Comparison of total polyphenol content, antioxidant activity, and antimicrobial potential among nine cactus pear (*Opuntia* spp.) cultivars and their by-products

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ABSTRACT

Nopal cacti (*Opuntia* spp.) are important natural resources in semi-arid zones throughout the world, where their fruits and stems are used as forage or human food depending on the variety and ripeness of the cladode. Members of the genus *Opuntia* show variable phytochemical composition as well as a high capacity for adaptation to extreme environmental conditions. One part of the process to prepare and use of "nopalitos" for human consumption includes removal of modified leaves or thorns, which is approximately 20% of the fresh weight of cladodes is considered as a by-product (production residue). The objective of this study was to evaluate the phytochemical composition, antioxidant capacity, and antibacterial potential of cladodes and cladode by-products from nine nopal cultivars. Young cladodes (<1 month, nopalitos) and their corresponding by-products were washed, dried, and macerated in ethanol. Ethanol extracts were isolated by evaporation and resuspended in water. Total phenols and flavonoids were quantified, and antioxidant capacity was assessed by spectroscopic assays [trolox equivalent antioxidant capacity (TEAC) and percent inhibition of diphenylpicrylhydrazyl (DPPH) radicals]. Antimicrobial activities of the extracts against *Vibrio cholerae* and *Clostridium perfringens* were determined by the well diffusion method and calculation of minimal bactericidal concentration (MBC) by agar plate assay. All extracts tested showed antioxidant and antibacterial activities. Extracts of cladodes and their corresponding by-products showed similar MBCs for *C. perfringens* (around 15 mg ml^{-1}) and *V. cholerae* (4.0 mg ml^{-1}). However, extracts of cladode by-products showed higher antioxidant capacity than those of cladodes according to both DPPH and TEAC assays. Higher concentrations of phenols and flavonoids were also found in the extracts of by-products (ranging from 91.23 to 853.78 mg gallic acid equivalents per gram dry weight and from 17.10 to 39.42 mg quercetin equivalents per gram dry weight, respectively). This study demonstrates that nopal cactus species exhibit higher antioxidant activity and contents of both phenols and flavonoids in cladode by-products than that in their corresponding cladodes. Our data indicate that discarded cladode by-products may be are a potentially valuable resource for nutraceutical and drug development.

Keywords: *Opuntia* spp., tender cladodes, antioxidant capacity, antimicrobial potential.

INTRODUCTION

There are 104 nopal cactus species belonging to the genus *Opuntia* growing in Mexico, of which 60 are considered endemic (Gallegos-Infante *et al*., 2009). In 2016 Mexico produced about 44,768 tons nopalitos generating 14 mmd, being USA the top export destination of this product (SIAP, 2017). These crops present high growth capacity in semi-arid ecosystems that do not favor other vegetal species and are also observed in family gardens and commercial plantations (Pérez-Cacho *et al*., 2006).

Cactus plants comprise succulent botanically articulated stems called nopal pads or cladodes composed of polysaccharides that allow the plant to retain water. Cladodes of nopal are of substantial commercial value as human food and cattle feed (Grünwaldt *et al.,* 2015). However, nopal cacti have other value-added uses, such as being sources of ingredients for cosmetics and pharmaceuticals, being substitutes for flour, and in the production of biofuels (El-Mostafa *et al*., 2014). These applications are possible due to the diversity of bioactive compounds in various nopal species, such as vitamin C, pigments (carotenoids and betalains), fiber, hydrocolloids (mucilage), polyphenols, and minerals (calcium, potassium) (Avila-Nava *et al*., 2014; Lira-Vargas *et al*., 2014; Matsuhiro *et al*., 2006). Reports indicate that the phytochemical composition of nopal cladodes (nopalitos) depends on the state of ripeness, harvest season, environmental conditions, and post-harvest treatments. Given this variability, it is important to compare cultivars grown under the same environmental conditions and at the same stage of ripeness (Astello-García *et al*., 2015).

At 3 to 4 weeks of age, “nopalitos” from cactus pear can be eaten fresh or cooked in several ways. Processing for human consumption includes removal of modified leaves or thorns that are usually discarded and have no economic value. Constituents of the cladode such as thorns, glochids, and some of the pulp that account for 20%–40% of their fresh weight are discarded during processing and considered as by-products or production residue (Bensadón *et al.,* 2010; Sánchez *et al.,* 2014). These by-products are considered to be rich in fiber, minerals, and bioactive antioxidant compounds and are widely used in the production of seed oil, cladode and mucilage fiber, pigments (contained in shells and fruits), and forage pasta (from seeds and shells) (Bensadón *et al*., 2010; Morales *et al.,* 2014); however, additional applications could represent aggregative value to cactus producers.

The most representative species of this genus, *O. ficus-indica*, is both ecologically and economically important because of its adaptability to different climatic conditions and its complex phytochemical composition (Griffith, 2004). Sánchez *et al*. (2014), reported the antimicrobial and antioxidant activities of eight nopal cacti extracts of fully grown (6-mo-old) cladodes from Mexican cultivars against *Campylobacter Jejuni*, *Vibrio cholera* and *Clostridium perfringens*, however, few studies have evaluated the antioxidant and antimicrobial potentials

of “nopalitos” and their by-products of *O. ficus-indica* and other species belonging to this genus. Therefore, the objective of this work was to compare the total polyphenol content, antioxidant capacity, and antimicrobial potential among the young cladodes (<1 month) and corresponding by-products of nine *Opuntia* spp. cultivars.

