By-product recovery of *Opuntia spp*. peels: betalainic and phenolic profiles and bioactive properties

Bruno Melgar[a,b], Maria Inês Dias[a], Ana Ciric[b], Marina Sokovic[b], Esperanza M. Garcia-Castello[c], Antonio D. Rodriguez-Lopez[d], Lillian Barros[a], Isabel C.F.R. Ferreira[a,*]

[a]*Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.*

[b]*University of Belgrade, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Bulevar Despota Stefana 142, 11000 Belgrade, Serbia.*

[c]*Institute of Food Engineering for Development, Universitat Politècnica de València, Camino de Vera, s/n CP, 46022 Valencia, Spain.*

[d]*Institute for Industrial, Radiophysical and Environmental Safety (ISIRYM), Universitat Politècnica de València, Camino de Vera, s/n CP, 46022 Valencia, Spain.*

*Corresponding author. Tel.+351 273 303219; fax +351 273 325405. E-mail address: iferreira@ipb.pt (I.C.F.R. Ferreira)

Abstract

Opuntia spp. are a tropical and subtropical plant that provides both edible green steams and fruits; however, the processing of this fruits results in the accumulation of enormous amount of by-products that can be a source of bioactive and pigmented compounds. Herein, three cactus pear from the species *Opuntia ficus-indica* var. sanguigna (OS) and gialla (OG) and *Opuntia engelmannii* (OE) were fully characterized regarding their phenolic and betalain composition and correlated with their antioxidant and antimicrobial activities. The hydroethanolic extracts of OE gave the highest amount of phenolic compounds (isorhamentin-*O*-(deoxyhexosyl-hexoside) and betacianins (betanin); however, no betaxanthins were identified in this sample. This sample also revealed the lowest EC_{50} values in all the antioxidant activity assays. Regarding antimicrobial activity, the hydroethanolic extracts of all species revealed to be more active than ampicillin. The pivotal objective of this work was to focus on exploring by-product biocompounds and possible outputs, thus, we could suggest the use of these natural colorants with intrinsic antioxidant and antimicrobial activity, which would grant industries to produce cleaner label products with functional benefits.

Keywords: *Opuntia ficus-indica*; *Opuntia engelmannii*; by-product; betalains; phenolic compounds; antimicrobial activity.

1. Introduction

Opuntia spp., including their several varieties, belong to the dicotyledonous angiosperm and are the largest genus within *Cactaceae* family. *Opuntia* species are tropical and subtropical plants, able to grow in arid and semi-arid environments, with easy geographic adaptation due to their crassulacean acid metabolism (CAM), which enables the plant to survive to extreme heat, low temperatures (-40 °C) and drought because of their highly efficiency in the use of water (Nobel, 1998; Sudzuki et al., 1993). This plant provides both edible green steams and fruits. Cactus fruit is also known as prickly pear, cactus fig, Indian fig, cactus pear, Barbary fig, and is available across the 5 continents from early summer until late autumn. Although *Opuntia* species are native of Mexico, where the steams and fruits are consumed fresh in the local diet, fruits, are also processed and commercialized in numerous countries as jams, sweats, ice cream and alcoholic and not alcoholic beverages (Martins et al., 2016).

The processing of the fruits results in the accumulation of several quantities of by-products, namely prickly pear peels. Proper utilization of this by-product could reduce waste disposal problems and serve as a potential new source of bioactive compounds and pigments. According to several authors (Belwal et al., 2016; Garcia-Castello et al., 2015; Pinela et al., 2016; Rao, 2010), one of the best ways to use this kind of by-products could be the application of an appropriate green solid-liquid extraction technology in order to obtain bioactive compounds with different properties and health benefits. Several studies marked fruits and fruits by-products as a rich source of natural molecules such as polyphenols (i.e. flavonoids), vitamins, colorants like betalains and carotenoids (Albuquerque et al., 2016; Alzate T et al., 2016; Ayala-Zavala et al., 2011; Fernandez-Rojas et al., 2010; Silva et al., 2016), which could have high potential interest in human health, medicine and production of new added-value products.

