Analytical evaluation of phenolic compounds and minerals of *Opuntia robusta* J.C. Wendl. and *Opuntia ficus-barbarica* A. Berger

Şeyda Kivrak*, Ibrahim Kivrak**|**| and Erşan Karababa*

*Department of Nutrition and Dietetics, Faculty of Health Sciences, Muğla Sitki Koçman University, Kötekli, Turkey; **Department of Chemistry and Chemical Treatment Technologies, Muğla Sitki Koçman University, Muğla, Turkey; *Research Laboratory Center, Food Analysis Laboratory, Muğla Sitki Koçman University, Kötekli, Turkey

**ABSTRACT**

In this study, 19 phenolic compounds were detected using high-throughput instrument ultra performance liquid chromatography with electrospray ionization tandem mass spectrometry (UPLC–ESI–MS/MS) in *Opuntia ficus-barbarica* A. Berger and *Opuntia robusta* J.C. Wendl. fruits. The five macro- and five micro-minerals determined in both species were analyzed using inductively coupled plasma–mass spectrometer. The phenolic compounds, mineral content, and the antioxidant capacity of the fruits of *O. robusta* and *O. ficus-barbarica* were analyzed. All phenolic compounds and minerals varied significantly between the two species. The total of phenolic compounds content was calculated as 69.237 and 66.385 mg kg$^{-1}$, respectively, in *O. ficus-barbarica* and *O. robusta*. Ferulic acid was the highest quantities, 31.620 and 26.931 mg kg$^{-1}$ in *O. robusta* and *O. ficus-barbarica*, in all phenolic contents, respectively. The macroelements calcium and potassium were the most abundant in both *Opuntia* species. The antioxidant activity of *O. ficus-barbarica* and *O. robusta* fruit samples was measured in the extracts of hexane, ethyl acetate, methanol, and water. The DPPH assay of *Opuntia* samples displayed a good radical scavenging inhibition, similar to butylated hydroxyanisole and butylated hydroxytoluene standards, as half maximal inhibitory concentration IC$_{50}$ = 69.32 and 67.57 µg mL$^{-1}$ in ethyl acetate extracts of *O. ficus-barbarica* and *O. robusta* fruits, respectively. This work presents a suitable method for the extraction, detection, and quantification of phenolic compounds by UPLC–ESI–MS/MS. MS/MS determination for multiclass determination was validated in *Opuntia* samples obtaining good results.

**Abbreviations:** ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DTNB, (5,50-Dithio-bis(2-nitrobenzoic)acid; ICP/MS, inductively coupled plasma/mass spectrometer; LoD, yhe limit of detection; MRM, multiple reaction monitoring; QEs, quercetin equivalents; PEs, pyrocatechol equivalents; $R^2$, correlation coefficients; $r$, Pearson’s correlation coefficient; SD, standard deviation; TIC, total ion chromatogram; UPLC–ESI–MS/MS, ultra performance liquid chromatography with electrospray ionization tandem mass spectrometry

**ARTICLE HISTORY**

Received 24 August 2017
Accepted 8 March 2018

**KEYWORDS** *Opuntia ficus-barbarica*; *Opuntia robusta*; phenolics; minerals; ferulic acid; Ultra performance liquid chromatography with electrospray ionization tandem mass spectrometry; Inductively coupled plasma-mass spectrometer

**Introduction**

The cactus pear is cultivated and grown commercially in countries such as Mexico, the United States, Italy, and Israel. On the other hand, this cactus is categorized as an underutilized crop in several
other Mediterranean countries, including Turkey. The cactus pear grows wild in regions comprising high humidity, as are the Mediterranean and Aegean regions in Turkey.\textsuperscript{[1]}

Cactus pear fruits have high commercial value. The \textit{Opuntia} genus are characterized by a high potential of biomass production.\textsuperscript{[2]} They are highly flavored and have excellent nutritional properties. The \textit{Opuntia} fruits are used for the manufacture of food products such as pulps, juices, alcoholic beverages, jams, and natural liquid sweeteners. Additionally, most portions of the cactus plants have been used as drug, capsules, drinks, pills, or powders.\textsuperscript{[3]}