MATERIALS AND METHODS

Plant material and bacterial strains

“Nopalitos” (not more than one month old) from nine cultivars of *Opuntia* spp. were collected from the nopal germplasm bank of the Facultad de Agronomía, Universidad Autónoma de Nuevo León (Escobedo, Mexico) and identified by Dr. Rigoberto Vázquez Alvarado. The cultivars analyzed included five of *O. ficus-indica* (L), [Jalpa (JL), Villanueva (VN), Copena F1 (CF1), Copena V1 (CV1), and Esmeralda (ES)] and one each of *O. streptacantha* [Cardón Blanco (CB)], *O. albicarpa Scheimvar*, [Cristalina (CR)], *O. rastrera* [Forrajero Mina (FM)], and *O. lindheimeri* [Cacanapo (CA)]. The cladodes were peeled as usual for human consumption, removing thorns, glochids, and epidermis (by-products). Ten grams of plant material (cladodes and by-products) were washed, dried, and macerated with 100 ml of 70% ethanol (CTR Scientific, Mexico) for 5 days at room temperature. The ethanol was evaporated using a rotary evaporator (Buchi, R3000-A24) and the residue resuspended in a small volume of water. An aliquot from each extract was used for dry weight (DW) determination and stock solution preparation (all 25 mg ml^{-1}). Stock extracts were stored in dark vials at 4°C and used within 5 days.

A Gram positive, sporeforming enterotoxin-positive strain of *C. perfringens* and a toxigenic Gram negative strain of *V. cholerae* were used for antimicrobial testing. The *C. perfringens* strain FD-1041 was provided by Stanley Harmon (Food and Drug Administration, Washington, D.C. USA) and maintained as a stock spore culture in cooked meat medium (Difco Laboratories, Detroit, MI, USA) at –20°C. Active cultures were obtained by transferring two drops of the stock culture into test tubes containing 5 ml of fluid thioglycollate medium (FTG; Difco, Laboratories, Sparks, MD, USA.), heat-activated at 75°C for 15 min, and incubated overnight (16 to 18 h) at 37°C (García-Alvarado *et al.,* 1992). The toxigenic *V. cholerae* strain 1837 Ogawa O139 was provided by Elisa Elliot (Food and Drug Administration, Washington, DC, USA) and maintained as a stock culture in Luria–Bertani (LB) agar (LB broth containing 1% NaCl, 1% pancreatic digest of casein, and 0.5% yeast extract plus1.5% agar) at room temperature. Active cultures were obtained by transferring an aliquot of the stock culture into test tubes containing 10 ml LB broth and incubating overnight (16 to 18 h) at 37°C. All experiments were performed at least in triplicate.

Determination of extract phenol, flavonoid, and tannin contents

Total phenols

Total phenols were measured by the Folin–Ciocalteu reagent colorimetric method reported by Singleton *et al*. (1999) with some modifications. In brief, aliquots of the extract (15 µl, adjusted to 10 mg ml^{-1}) were added to wells of 96-well microplates containing 240 µl of distilled water. Then, 15 µl of 2N Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) was added and the mixture allowed to sit for 3 min at room temperature. The reaction was stopped by addition of 30 µl of 4N Na_2CO_3 (Sigma-Aldrich). After incubation for 2 h at room temperature, absorbance was measured at 760 nm on a spectrophotometer (UV/Vis Epoch™ 2C, BioTek Instruments Inc., USA). Total phenolic content was quantified by a gallic acid (0 to 0.4 mg ml^{-1}) standard curve. Results are reported as mg of gallic acid equivalents (GAE) g^{-1} of extract DW.

Total flavonoids

Total flavonoids were measured by the method reported by Ghasemi *et al.* (2009), with slight modifications. In brief, 20 µl of extract adjusted to 10 mg ml^{-1} was mixed with 112 µl distilled water, 60 µl of 80% methanol (Sigma-Aldrich, St. Louis, MO, USA), 4 µl of 10% $AlCl_3$ (Fermont, MTY, MEX), and 4 µl of 1M $C_2H_3KO_2$ (Fermont) in each well of 96-well flat-bottom microplates (Thermo Fisher Scientific). Microplates were incubated in the dark for 30 min at room temperature, and absorbance was measured at 415 nm. Total flavonoids are expressed in mg of quercetin equivalents (EQ) g^{-1} DW.

Total tannins

Total condensed tannins (CTs) was determined by the vanillin–H_2SO_4 reaction as described by Sun *et al.* (1998). Twenty-five microliters of extract (adjusted to 10 mg ml^{-1}) was mixed with 65 µl of 1% vanillin (Sigma-Aldrich) in methanol (Sigma-Aldrich) and 65 µl of 25% H_2SO_4 in methanol in 96-well microplates (Thermo Fisher Scientific). Plates were incubated for 15 min at 30°C, and absorbance was measured at 500 nm. Catechin was used as the standard, and results are expressed as mg equivalents of catechin per gram of DW (ECat g^{-1} DW).