Scientists have lately reported multiple properties regarding to phenolic compounds as antioxidants endowed with anticancer, anti-inflammatory, antimicrobial and antidiabetic activities (Dias et al., 2016; Kaur Kala et al., 2016), among the most cited properties attributed to polyphenols due to their protective effect against damage of the human body caused by reactive oxygen, nitrogen and sulfur species (ROS, RNS and RSS, respectively) (Ambigaipalan, 2015; Carocho and Ferreira, 2013).

Additionally to phenolic compounds, other relevant components in the cactus pear are the betalains pigments, which are present in most of the Caryophyllales family including the *Cactaceae* and replace the anthocyanins in flowers and fruits of plants within this family. Betalains are water soluble nitrogen-containing pigments situated in the vacuoles of the plant tissue responsible for the red-violet (betacyanins) and yellow-orange (betaxanthins) colors, showing a stable appearance in the range of pH 3 to 7 (Stintzing et al., 2002). Therefore, betalains could be used as a natural colorant alternative to synthetic dyes used in a broad range of food products.

The present study intends to contribute to the characterization of the bioactive compounds profile, antioxidant and antimicrobial activities of by-products, such as peels, of the cactus pear from the species *Opuntia ficus-indica* var. sanguigna (OS) and gialla (OG) and *Opuntia engelmannii* (OE). The results of this study might be useful to maximize the potential of the fruits by-products for their colorant and functional added value.

2. Material and Methods

2.1. Samples preparation

Cactus pear fruits (*Opuntia ficus-indica* var. sanguigna -OS and gialla -OG) were collected in July-August 2016 in Sicily, Italy and purchased from a local market in Bragança, Portugal. Fruits from this specie were separated regarding the maturation colour orange-red (*Opuntia ficus-indica* var. gialla) and red-violet (*Opuntia ficus-indica* var. sanguigna), obtaining two different samples. Prickly pear wild fruits (*Opuntia engelmannii*- OE) were collected in Bragança, Portugal (GPS location: 41.797344, -6.772735) on early September 2016. Dr. Carlos Aguiar of the School of Agriculture, Polytechnic Institute of Bragança (Trás-os-Montes, Portugal), confirmed the botanical identifications and voucher specimens were deposited in a herbarium.

Within 24 hours, fruits were washed with distilled water in order to remove glochids and then further air-dried. Afterwards, all the fruits were peeled and the resulting peel was lyophilized (LabConco, Frezone -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. Extraction procedure

The hydroethanolic (ethanol: water, 80:20 *v/v*) extract was obtained from the lyophilized peels. The sample (1 g) was extracted twice by stirring with 25 mL of hydro-alcoholic solution (25 ºC at 150 rpm) for 1 h and subsequently filtered through the Whatman no. 4 paper. The obtained extracts were frozen, lyophilized and re-dissolved in: (i) hydroethanolic solution (ethanol: water, 80:20 *v/v*) for phenolic characterization (final concentration 5 mg/mL) and antioxidant activity evaluation (final concentration 40 mg/mL); or (ii) water for cytotoxicity evaluation (final concentration 8 mg/mL) and betalain characterization (final concentration 30 mg/mL); or (iii) 5%

DMSO in distilled water (final concentration 10 mg/mL) for antimicrobial assays. The final solutions were further diluted to different concentrations to be submitted for distinct *in vitro* bioactivity evaluation assays.

2.3. Phenolic compounds

The lyophilized hydroethanolic extracts were analysed for their content in phenolic compounds, re-dissolve in ethanol:water (80:20, *v/v*) to a final concentration of 5 mg/mL. LC-DAD–ESI/MSn analyses were performed using a Dionex Ultimate 3000 UPLC instrument (Thermo Scientific, San Jose, CA, USA), coupled to a diode-array detector (using several wavelengths, 280, 330 and 370 nm) and to a mass detector (Linear Ion Trap LTQ XL mass spectrometer, equipped with an ESI source, ThermoFinnigan, San Jose, CA, USA). The elution gradient, chromatographic and mass spectrometer conditions were performed according to (Bessada et al., 2016). Identification was performed by comparing their fragmentation pattern, retention times and UV–vis spectra with authentic standards, when available, or by comparing the obtained information with available data from literature. When a standard was not available to quantify a phenolic compound a calibration curve of similar compound from the same phenolic group was used.