There are increasing concerns and recommendations for consumers to use natural antioxidants from plant sources since the use of synthetic antioxidants has been restricted because some of them have been found to be toxic and carcinogenic. The frequent consumption of fruits and vegetables high in natural antioxidants was reported in many epidemiological studies to lower the incidence of certain types of cardiovascular diseases, diabetes, and cancer.\textsuperscript{[3–6]} These beneficial effects are related to bioactive compounds like phenolic acids, flavonoids, anthocyanins, and carotenoids possessing antioxidant activity.\textsuperscript{[7–11]}

Antioxidants are the compounds that can delay, inhibit, or prevent the oxidation of biomolecules like lipids, proteins, or nucleic acids. Antioxidants may scavenge the free radicals or break the chain reaction due to their redox properties.\textsuperscript{[9,12,13]} Antioxidants are classified as natural and synthetic. Recently, there is an increasing interest in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are restricted due to their carcinogenicity and toxicity.\textsuperscript{[14]} Phenolic compounds, as secondary metabolites, have ability to reduce oxidative damage joint with many diseases including cancer, cardiovascular diseases, cataract, arthritis, and diabetes.\textsuperscript{[9,15]} The antioxidant properties of the phenolic compounds in cactus pear plants make them an important product for preventing human health against degenerative diseases such as cancer, diabetes, hypercholesterolemia, arteriosclerosis, or cardiovascular and gastric diseases.\textsuperscript{[3,16–18]}

\textit{Opuntia} genus also could be a good source of minerals mainly calcium in the common diet.\textsuperscript{[19]} Recent studies highlight the presence of sugars, dietetic fiber, ascorbic acid, phenolic compounds, and pigments (betalains), vitamins, and minerals.\textsuperscript{[20,21]} Phenolic compounds are identified in some fruits of the \textit{Opuntia} genus\textsuperscript{[22,23]} that protect plants from oxidative stress, and in human food, they contribute to preventing disease. Few studies describe the presence of flavonoids in cactus fruits.\textsuperscript{[24]} These metabolites are also important as they have antioxidant, anti-inflammatory, and anticancer properties.\textsuperscript{[25,26]}

Phenolic acids and flavonoids from the genus \textit{Opuntia} have been identified as antioxidants. Phenolic acids such as vanillic acid, ferulic acid, \textit{p}-coumaric acid, \textit{p}-hydroxybenzoic acid, syringic acid, protocatechuic acid, caffeic acid, salicylic acid, gallic acid, and sinapinic acid and flavonoids such as rutin, iso quercitrin, kaempferol, and narcissi are found in plants from the genus \textit{Opuntia}.\textsuperscript{[27,28]}

However, little information is available on the phenolic composition and mineral content of \textit{Opuntia robusta} and \textit{Opuntia ficus-barbarica} fruit samples. The objective of the present study was to investigate the individual phenolic compounds, elemental content, and the antioxidant capacity of \textit{O. robusta} and \textit{O. ficus-barbarica} fruit samples.

\textbf{Material and methods}

\textbf{Opuntia species}

Natural samples of \textit{O. ficus-barbarica} A.Berger and \textit{O. robusta} J.C. Wendl. originating from southwest of Fethiye, Muğla, Turkey, 36°41′ 51.96″N–29°02′ 46.02″E 62 ft and 36°41′ 51.56″N–29°02′44.68″E 58 ft, respectively, were collected in August 2015 (Fig. 1). Samples are identified in the Department of Molecular Biology and Genetic, Faculty of Science, Muğla Sıtkı Koçman University, Muğla (Turkey). The \textit{Opuntia} samples were stored at +4°C and protected from light until further analyses.
Standards and reagents