Antioxidant activity

Scavenging of radical activity using DPPH method.

Antioxidant capacity of extracts was measured by the ability to inactivate the stable radical $DPPH^{\bullet}$ according to the method described by Kedare and Singh (2011) with some modifications. An aliquot (2.5 mg) of $DPPH^{\bullet}$ radical (Sigma-Aldrich) was solubilized with 100 ml absolute methanol and adjusted to $A_{515\,nm}$ ($A_{Initial}$) of 0.7 ± 0.02. An aliquot (280 µl) of this solution was mixed with 20 µl of the extract (adjusted to 10 and 1 mg ml^{-1} for cladodes and by-products, respectively), stirred for 10 s, and incubated for 30 min in darkness at room temperature.

Absorbance was measured at 515 nm (A_{Final}). The free radical capture was expressed as the percentage inhibition of DPPH$^{\bullet}$ radical using the formula:

$$\% \, Inh_{DPPH\bullet} = [(A_{Initial} - A_{Final})/Abs_{Initial}] \times 100$$

In addition, the antioxidant capacities of cladode and by-product extracts were measured by the trolox equivalent antioxidant capacity (TEAC) assay. A 1-ml working solution of 7.4 mM 2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$, Sigma-Aldrich) plus 1 ml of 2.6 mM $K_2S_2O_8$ was prepared and adjusted to $A_{734\ nm}$ 0.7. Aliquots (285 µl) of this solution were mixed with 15 µl of extract (adjusted to 10 mg/ml for cladode and 1 mg ml^{-1} for by-product extract) or trolox standard solution dissolved in methanol. At minutes 1 and 6 of the reaction, $A_{734\ nm}$ was measured (Thaipong *et al*., 2006). Percentage inhibition of $ABTS^{\bullet+}$ and test samples were compared to trolox standards and results were expressed in µmol of TE g^{-1} DW.

Determination of Antibacterial Activity

A preliminary assay to determine the antimicrobial activity of extracts against *C. perfringens* and *V. cholerae* strains was performed using the well diffusion method (García *et al*., 2002). For *C. perfringens,* an aliquot (100 µl) of the activated strain adjusted to 1.5×10^8 CFU ml^{-1} was pipetted into a sterile Petri dish, immediately covered with 15 ml of melted plate count agar (PCA, Difco, Laboratories, Sparks, MD, USA) at 50°C, and uniformly distributed by stirring in a circular motion. Once the agar solidified, 5 ml PCA was added over the inoculated agar to favor anaerobiosis. For *V. cholera* tests, an aliquot (1.5×10^7 UFC) was inoculated on LB agar in Petri dishes using a Driglasky loop.

Five holes (12 mm in diameter) were made in the seeded agar and filled with 100 µl of each extract. Plates were incubated at 37°C for 24–48 h [under anaerobic conditions (5% CO_2 and 95% N_2) for *C. perfringens*]. Growth inhibitory activity was then visualized as loss of bacterial growth in the area surrounding the holes (halo). The relative inhibition of bacterial growth by the extract was quantified by measuring the diameter of the resulting inhibition zone.

Minimal Bactericidal Concentration (MBC)

The MBC was determined by the method described by García *et al*. (2002). In brief, cells (1×10^8 CFU) from activated cultures of *C. perfringens* or *V. cholerae* were grown in wells of microtiter plates (Thermo Fisher Scientific, Marietta, OH, USA) containing either 125 µl of 2× FTG (for *C. perfringens*) or 2× LB broth (for *V. cholerae*) in the presence of various concentrations of extract (in aliquots of 125 µl). Cultures were then incubated (anaerobically for C*. perfringens* or aerobically for *V. cholerae*) at 37°C for 48 h. Bacterial survival was determined by plating *C. perfringens* on CPA and *V. cholerae* on LB agar and incubating as described above. MBC was regarded as the lowest concentration of the extract that did not

permit any visible bacterial colony growth on the agar plate after the period of incubation. Sterile distilled water was used as a negative control (García-Heredia *et al*., 2016).

Statistical analyses

Data were analyzed using the general linear model procedure of Number Cruncher Statistical System version 6.0 (NCSS, LLC). Significant differences between cultivars were determined using Tukey's comparison test. $P \leq 0.05$ was considered significant. Each experiment was performed in triplicate.

RESULTS

Phenol, flavonoid, and tannin content of extracts

Total extractable phenol, flavonoid, and CT contents differed significantly ($P \leq 0.05$) among nopal cactus cultivar extracts (Fig 1 and 2). Cladodes of the CB cultivar exhibited the highest total phenolic content (86.36 ± 5.8 mg GAE g^{-1} DW), followed by ES (74.94 ± 10.8), FM (53.61 ± 8.6), and CA (24.65 ± 3.1), whereas cladode extracts of the cultivars CR, JL, VN, CF1, and CV1 did not differ significantly in phenol content (Fig. 1A).