2.4. Betalain compounds

The profile in betalain compounds was determined by LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA). Chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column working at 35 °C. The solvents used were: (A) 0.1% trifluoroacetic acid (TFA) in water, (B) acetonitrile. The gradient elution

followed these parameters: from 0% to 10% B for 15 min, from 10% to 15% B for 5 min, from 15 to 18% B for 5 min, from 18 to 50% B for 8 min, and from 50 to 0% B for 12 min. The resulting total run time was 45 minutes, using a flow rate of 0.5 mL/min. Double detection was carried out in the DAD using 480 nm (betaxanthins) and 535 nm (betacyanins), as the preferred wavelength, and in a mass spectrometer (MS). MS detection was performed using positive mode, with a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source. Nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage of 4.8 kV, a source temperature of 320 °C, a capillary voltage of 39 V. The tube lens offset was kept at a voltage of 140 V. The full scan covered the mass range from *m/z* 100 to 1500, collision energy used was 24 (arbitrary units) and data acquisition was carried out with Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

The identification of the betalain compounds (betacyanins and betaxanthins) were performed by comparing the obtained information with available data reported in the literature giving a tentative identification. For quantitative analysis, a calibration curve using an isolated compound gomphrenin III (isolated from *Gomphrena globosa* L.) was constructed based on the UV signal (y = 14670x - 19725, R^2 = 0.9997) (Roriz et al., 2017). The results of betacyanins were expressed as mg per 100 g of pulp fresh weight (FW), and the results of betaxanthins were expressed as relative percentage (%) of their areas recorded at 480 nm.

2.5. Bioactive properties evaluation

2.5.1. Antioxidant activity assays

Successive dilutions were made from the stock solution and submitted to *in vitro* assays already described by (Heleno et al., 2010) to evaluate the antioxidant activity of the

samples. DPPH radical-scavenging activity was evaluated using an ELX800 microplate reader (Bio-Tek Instruments, Inc.; Winooski, VT, USA) and calculated in percentage after 1 h of reaction in the dark. The reducing power (RP) assay evaluated the capacity of the extracts to reduce Fe3+ to Fe2+, measuring the absorbance at 690 nm. In β-carotene bleaching assay, the presence of antioxidants in the samples and their capacity to neutralize the linoleate free radicals, avoids β-carotene bleaching, which can be evaluated measuring the absorbance at 470 nm. Therefore, β-carotene bleaching inhibition was calculated using the following formula: (Absorbance after 2 h of assay/initial absorbance) × 100. The results were expressed as EC_{50} values (sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay) for antioxidant activity. Trolox was used as positive control.

2.5.2. Antimicrobial activity assays

The minimum inhibitory (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) were determined by methodologies, described by (Vieira et al., 2016).

Antibacterial activity was assayed using the following, Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC10240), and *Listeria monocytogenes* (NCTC7973) and Gram-negative bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), and *Enterobacter cloacae* (ATCC 35030) were used. Whilst for antifungal assays, the following microfungi were used: *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). Bacterial and fungal organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Sinisa Stanković", University of Belgrade, Serbia. Standard drugs, namely streptomycin, ampicillin, bifonazole and ketoconazole were used as positive controls.

2.5.3. Cytotoxicity assays

Both, antitumor activity and hepatotoxicity was evaluated by the Sulphorhodamine B (SRB) assay that has been previously described by (Gomes-Correa et al., 2015; Pereira et al., 2014). Four human tumour cell lines were assayed: HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma) and NCI-H460 (non-small cell lung cancer), while PLP2 cells were used for hepatotoxicity. The five cell cultures were treated for 48 h with the different diluted sample solutions and assayed according to the SRB colorimetric methodology. Ellipticine was used as positive control and results were expressed in GI_{50} values in μg/mL (sample concentration that inhibited 50% of the net cell growth).

2.6. *Statistical analysis*

All the extractions and assays were performed in triplicate. Results were expressed as mean values and standard deviation (SD), being analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $p = 0.05$. In the case of only existing two samples, the results were analysed using a Student´s t-test, in order to determine the significant difference between the two samples, with $p = 0.05$. The treatment was carried out using IBM SPSS Statistics for Windows, version 23.0. (IBM Corp., Armonk, New York, USA).