Phenolic standards (pyrogallol, homogentisic acid, gentisic acid, pyrocatechol, galantamine hydrobromide, \( p \)-hydroxy benzoic acid, 3,4-dihydroxybenzaldehyde, catechin hydrate, vanillic acid, caffeic acid, syringic acid, vanillin, epicatechin, catechin gallate, \( p \)-coumaric acid, ferulic acid, rutin, \textit{trans}-2-hydroxy cinnamic acid, myricetin, resveratrol, \textit{trans}-cinnamic acid, luteolin, quercetin, naringenin, genistein, apigenin, kaempferol, hesperetin, and chrysin) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Quercetin, pyrocatechol, \( \beta \)-carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), ammonium acetate, butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U mg\(^{-1}\)), horse serum butryrycholinesterase (BChE, EC 3.1.1.8, 11.4 U mg\(^{-1}\)), 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB), acetyltiocholine iodide, and butyrylothiocholine chloride were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH, Steinheim, Germany). 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was obtained from Fluka Chemie (Fluka Chemie GmbH, Steinheim, Germany). Hexane, ethyl acetate, and methanol were supplied from Merck KGaA (Darmstadt, Germany). All solvents and other chemicals were of analytical grade purity and were supplied from Merck KGaA (Darmstadt, Germany). HPLC-grade water (18.2 m\( \Omega \)) was purified using a Millipore Elix Advantage 10 and Milli-Q Advantage A10 (Molsheim, France) system that comprise reverse osmosis, ion exchange, and filtration steps.

Determination of individual phenolic compounds

The peel and pulp manually removed at first. Edible portions, which were separated from the seeds, were frozen at \(-18^\circ\text{C}\). Then, the samples were lyophilized (Christ Freeze Dryer Alpha 1-4 LD plus, Germany) in order to remove the water completely and to reduce to a fine dried powder. The lyophilized powder (3 g) was extracted by 30 mL of 80% (v/v) acetone at 25°C for 6 h, and then, ultrasonic extraction was applied for 15 min. The extract was centrifuged at 4000 rpm for 10 min and then filtered using Whatman No. 4. After that, the residue was extracted with three additional 30 mL portions of 80% (v/v) acetone, then the extracts were combined, and the solvent in the extracts was evaporated at 40°C. That extract was treated with 30 mL of hexane for tree times in order to remove fatty acid content and that of hexane phase is discarded. The solid residue was redissolved in methanol and filtered through a PTFE-20/25 LC filter disk as reported by a previous method with slight modification.\(^{[29]}\) Phenolic compounds of \textit{Opuntia} samples were analyzed using high-throughput instrument a Waters ultra performance liquid chromatography with electrospray ionization tandem mass spectrometry (UPLC–ESI–MS/MS) and C18 column (Acquity UPLC BEH C18 100 mm \( \times \) 2.1 mm, 1.7-\( \mu \)m particle size) and the separation of compounds was performed by gradient elution. The mobile phases were composed of solvent A (0.1% acetic acid in water) and
solvent B (0.1% acetic acid in acetonitrile), and the flow rate was 0.650 mL/min, and at 40°C column temperature. The liquid chromatography and mass spectrometry conditions of the analysis were displayed in Table 1.

**Determination of metal contents using ICP–MS**

*Opuntia* fruit samples were washed with ultrapure water in order to clean residual portions of soil, and then samples were dried. Samples (0.2 g) were weighted into teflon tube and mixed with nitric acid (3 mL), hydrochloric acid (0.5 mL), and hydrogen peroxide (0.5 mL) and then extracted with microwave method (Cem Mars 5, Matthews, NC, USA) at a 1200-W power. After the extract solution was cooled down, it was added to flask (20 mL) by completing volume with ultrapure water. *Opuntia* fruit samples were analyzed for the assessment of metal and mineral content using an Agilent inductively coupled plasma–mass spectrometer (ICP–MS) (Agilent 7700×, Tokyo, Japan). Calibration ranges for the analysis were 0.5–200 ppb for microelements and 0.5–25 ppm for macromelements. Operation parameters for the analysis were as follows: plasma power, 1550 W; plasma mode, normal; plasma gas flow rate, 15.0 L min⁻¹; auxiliary gas flow rate, 1.0 L min⁻¹; carrier gas flow rate, 0.89 L min⁻¹; dilution gas flow rate, 0.15 L min⁻¹; sample depth, 8.0 mm; spray chamber temperature, 2°C; kinetic energy discrimination, 3 V; helium gas flow rate, 4.5 mL min⁻¹.