The phenol contents of by-product extracts varied markedly, from 91.2 ± 8.2 to 853.8 ± 52.7 mg GAE g^{-1} DW, with highest concentration detected in ESH extract, followed by CRH, CBH, FMH, VNH, CF1H, CAH, JLH, and CV1H extracts (Fig 1B). Extracts from the CV1H cultivar contained 89.3% less total phenol than those from the ESH cultivar (Fig. 1A).

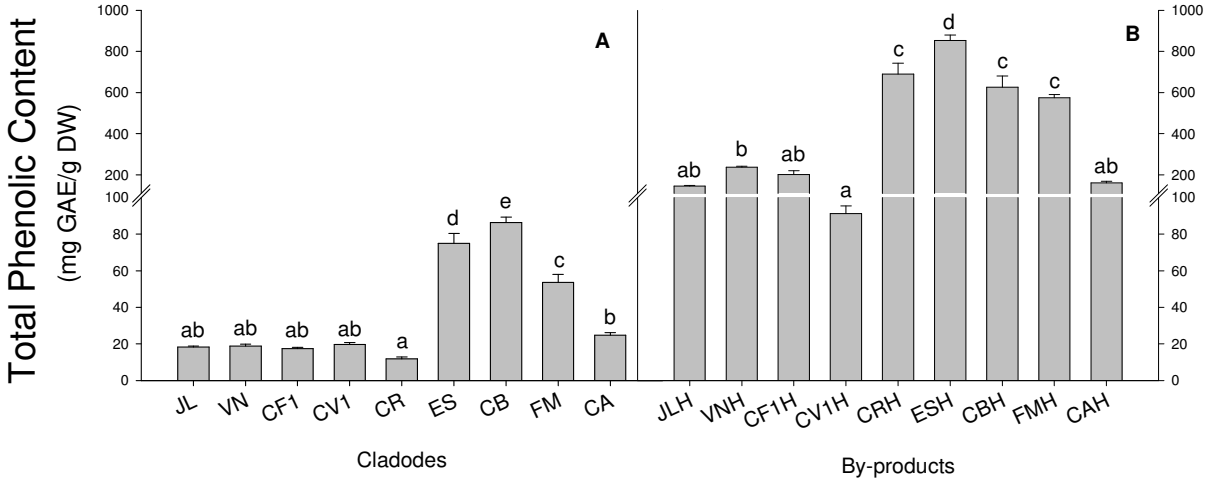

Figure 1 (A, B). Total phenol content of extracts from nine *Opuntia* spp. cultivars

and their by-products (B). Different letters represent significant differences (P<0.05) between cultivars.

Total flavonoid concentration also differed significantly among cultivars (P≤0.05) (Fig. 2). The highest total flavonoid content (in mg QE g^{-1} DW) was detected in the by-product extract from CBH (45.1 ± 3.9), followed by CRH (39.4 ± 16.3), ESH (36.1 ± 1.7), CV1H (35. 9 ± 1.0), CF1 (26.4 ± 2.0), FMH (25.5 ± 1.2), JLH (17.2 ± 0.8), CAH (17.1 ± 1.4), and VNH (16.4 ± 7.3) (Fig. 2B). For cladode extracts, the highest flavonoid content (in mg QE g^{-1} DW) was detected in the by-product extracts from the ES cultivar (4.8 ± 0.2), followed by VN (4.0 ± 0.4), CF1 (4.0 ± 0.5), CB (3.3 ± 1.6), JL (3.2 ± 0.3), CA (2.6 ± 2.9), FM (2.4 ± 0.4), CV1 (2.2 ± 0.3), and CR1 (1.8 ± 0.1) (Fig. 2B).

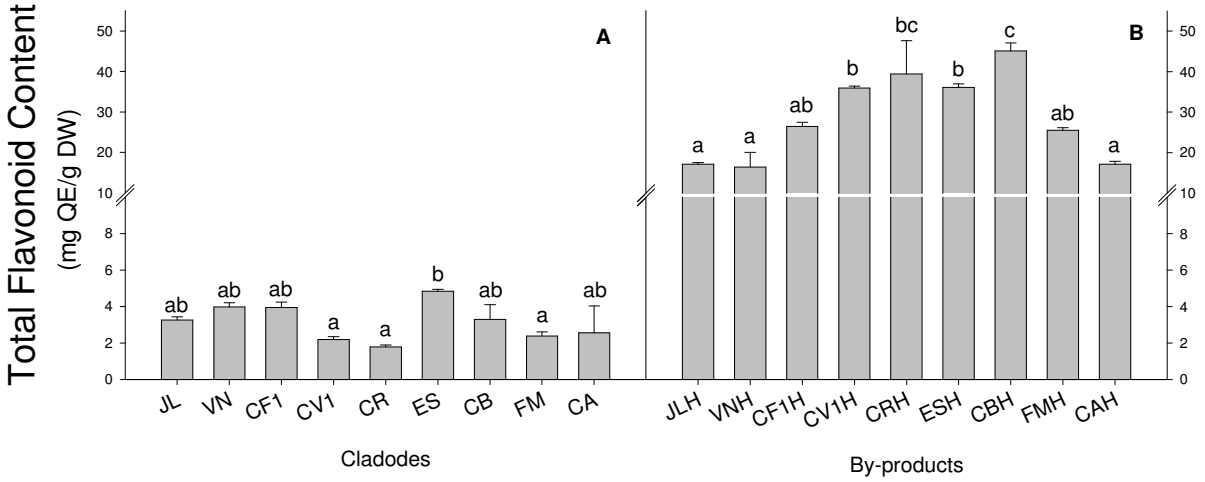

Figure 2 (A, B). Total flavonoid content of extracts from nine *Opuntia* spp. cultivars and their by-products (B). Different letters represent significant differences (P<0.05) between cultivars.