3. Results and discussion

3.1. Phenolic and betalain composition

Tables 1 and **2** present the peak characteristics (retention time, wavelength of maximum absorption and mass spectral data), tentative identification and quantification of the phenolic compounds and betalains, respectively, present in the hydroethanolic extracts of *Opuntia ficus-indica* var gialla (*OG), Opuntia ficus-indica* var sanguigna (OS) and *Opuntia engelmanniii* (OE) peels (**Figure 1**).

Twelve different phenolic compounds (**Table 1**) were found, two phenolic acids, piscid acid (peaks 1) and eucomic acid (peaks 2), and ten flavonoids (isorhamnetin, quercetin and kaempferol derivatives, peaks 3 to 12). All of the compounds have been previously described by other authors in *Opuntia* spp samples(Chahdoura et al., 2014; Chougui et al., 2015; Mata et al., 2016; Morales et al., 2015, 2014) Overall, OE sample presented higher concentration of phenolic compounds, being peak 11 (isorhamnetin-*O*-(deoxyhexosyl-hexoside)) the major compound found (5.99 mg/g).

Regarding betalain fractions (**Table 2**), seven compounds were identified; two betaxanthins, indicaxanthin isomer I and II (peaks 1 and 2), previously described by Castellanos-Santiago & Yahia (2008) in *Opuntia* spp. cultivars and also by Svenson et al., (2008) in *Ullucus tuberosus* fruits. Betalain molecules displays wide range of hues, betacyanins have reddish-violet colour while betaxanthins have a general yellow-orange colour (Albano et al., 2015; Esquivel, 2016; Gandía-herrero et al., 2013; Gandía-Herrero et al., 2010), the latter being only identified in OG and OS samples. Five betacyanins were identified, betanidin-5-*O*-β-sophoroside, betanidin-5-*O*-β-glucoside (betanin), isobetanin, gomphrenin I and betanidin (peaks 3, 4, 5, 6 and 7, respectively), having been previously identified by other authors (Castellanos-Santiago and Yahia,

2008; Morales et al., 2015, 2014; Stintzing et al., 2002; Svenson et al., 2008). On the contrary of betaxanthins, betacianins fraction was found in higher quantities in OE samples, being peak 4 (betanin) the main compound found (14.9 mg/g extract), This characteristic can be easily predicted due to the strong violet hue present in OE and also because betanin (betanidin-5-O-β-glucoside) is the most widespread betacyanin among plants (Esatbeyoglu et al., 2014). In this matter, betanin concentration in the analysed samples was in concordance with the previous statement commented. OE displayed differences of around 15 and 5-fold higher than OG and OS, respectively. The second highest concentration of betacyanins was conferred by isobetanin in OE and OS extracts. Only OE showed other 3 different betacyanins (betanidin, betanidin-5-O-β-sophoroside and gomphrenin I). Although, OG has this name from the Italian word "yellow", betacyanin molecule betanin, was also present but only around 10% of the total betalains detected, this pigment can be spotted as a reddish-orange hue intrinsic in OG peels.

3.2. Antioxidant activity

The results on antioxidant activity of the hydroethanolic extracts are shown in **Table 3**. There is a clear pattern in the antioxidant activity of each *Opuntia* spp., at first glance, OE exhibits the best EC_{50} values, followed by OS and lastly OG, nonetheless, the difference within OE *vs* OS and OG is around 2-fold higher, while the difference among OS and OG is barely perceptible. At this point, it is worth noting the high EC_{50} values presented by OS in the β-carotene bleaching inhibition our hypothesis on this value could be due to an interference with the sample colour.

Independently of the EC_{50} values that OS shown in the and β-carotene assay, all the antioxidant activities of all samples, can be inversely correlated with the concentration

of the betacyanins and phenolic compounds previously described in **Table 2** and **Table 1**, respectively. The relationship between betacyanins (Bc) or phenolic compounds (PC) exhibited high correlation pattern with DPPH radical scavenging activity (R^2=-0.99 for both Bc and PC), reducing power (R^2=-0.97 also for Bc and PC) and β-carotene bleaching inhibition (R^2=-0.75 for Bc and R^2=-0.76 for PC). Therefore, with these correlation values, very similar among the samples and there composition, can lead to possible synergistic effect between these molecules on the antioxidant activity. Several authors have suggested the positive influence of both betalains and phenolic compounds on the antioxidant activity. Cai et al., (2003) and Gandía-herrero et al., (2013) for instance, have reported that the radical scavenging properties of betalains are increased with the number of hydroxyl and imino groups. Albano et al. (2015) have also provided data related to the antioxidant activity of betalainic colorants. On the other hand, there is plethora of information related with the antioxidant effect and mechanisms of action of the phenolic compounds (Ambigaipalan, 2015; Carocho and Ferreira, 2013; Dias et al., 2016; Kaur Kala et al., 2016).

