2 ± 0.3	< 0.001	< 0.001
Tocopherols (mg/100 g dw)						
α-tocopherol	46 ± 1	0.19 ± 0.01	8.4 ± 0.3	0.80 ± 0.05	< 0.001	< 0.001
β-tocopherol	1.37 ± 0.05	nd	0.12 ± 0.01	0.060 ± 0.003	< 0.001	–
γ-tocopherol	0.94 ± 0.05	0.055 ± 0.004	0.24 ± 0.04	0.24 ± 0.02	< 0.001	< 0.001
Tocopherols	48 ± 1	0.24 ± 0.01	8.7 ± 0.3	1.10 ± 0.05	< 0.001	< 0.001

Tocopherol contents were higher in skins (Table 4), which is in agreement with the protective nature of these compounds, especially considering the percentages of PUFA detected in *Opuntia* skins, and the tocopherols' action against lipid peroxidation (Kanu et al., 2007). The main vitamer in both plant parts and species was, by far, α-tocopherol. The fruit skins of *O. microdasys* gave higher tocopherol quantities than *O. macrorhiza* (48 mg/100 g dw *vs*. 8.4 mg/100 g dw), but an opposite result was obtained for pulps (1.10 mg/100 g dw in *O. macrorhiza* against 0.24 mg/100 g dw in *O. microdasys*). The detected differences were significant ($p < 0.050$) in all cases.

3.4. Bioactive properties

Two types of bioactivity (Table 5) were assessed: antioxidant activity and cytotoxicity (against tumoral and non-tumoral cells).

In what concerns antioxidant activity, the highest capacity (lowest EC_{50} values) was measured in the inhibition of thiobarbituric acid reactive substances (TBARS) formation, followed by reducing power, β-carotene bleaching inhibition and DPPH scavenging activity. The botanical parts of *O. macrorhiza* showed slightly higher antioxidant activity ($p < 0.050$) than their counterparts in *O. microdasys*, but the antioxidant power was expected to be more dissimilar, considering the high differences among the bioactive compounds contents quantified in each species.

Considering the results obtained in cytotoxicity assays, the assayed extracts revealed low toxicity, either against tumor cell lines (HeLa cell line was nonetheless the most sensitive), as well as against the non-tumor cell line (PLP2). In fact, in the case of MCF7 and PLP2, no acute toxicity was observed at all (up to the maximum assayed concentrations of 400 μg/mL).

The antimicrobial activity, which was measured against several species of bacteria and fungi, was only detected in *Opuntia* skins; up to the assayed concentrations, fruit pulps had no antibacterial, neither antifungal activity (Table 5). In general, the fruit skins from *O. macrorhiza* demonstrated higher antimicrobial activity than those from *O. microdasys*, which is also in agreement with their higher concentration in bioactive compounds. In fact, the MIC and MBC values obtained for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* were similar to the ones obtained with ampicillin (and streptomycin in the case of *S. typhimurium*).

Surprisingly, the fungistatic and fungicidal activity was higher among *O. microdasys* skins, but not comparable to that measured with bifonazole or ketoconazole. The fungal strains more sensitive to *O. microdasys* fruit skins extracts were *Aspergillus versicolor* and *Penicillium funiculosum*.

4. Conclusion

The skins and pulps of *Opuntia microdasys* and *O. macrorhiza* were thoroughly studied regarding their phytochemical profiles as well as their antioxidant and antimicrobial activity, revealing high and differentiated potential for alternative food or pharmaceutical-related applications. The identified capacity might represent a strong incentive to increase the industrial application of this important natural resource.

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Table 5
Bioactivity (antioxidant activity: EC_{50}, mg/mL; antiproliferative activity: GI_{50}, μg/mL; hepatotoxicity: GI_{50}, μg/mL; antimicrobial activity: MIC, MBC, and MFC, mg/mL) of skins and pulps from *Opuntia microdasys* and *Opuntia macrorhiza*.

	O. microdasys		*O. macrorhiza*		*t*-Student test	
	skin	pulp	skin	pulp	skins	pulps
Antioxidant activity						
DPPH scavenging activity	1.34 ± 0.01	1.28 ± 0.01	0.99 ± 0.05	1.11 ± 0.01	< 0.001	< 0.001
Reducing power	1.20 ± 0.01	0.83 ± 0.02	0.52 ± 0.01	0.76 ± 0.01	< 0.001	< 0.001
β-carotene bleaching inhibition	0.86 ± 0.05	0.42 ± 0.01	0.27 ± 0.03	0.23 ± 0.02	< 0.001	< 0.001
TBARS inhibition	0.40 ± 0.05	0.28 ± 0.02	0.08 ± 0.01	0.13 ± 0.01	< 0.001	< 0.001
Antiproliferative activity						
MCF7	>400	>400	>400	>400	–	–
HCT15	245 ± 6	396 ± 8	234 ± 7	296 ± 3	0.004	< 0.001
HeLa	206 ± 4	237 ± 3	121 ± 5	218 ± 11	< 0.001	0.001
HepG2	359 ± 12	374 ± 4	359 ± 12	373 ± 4	0.963	0.636
Hepatotoxicity						
PLP2	>400	>400	>400	>400		

Antibacterial activity	**O. microdasys skin**		**O. macrorhiza skin**		**Streptomycin**		**Ampicillin**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Staphylococcus aureus	1.85	2.50	0.45	0.62	0.04	0.10	0.25	0.40
Bacillus cereus	1.25	2.50	0.62	1.25	0.10	0.20	0.25	0.40
Micrococcus flavus	3.75	5.00	0.45	0.62	0.20	0.30	0.25	0.40
Listeria monocytogenes	2.50	5.00	1.85	2.50	0.20	0.30	0.40	0.50
Pseudomonas aeruginosa	1.85	2.50	0.45	0.62	0.20	0.30	0.75	1.20
Escherichia coli	1.85	2.50	0.95	1.25	0.20	0.30	0.40	0.50
Enterobacter cloacae	1.25	5.00	1.85	2.50	0.20	0.30	0.25	0.50
Salmonella typhimurium	1.85	2.50	0.40	0.62	0.25	0.50	0.40	0.75

Antifungal activity	***O. microdasys* skin**		***O. macrorhiza* skin**		**Bifonazole**		**Ketoconazole**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus fumigatus	2.50	5.00	10.00	12.50	0.15	0.20	0.20	0.50
Aspergillus versicolor	1.25	2.50	10.00	11.25	0.10	0.20	0.20	0.50
Aspergillus ochraceus	2.50	5.00	10.00	12.50	0.15	0.20	1.50	2.00
Aspergillus niger	2.50	5.00	10.00	12.50	0.15	0.20	0.20	0.50
Trichoderma viride	1.25	5.00	10.00	11.25	0.15	0.20	1.00	1.00
Penicillium funiculosum	1.25	2.50	5.00	10.00	0.20	0.25	0.20	0.50
Penicillium ochrochloron	5.00	10.00	2.50	10.00	0.20	0.25	2.50	3.50
Penicillium verrucosum	5.00	10.00	5.00	10.00	0.10	0.20	0.20	0.30

EC_{50} values correspond to the sample concentration achieving 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in human tumor cell lines or in liver primary culture PLP2. MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; MFC: minimal fungicidal concentration.

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