Similarly, the concentration of tannins varied significantly (P≤0.05) among extracts from cladodes (average of 14.8 mg ECat g^{-1} DW) and by-products (average of 39.8 mg ECat g^{-1} DW), with highest cladode extract content from cultivar CR (35.1), followed by ES, FM, CF1, VN, JL, CB, CV1, and CA (17.4 ± 0.0, 13.9 ± 0.0, 13.8 ± 0.0, 12.8 ± 0.0, 12.5 ± 0.0, 11.4 ± 0.0, 10.3 ± 0.0, and 6.3 ± 0.0 mg ECat g^{-1} DW, respectively, Fig. 3A). Of by-product extracts, those from CAH showed the highest tannin concentration (51.4 mg GAE g^{-1} DW), followed by CV1H (50.0 ± 0.0), CRH (43.9 ± 0.0), ESH (40.8 ± 0.0), FMH (40.3 ± 0.0), CBH (37.0 ± 0.0), CFH (34.4 ± 0.0), JLH (32.0 ± 0.0), and VNH (28.7 ± 0.0) (Fig. 3B).

Significant differences (P≤0.05) in antioxidant capacity as measured by DPPH and TEAC assays were found among both cladode and by-product extracts (Figs. 4 and 5). Like tannin content, by-product extracts showed generally higher antioxidant capacities than cladode extracts as measured by DPPH radical elimination, with highest percentage radical scavenging activities (%RSAs) for cultivars JLH (47.57% ± 2.9%), CV1H (43.48% ± 8.8%), and CF1H (40.59% ± 3.2%). These activities were significantly higher than those for the corresponding cladodes. Alternatively, %RSAs for the remaining by-products range from 18.1% ± 1.9% to 35.9% ± 6.9% and did not differ from the corresponding cladodes (%RSAs from 19.3% ±1.4% to 24.1% ±3.1%) with the exception of the VN cultivar (33.12% ± 6.9%).

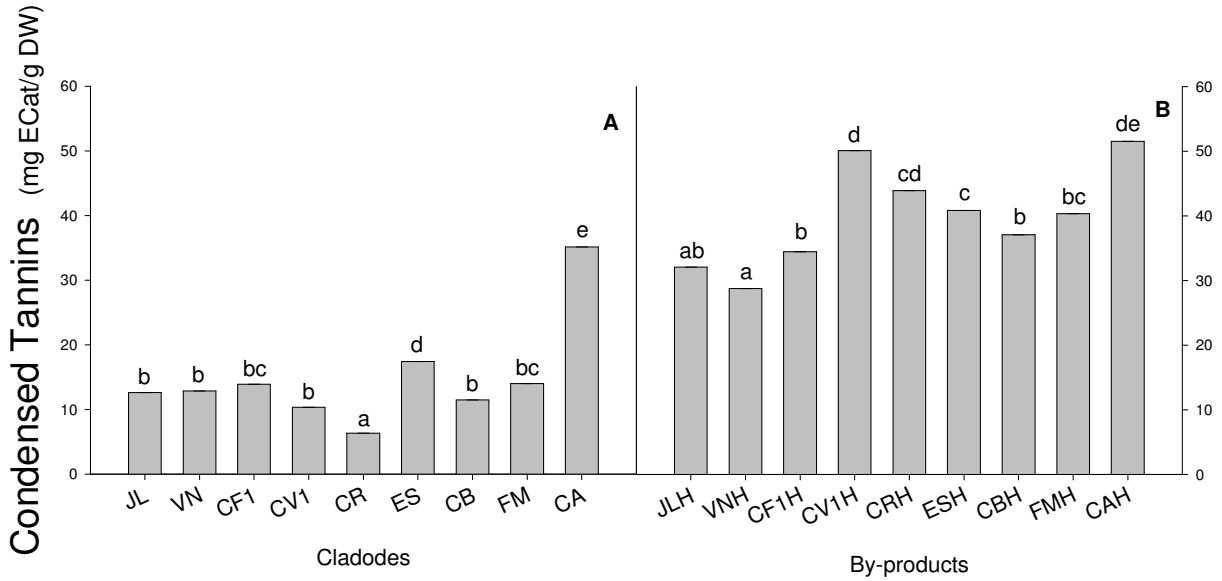

Figure 3. (A, B). Condensed tannin content of extracts from nine *Opuntia* spp. cultivars and their by-products (B). Different letters represent significant differences ($P < 0.05$) between cultivars.