3.3. Antibacterial and antifungal activity

The bacteriostatic and bactericidal effects of *Opuntia* spp. extracts were tested against 8 different pathogenic strains (**Table 4)**. The hydroethanolic extract exhibited effect on 6 of the 8 strains tested, being *Micrococcus flavus* and *Escherichia coli* the only resistant strains. The comparison of hydroalcoholic extracts *vs* commercial antibiotics (streptomycin and ampicillin) were also performed and analysed. It is important to stand out the high efficiency of streptomycin *vs S. aureus* and the better efficiency shown of streptomycin *vs* ampicillin. Nonetheless, *Opuntia* extracts display a higher performance against the pathogenic strains when compared with ampicillin. Although, confronting

the extract *vs* streptomycin, it was not possible to notice a good performance shown by the *Opuntia* extract, OG and OS were better bacteriostatic in 5 of out of 8, while OE display equal or better strength in 3 out of 8 strain tasted. The bactericidal activity of the extracts was equal or higher in 3 out of 8 strains. OG exhibited the best performance against *Bacillus cereus*, while OE had stronger activity *vs Enterobacter cloacae*.

Regarding antifungal activity, the results obtained proved to be consistent and reproducible with the hydroethanolic extracts (**Table 4)**. The results revealed an equal or higher performance in 4 (OS extract) and 3 (OG and OE extract) strains out of the 8 strains tested. All three samples had higher activity on *Penicillium ochrochloron* compared with the antifungal controls used. However, the fungicidal activity could not improve compared with fungistatic activity mention before. The *Opuntia* peel extracts showed equal or higher activity regarding ketoconazole or bifonazole drugs. A higher activity performance of the extract was attained on *Trichoderma viride* and *Penicillum ochrochloron* strains.

Studies from different authors attributed the inhibitory effect of plant extracts against microbial pathogens to their phenolic composition (Balouiri et al., 2016; Song et al., 2010). The inhibitory effect of these phenolics could be explained by adsorption to cell membranes, interaction with enzymes or deprivation of substrate and metal ions (Baydar et al., 2004).

3.4. Cytotoxic activity

Hepatotoxicity analysis were performed on the *Opuntia* spp. peel extracts treating PLP2 cells in order to guarantee the safety of the extracts and no toxicity was found in none of the extracts (GI_{50} values > 400 µg/mL). The results obtained will allow to continue studying different application of the by-product extracts in depth. Porcine liver cells

were employed as the *in vitro* model, due to analogous physiological functioning to the human cells. Concurrently, antitumor activity was tested with four different human tumour cell lines (breast adenocarcinoma - MCF-7, non-small cell lung cancer - NCI-H460, cervical carcinoma - HeLa and hepatocellular carcinoma - HepG2), although the extracts displayed beneficial antitumor activity, the strength of the extracts could not overcome the antitumor activity of the ellipticine control tested (data not shown).

4. Conclusion

Overall, *Opuntia* spp. chemical characterization was able to identify the phenolic and betalainic profile inherent to the by-products (peels) of *Opuntia ficus-indica* and *Opuntia engelmanni*. This recompilation of data will bring deeper comprehension on the specific compounds related to the antioxidant and antimicrobial capacity of the extracts analysed and the absence of toxicity. The mentioned benefits make them of huge interest as a new source of functional ingredients. The pivotal objective of this work was focused on exploring by-product biocompounds and possible outputs, thus, we could suggest their use as natural colorants with intrinsic antioxidant and antimicrobial activity, which would grant industries to produce cleaner label products with functional benefits.

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Table 1. Retention time (Rt), wavelengths of maximum absorption in visible region (λmax), mass spectral data, tentative identification and quantification of phenolic compounds in *Opuntia* peels.