**Measurement of in-vitro antioxidant capacity**

**Inhibition of β-carotene bleaching assay**

The total antioxidant activity was determined using β-carotene-linoleic acid test system based on the detection of inhibition of conjugated dien hydroperoxides due to oxidation of linoleic acid. [31] β-Carotene (0.5 mg), dissolved in 1 mL of chloroform, was mixed with linoleic acid (25 μL) and Tween 40 emulsifier (200 mg). Chloroform was evaporated under vacuum; 50 mL of distilled water saturated with oxygen was added by vigorous shaking. Aliquots (160 μL) of this emulsion were added to 40 μL of the extract solutions at different concentrations. As soon as the emulsion was added to each tube, the zero time absorbance was initially measured at 470 nm using 96-well microplate reader Spectra Max 340PC Molecular Device, and then, the absorbance measurements were done for every 30 min until 120 min. The results were given as 50% inhibition concentration (IC₅₀). The sample concentration inhibiting 50% antioxidant activity (IC₅₀) was calculated from the graph of activity percentage against sample concentration.

**DPPH radical scavenging activity**

The free radical scavenging activity of *Opuntia* extract was determined using DPPH free radical according to literature. [29] The extract solutions with different concentrations (40 μL) and ethanolic solution (120 μL) containing DPPH radicals (0.4 mM) were incubated at room temperature in darkness for 30 min. Absorbance was measured at 517 nm using 96-well microplate reader Spectra Max 340PC Molecular Device.

**ABTS cation radical decolorization assay**

ABTS cation radical decolorization assay was analyzed according to Thaipong et al. [32] The ABTS (7 mM) in water and potassium persulfate (2.45 mM) reacted to give ABTS⁺, stored in the dark at room temperature for 12 h, and oxidation of ABTS appeared immediately; however, the stability of absorbance was gained after 6 h. Then, the sample solution (40 μL) in ethanol at different concentrations was mixed with ABTS⁺ solution (160 μL), giving the absorbance at 734 nm using 96-well microplate reader Spectra Max 340PC Molecular Device.
Table 1. Accuracy, precision, linearity, sensitivity of evaluation of phenolic compounds, and chromatography–mass spectrometry conditions\(^{[200]}\).