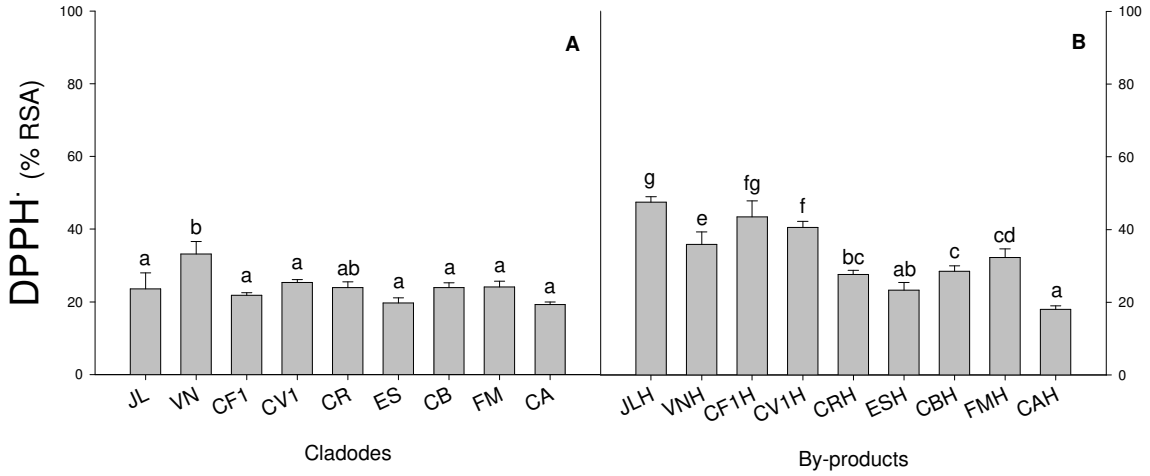

Figure 4. (A, B). Radical scavenging activities of extracts from nine *Opuntia* spp. cultivars and their by-products (B). Different letters represent significant differences (P<0.05) between cultivars.

By-product extracts also generally showed higher antioxidant capacities as measured by TEAC assay. Highest activities (in μMET g^{-1} DW) were found in by-product extracts from cultivars FMH (2,646.5 ± 273.5) and CBH (2,317.7 ± 257.5), with TEAC values for the remaining extracts (from CAH, JLH, VNH, CF1H, CV1H, ESH, and CRH) ranging from 271.2 ± 127.6 to 868.6 ± 118.9 μM TE g^{-1} DW (Fig. 5B).

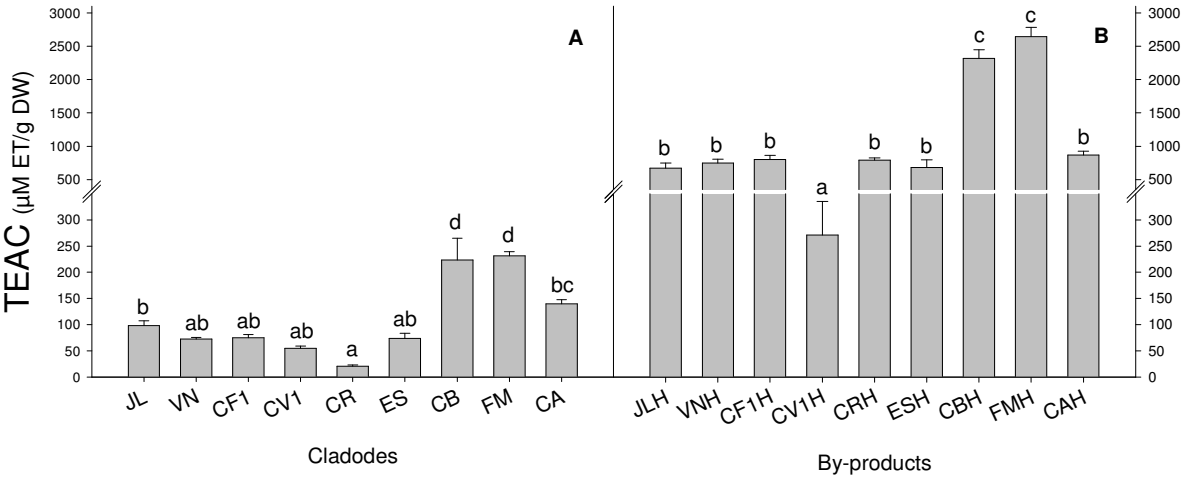

Figure 5. (A,B). Trolox equivalent antioxidant capacity (TEAC) of extracts from nine *Opuntia* spp. cultivars and their by-products (B). Different letters represent significant differences (P<0.05) between cultivars.

The TEAC values in extracts from young cladodes were highest (P≤0.05) for cultivars FM (231.5 ± 15.5) and CB (223.1 ± 83.1), whereas values in the rest ranged from 139.5 ± 15.5 to 20.3 ± 4.6 (Fig. 5A).