Peak	Tentative identification	Rt (min)	λmax (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	OG	OS	OE	*t*-Student *p*-value
1	Piscidic acid[1]	4.65	222,276	255	193(34),179(6),165(100),149(3)	1.061 ± 0.04	nd	nd	-
2	Eucomic acid[1]	6.71	223,276	239	179(100),149(62)	1.40 ± 0.01	2.2 ± 0.2	nd	<0.001
3	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside)[2]	17.09	354	769	315(100)	0.22 ± 0.01	0.56 ± 0.06	nd	<0.001
4	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside) [2]	17.4	353	769	315(100)	tr	0.21 ± 0.03	nd	-
5	Isorhamentin-*O*-(deoxyhexosyl-pentosyl-hexoside) [2]	17.7	354	755	315(100)	0.77 ± 0.02	1.3 ± 0.1	nd	<0.001
6	Quercetin-3-*O*-rutinoside[2]	17.98	354	609	301(100)	nd	nd	tr	-
7	Isorhamentin-*O*-(deoxyhexosyl-pentosyl-hexoside)[2]	18.14	354	755	315(100)	tr	tr	nd	-
8	Isorhamentin-*O*-(pentosyl-hexoside)[2]	19.58	354	609	315(100)	0.100 ± 0.001	0.67 ± 0.06	nd	<0.001
9	Kaempferol-3-*O*-rutinoside[2]	21.31	348	593	285(100)	nd	nd	tr	-
10	Isorhamentin-*O*-(deoxyhexosyl-hexoside)[2]	21.67	354	623	315(100)	tr	tr	0.48 ± 0.01	-
11	Isorhamentin-*O*-(deoxyhexosyl-hexoside)[2]	22.25	354	623	315(100)	0.77 ± 0.01^c	1.7 ± 0.1^b	5.99 ± 0.04^a	-
12	Isorhametin-3-*O*-glucoside[2]	23.08	354	447	315(100)	nd	nd	tr	-
	Total phenolic compounds					3.26 ± 0.04^c	3.7 ± 0.3^b	6.5 ± 0.1^a	-

Phenolic compounds were expressed in mg/g extract; nd- not detected; Calibration standards: 1- *p*-hydroxibenzoic acid ($y = 208604x + 173056$, $R^2 = 0.9995$); 2-quercetin-3-*O*-glucoside ($y = 34843x - 160173$, $R^2 = 0.9998$). OG, *Opuntia ficus-indica* var gialla; OS, *Opuntia ficus-indica* var sanguigna; OE, *Opuntia engelmannii;* nd, not detected; tr, traces.
In each row different letters mean significant differences ($p < 0.05$). *Statistical differences (<0.001) were observed when t-student test was applied.

Table 2. Retention time (Rt), wavelengths of maximum absorption in visible region (λmax), mass spectral data, tentative identification and quantification (mg/g of extract) of betalains detected in *Opuntia* peels.

Peak	Tentative identification	Rt (min)	λmax (nm)	$[M+H]^+$ (*m/z*)	MS^2 (*m/z*)	OG	OS	OE
Betaxanthins								
1	Indicaxanthin isomer I	24.47	477	309	263(100),219(43),188(15)	nq (25.3±0.4)	nq (56±6)	nd
2	Indicaxanthin isomer II	25.63	477	309	263(100),219(43),188(15)	nq (64.5±0.5)	nq (2.8±0.1)	nd
Betacyanins								
3	Betanidin-5-*O*-β-sophoroside[1]	22.76	534	713	551(34),389(100),345(3),150(3)	nd	nd	0.98 ± 0.02 (4.88±0.01)
4	Betanidin-5-*O*-β-glucoside (betanin)[1]	23.26	534	551	389(100),345(50),150(28)	1.25 ± 0.01[c] (10.2±0.1)	3.44 ± 0.03[b] (36.4)	14.9 ± 0.3[a] (77.7±0.3)
5	Isobetanin[1]	24.62	534	551	389(100),345(73),150(46)	nd	0.5439± 0.003 (5.3)	1.69 ± 0.06* (8.6±0.1)
6	Gomphrenin I[1]	25.73	535	551	507(3),389(38),345(100),301(21)	nd	nd	0.423 ± 0.002 (1.97±0.05)
7	Betanidin[1]	28.16	523	389	343(97),150(91)	nd	nd	1.36 ± 0.07 (6.9±0.2)
	Total betacyanin compounds					1.25 ± 0.01[c]	3.97±0.03[b]	19.4±0.4[a]

Amounts in samples outside brackets in mg/g of extract, amounts inside brackets are the relative percentages of both betaxanthins and betacyanins. OG, *Opuntia ficus-indica* var gialla; OS, *Opuntia ficus-indica* var sanguigna; OE, *Opuntia engelmannii.*

nq, not quantified; nd, not detected. Calibration curve: [1]gomphrenin III (y=14670x–19725, R^2=0.9997). In each row different letters mean significant differences (p < 0.05). *Statistical differences (<0.001) were observed when t-student test was applied.

Table 3. Antioxidant activity of *Opuntia* peels.

EC_{50} (mg/mL extract)	OG	OS	OE
DPPH scavenging activity	4.6 ± 0.1^{a}	4.1 ± 0.3^{a}	1.96 ± 0.04^{b}
Reducing power	2.65 ± 0.05^{a}	2.08 ± 0.06^{b}	1.08 ± 0.04^{c}
β-carotene bleaching inhibition	3.87 ± 0.01^{b}	6.49 ± 0.02^{a}	1.73 ± 0.01^{c}

EC_{50} values, in mg/mL extract; OG, *Opuntia ficus-indica* var gialla; OS, *Opuntia ficus-indica* var sanguigna; OE, *Opuntia engelmannii*. In each row different letters mean significant differences ($p < 0.05$). Trolox EC_{50} values: 62.98 μg/mL (DDPH), 45.71 μg/mL (reducing power), and 10.25 μg/mL (β-carotene bleaching inhibition).

Table 4. Antibacterial and antifungal activity (mg/mL of extract) of *Opuntia* peels.

Bacteria	OS		OG		OE		C1		C2	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacillus cereus	0.075	0.15	0.05	0.075	0.10	0.15	0.10	0.20	0.25	0.40
Micrococcus flavus	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.20	0.30	0.25	0.40
Staphylococcus aureus	0.15	0.45	0.15	0.30	0.30	0.45	0.04	0.10	0.25	0.45
Listeria monocytogenes	0.15	0.60	0.15	0.45	0.30	0.45	0.20	0.30	0.40	0.50
Escherichia coli	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.20	0.30	0.40	0.50
Enterobacter cloacae	0.075	0.30	0.075	0.30	0.10	0.15	0.20	0.30	0.25	0.50
Pseudomonas aeruginosa	0.15	0.45	0.15	0.45	0.30	0.45	0.20	0.30	0.75	1.20
Salmonella typhimurium	0.15	0.30	0.15	0.45	0.15	0.30	0.25	0.50	0.40	0.75
Mold	OS		OG		OE		C3		C4	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus fumigatus	0.30	0.45	0.30	0.60	0.30	0.60	0.25	0.50	0.15	0.20
Aspergillus versicolor	0.30	0.45	0.30	0.45	0.30	0.45	0.20	0.50	0.10	0.20
Aspergillus ochraceus	0.10	0.15	0.15	0.30	0.15	0.30	1.50	2.00	0.15	0.20
Aspergillus niger	0.30	0.45	0.30	0.45	0.30	0.45	0.20	0.50	0.15	0.20
Trichoderma viride	0.15	0.30	0.075	0.15	0.10	0.15	1.00	1.00	0.15	0.20
Penicillium funiculosum	0.15	0.30	0.30	0.45	0.30	0.45	0.20	0.50	0.20	0.25
Penicillium ochrochloron	0.075	0.15	0.075	0.15	0.10	0.15	2.50	3.50	0.20	0.25
Penicillium verrucosum var. *cyclopium*	0.30	0.45	0.30	0.45	0.30	0.60	0.20	0.30	0.10	0.20

C1, Streptomycin; C2, Ampicillin; C3, Ketoconazole; C4, Bifonazole; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentrations; MBC, minimum fungicidal concentrations; N.A., No activity.