Antibacterial Activity

The antimicrobial activities of ethanol extracts from nopal cactus cultivars and their by-products against *C. perfringens* and *V. cholerae* as measured by the well diffusion method are presented in Table 1. All cladode extracts produced halos indicative of *V. cholerae* growth inhibition (2.41 ± 0.7 to 6.08 ± 1 mm), as did the corresponding by-product extracts (1.33 ± 0.5 to 6.28 ± 2.1 mm) with significant differences (P≤0.05) in potency according to the halo diameter. While, no differences in *C. perfringens* growth inhibition were observed between cladode extracts (19.1 ± 9.6 to 27.8 ± 3.7 mm) and their corresponding by-product extracts (4.6 ± 2.3 to 26.6 ± 6.7 mm). In general, cladode and by-product extracts from cultivars VN, JL, CV1, and CF1 inhibited *C. perfringens* growth at concentrations of 15 mg ml^{-1} or higher, whereas they inhibited *V. cholerae* growth at concentrations of 4 mg ml^{-1} or higher. Cladode and by-product extracts from these cultivars also exhibited similar MBCs, ranging from 10 to 15 mg ml^{-1} against *C. perfringens* and 4.0 or 5.0 mg ml^{-1} against *V. cholerae*.

Table 1. Growth inhibition halo sizes and minimal bactericidal concentrations (MBCs) of extracts from nine cultivars of *Opuntia* spp. and by-products against *Clostridium perfringens* and *Vibrio cholerae*

	Cladodes				By-products			
	C. perfringens		*V. cholerae*		*C. perfringens*		*V. cholerae*	
Nopal Cactus Cultivar**	FD-1041 Halos of inhibition (mm)	MBC (mg ml^{-1})	1837 Halos of inhibition (mm)	MBC (mg ml^{-1})	FD-1041 Halos of inhibition (mm)	MBC (mg ml^{-1})	1837 Halos of inhibition (mm)	MBC (mg ml^{-1})
JL	25.8 ± 7.2^{a}	15	3.66 ± 0.28*^{ab}	4	24.5 ± 9.5^{ab}	15	3.16 ± 0.28*^{abc}	4
VN	27.8 ± 3.7^{a}	15	4 ± 0^{ab}	4	25 ± 8.6^{ab}	15	3.91 ± 0.15^{abcd}	4
CF1	25.0 ± 8.6^{a}	15	2.58 ± 1^{a}	4	20.3 ± 8.4^{ab}	10	4.5 ± 0.86^{bcd}	4
CV1	22.6 ± 6.3^{a}	ND	4 ± 1.73^{ab}	4	ND	ND	1.33 ± 0.57^{a}	4
CR	24.8 ± 5.0^{a}	10	2.66 ± 1.15^{a}	4	11.8 ± 3.2^{ab}	10	4.91 ± 0.87^{cd}	5
ES	19.1 ± 9.6^{a}	10	4.16 ± 2^{ab}	4	22.0 ± 11.3^{ab}	15	1.66 ± 0.57^{ab}	4
CB	20.8 ± 8.9^{a}	15	2.41 ± 0.71^{a}	4	27.2 ± 3.8^{ab}	10	3.74 ± 0.65^{abcd}	4
FM	26.1± 6.6^{a}	15	5 ± 1^{ab}	4	26.6 ± 5.7^{b}	ND	3.5 ± 1.32^{abcd}	5
CA	27.3 ± 4.6^{a}	15	6.08 ± 1^{b}	4	4.6 ± 2.3^{a}	10	6.28 ± 2.19^{d}	4

** JL, Jalpa (*Opuntia ficus-indica*); VN, Villanueva (*O. ficus-indica*); CF1, Copena F1 (*O. ficus-indica*); CV1, Copena V1 (*O. ficus-indica*); CR, Cristalina *(O. albicarpa Scheimvar*); ES, Esmeralda (*O. ficus-indica*); CB, Cardón Blanco (*O. streptacantha*); FM, Forrajero Mina (*O. rastrera*); CA Cacanapo (*O. lindheimeri*). Different letters represent significant differences (*P*<0.05) among cultivars.

DISCUSSION

Cactus species of the genus *Opuntia* are important natural and economic resources in desert environments due to their adaptability and rich phytochemistry (Stintzing and Carle, 2005). Indeed, these plants are rich in vitamin C, β-carotene, and secondary metabolites such as polyphenols (Stintzing and Carle, 2005). These polyphenols (mainly phenolic acids, flavonoids, tannins, and in lower concentrations, stilbenes and lignans) are involved in numerous physiological and developmental processes (Daglia, 2012). Moreover, these compounds possess a variety of therapeutic properties in humans and animals, including antiallergic, antiartherogenic, anti-inflammatory, antimicrobial, and antioxidant activities. The antioxidant capacity is mainly due to the ability of plant polyphenols to scavenge free radicals, thereby reducing further free radical reactions (Balasundram *et al*., 2006; Daglia, 2012).

The cultivars of *Opuntia* analyzed in this study have a variety of commercial uses. For instance, the cultivars JL, VN, and CV1 are grown for human cladode consumption, whereas the cultivars CF1, CA, and FM are used as forage and CR, ES, and CB for fruit production (Mondragón-Jacobo, 2003; Vázquez-Alvarado *et al*., 2008, 2009). However, although the phytochemical compositions and antioxidant properties of cactus pear cladodes are known, few studies have compared the antioxidant and antimicrobial properties of different cultivars (Sánchez *et al*., 2014).

Total phenolic content of nopal cactus plants varies markedly among regions. Chougui *et al.* (2015) reported 1,512 ± 31 mg GAE 100 g^{-1} DW in by-products of the prickly pear cactus *O. ficus-indica* from Algeria, whereas those growing in Italy contained 760 mg GAE 100 g^{-1} DW, those in Mexico 194–362 mg GAE 100 g^{-1} DW, and those in Tunisia 1,650–1,850 mg GAE 100 g^{-1} DW. The mean content found in this study (1,512 ± 31 mg GAE 100 g^{-1} DW) is consistent with that reported in Tunisia but substantially higher than that previously reported in Mexico. This disparity could be attributed to differences in cultivar, geographical origin, stage of maturity, storage conditions, and (or) the methods and solvents used for extraction (Kim *et al.,* 2015).

The total phenolic contents of cladodes at 6 months were recently compared among *Opuntia* cultivars (Sánchez *et al*., 2014). In the present study, total phenolic contents were higher, possibly due to stage of ripeness or inherent differences among cultivars. Similar differences among cladodes of different ripeness (Rodríguez-García *et al.,* 2007) suggest that physicochemical composition changes substantially with state of maturation. Total phenolic content of young cladodes from cactus pear (*O. ficus-indica*) was reported to range from 17 to 40 mg DW (Guevara-Figueroa *et al.,* 2010; Medina-Torres *et al.,* 2011) in accordance with the values obtained in the current study. However, these values are considerably lower than those obtained in by-product extracts.

Previous reports on cactus pear also found higher phenolic contents in the colorless spines than those in the chlorophyll-containing tissue (Stintzing and Carle, 2005). In the present study,

the young spines examined were still in the green state but similarly high values were found. It would be important to compare the total content of phenols of the green-young spines with that of the colorless spines.

Some condensed tannins (CTs) have been reported to possess antibacterial or bacteriostatic activity and anticarcinogenic properties. Furthermore, these compounds can inhibit lipid peroxidation and platelet aggregation. The tannins present in cranberry juice induce acidification of the urinary tract, whereas the proanthocyanidins prevent adhesion of *Escherichia coli* to cell surfaces (Vázquez-Flores *et al.,* 2012). Tannin contents appear to differ substantially among cultivars. Mendez *et al.* (2012) reported 30.177 mg g^{-1} DW of total tannins in *O. ficus-indica* (29.0 mg g^{-1} DW as CTs and the remainder as soluble tannins), whereas in the present study, levels ranged from 6.23 to 35.11 mg ECAT g^{-1} DW in cladode extracts and from 28.73 to 51.49 ECAT g^{-1} DW in by-products ex tracts from different cultivars. The radical capture activities (%RSAs) of different cactus pear cultivars reported by Sánchez *et al.* (2014) are similar to the cladode extract values obtained in the present study; however, values were higher in by-product extracts (18.10.7 to 47.65.7 %RSA). Thus, as is the case for total phenols, tannin content may be enriched in cladode by-products, conferring higher antioxidant activity.

Cactus pear cultivars and by-products have also been examined for antimicrobial activities. Methanolic extracts of mucilage and flowers of *O. ficus-indica* showed activity against some gram-negative bacteria (*E. coli* and *P. aeruginosa*) and gram-positive bacteria (*S. aureus*, *B. subtilis*, and *L. monocytogenes*) (Ammar *et al.,* 2015). Aceto-aqueous extracts of the Tunisian syrup variety of O*. ficus-indica* showed activity against *E. coli* (MBC 0.6 mg mL^{-1}), *S. aureus* (MBC 1.3 mg mL^{-1}), *S. epidermidis* (1.3 mg mL^{-1}), and *Salmonell*a (MBC 6.65 mg mL^{-1}) (Dhaouadi *et al.,* 2013) as well as *Campylobacter jejuni* and *C. coli* (El-Mostafa *et al.,* 2014), and *V. cholerae*. In these studies, it was suggested that antimicrobial effects could be due to membrane rupture or increased membrane permeability and consequent decreases in intracellular pH and ATP (El-Mostafa *et al.,* 2014). Mendez *et al.* (2012) reported that gram-positive *S. aureus* strains are more susceptible to inhibition by these vegetables than gram-negative bacteria such as *E. coli, Salmonella*, and *Enterobacter*. In the current study, we also found a tendency for greater activity against gram-positive strains.

CONCLUSION

This study contributes to the knowledge of the antioxidant and antimicrobial properties of different varieties of nopal and their possible correlation. Nopal extracts appear to be excellent candidates for their inclusion in food systems as nutraceuticals with antimicrobial potential. There were significant differences ($P < 0.05$) between total polyphenol content and antioxidant capacity of the extracts of “nopalitos” and their by-products. Generally, by-products extracts showed higher antioxidant capacities and total polyphenol contents than the corresponding

cladode extracts, suggesting additional value-added applications that are currently not exploited.

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