OG, *Opuntia ficus-indica* var gialla; OS, *Opuntia ficus-indica* var sanguigna; OE, *Opuntia engelmannii*.

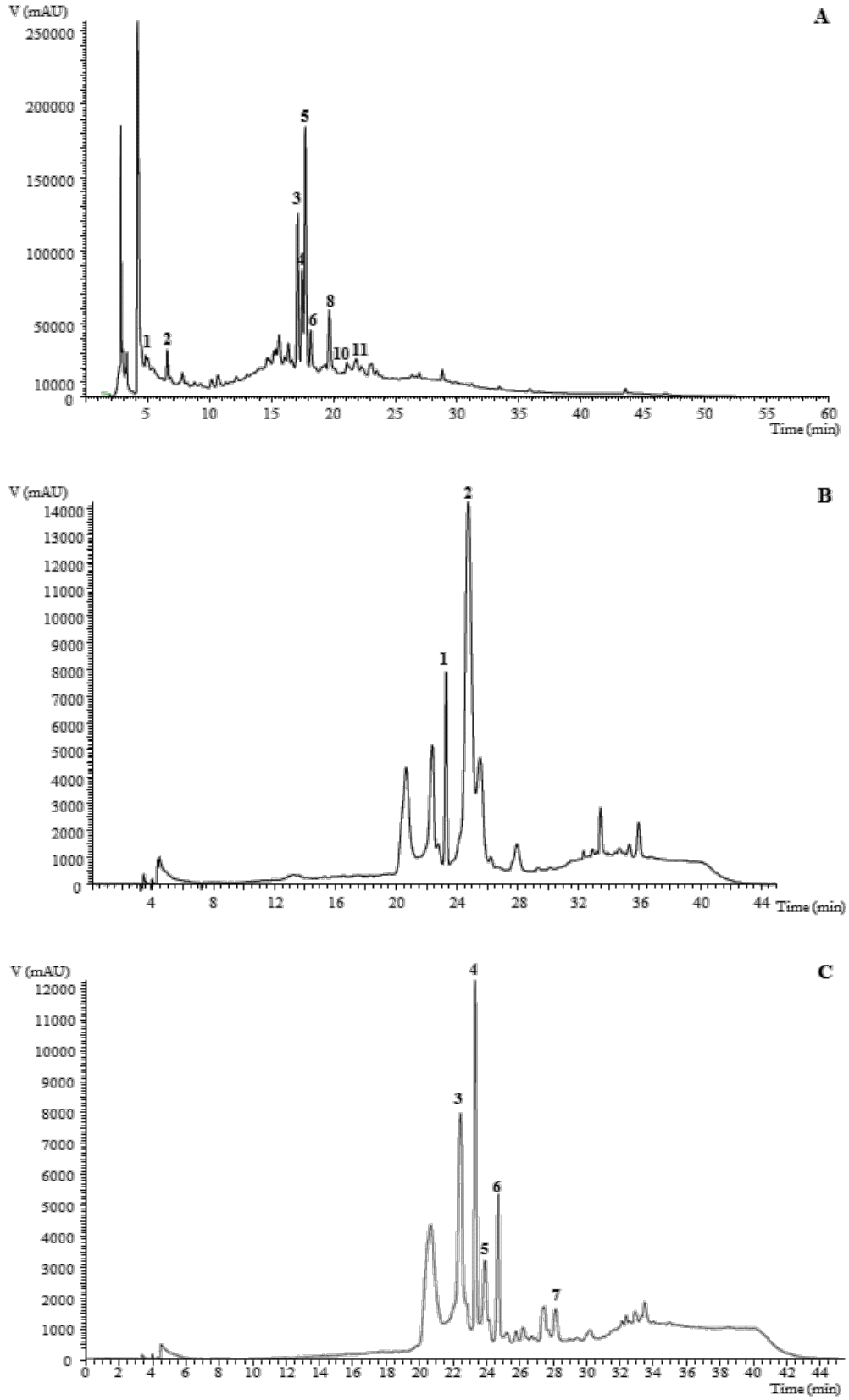

Figure 1. HPLC chromatogram of OG phenolic profile recorded at 370 nm (A), betaxanthins profile of OS recorded at 484 nm (B) and betacyanins profile of OE recorded at 535 nm (C).