| Phenolic compounds | Retention time (min) | Transitions (m/z) | Cone voltage (V) | Collision energy (V) | LoD (mg kg\(^{-1}\)) | Recovery (%) | \(R^2\) | Calibration equations |
|--------------------|----------------------|------------------|-----------------|---------------------|---------------------|--------------|-------|----------------------|
| Homogentisic acid  | 1.92                 | 167.03 > 123.03, 122.08, 108.00 | 10 20, 20, 10 | 0.012 96.3–98.1 | 0.999430 | y = 230.94x + 1003.10 |
| Protocatechuic acid| 2.39                 | 153.06 > 108.00, 81.01, 91.01 | 10 20, 20, 20 | 0.010 95.9–97.6 | 0.995324 | y = 1247.22x + 14883.80 |
| Gentic acid        | 2.39                 | 153.05 > 109.04, 108.03, 81.00 | 10 20, 20, 12 | 0.015 98.1–99.3 | 0.999451 | y = 1012.15x + 2260.24 |
| Pyrocathecol        | 2.96                 | 153.06 > 81.01, 108.00, 109.04 | 8 20, 20, 20 | 0.012 96.5–98.4 | 0.999639 | y = 1077.80x + 1389.91 |
| Galanthamine       | 4.91                 | 288.10 > 198.00, 213.09, 230.95 | 20 32, 23, 17 | 0.010 96.3–99.9 | 0.999225 | y = 1966.17x + 4474.31 |
| \(p\)-Hydroxybenzoic acid | 4.51        | 136.98 > 93.03, 65.10 | 10 25, 14 | 0.010 93.7–96.7 | 0.999702 | y = 1244.52x + 2044.98 |
| 3,4-Dihydroxybenzaldehyde | 4.64 | 137.00 > 91.93, 107.94, 136.00 | 8 21, 20, 18 | 0.012 96.4–99.5 | 0.996602 | y = 647.36x + 3851.16 |
| Catechin hydrate   | 5.55                 | 288.88 > 109.15, 124.99, 245.26 | 30 25, 20, 15 | 0.011 92.7–99.4 | 0.994964 | y = 187.27x + 4499.99 |
| Vanillic acid      | 5.61                 | 166.98 > 151.97, 108.03, 123.03 | 20 18, 12, 14 | 0.012 95.6–98.8 | 0.999753 | y = 787.56x – 2152.85 |
| Caffeic acid       | 5.64                 | 179.10 > 135.14, 107.10, 133.9 | 32 23, 23, 24 | 0.011 98.9–99.3 | 0.998810 | y = 1191.68x + 578.71 |
| Syringic acid      | 6.11                 | 197.20 > 123.00, 167.00, 182.00 | 15 22, 18, 14 | 0.010 97.7–99.7 | 0.998818 | y = 1255.04x + 3501.43 |
| Vanillin           | 6.50                 | 150.95 > 135.94, 91.90, 107.97 | 30 20, 20, 14 | 0.010 92.1–98.1 | 0.969097 | y = 2.8350x + 378.37 |
| Epicatechin        | 6.42                 | 189.18 > 151.00, 203.00, 205.00 | 20 20, 20, 20 | 0.009 96.8–100.7 | 0.993134 | y = 1065.97x + 9349.89 |
| \(p\)-Coumaric acid | 6.59            | 163.01 > 119.04, 93.00, 117.01 | 5 27, 27, 15 | 0.005 92.3–98.3 | 0.999939 | y = 969.50x + 200.96 |
| Ferulic acid       | 7.35                 | 193.03 > 134.06, 178.00, 149.02 | 20 16, 12, 13 | 0.010 92.4–97.6 | 0.965500 | y = 582.30x – 128.89 |
| Catechin gallate   | 7.88                 | 441.00 > 168.98, 288.97 | 30 20, 20 | 0.009 94.6–98.7 | 0.999938 | y = 434.33x + 66.57 |
| Rutin              | 7.86                 | 609.00 > 254.99, 270.93, 299.90 | 17 55, 55, 40 | 0.006 93.5–96.9 | 0.999169 | y = 158.89x – 100.26 |
| trans-2-Hydroxycinnamic acid | 6.61 | 163.04 > 119.04, 117.01, 93.07 | 10 25, 22, 13 | 0.010 90.8–99.9 | 0.966490 | y = 32.40x + 1338.53 |
| Myricetin          | 10.23                | 316.90 > 107.07, 137.01, 150.97 | 30 30, 25, 25 | 0.010 96.2–98.9 | 0.998016 | y = 2746.02x + 12279 |
| Resveratrol        | 9.16                 | 227.01 > 143.01, 159.05, 185.03 | 30 25, 18, 18 | 0.010 95.0–96.4 | 0.999205 | y = 1450.73x + 4459.96 |
| trans-Cinnamic acid | 10.22            | 146.98 > 103.03, 62.18 | 30 10, 10 | 0.009 98.2–99.5 | 0.995544 | y = 702.57x – 5054.70 |
| Rutelin            | 10.28                | 284.91 > 107.01, 133.05, 151.02 | 20 30, 33, 30 | 0.015 90.7–95.7 | 0.999608 | y = 885.18x + 191.21 |
| Quercetin          | 10.97                | 303.00 > 137.00, 153.00, 229.00 | 20 30, 32, 30 | 0.010 98.9–100.1 | 0.998855 | y = 1208.91x + 1135.95 |
| Naringenin         | 10.8                 | 270.98 > 107.00, 119.04, 150.97 | 20 25, 25, 20 | 0.010 96.3–99.9 | 0.999133 | y = 344.66x + 1446.87 |
| Genistein          | 10.87                | 271.00 > 153.00, 215.00, 243.00 | 20 27, 25, 24 | 0.010 94.8–99.7 | 0.999140 | y = 84.47x – 223.41 |

(Continued)
Table 1. (Continued).

| Phenolic compounds   | Retention time (min) | Transitions (m/z) | Cone voltage (V) | Collision energy (V) | LoD (mg kg\(^{-1}\)) | Recovery (%) R\(^2\) Calibration equations |
|----------------------|----------------------|-------------------|------------------|----------------------|----------------------|---------------------------------------------|
| Apigenin             | 10.86                | 269.10 > 107.00, 117.00, 149.00 | 20               | 30, 30, 25           | 0.008                | 98.2–100.0 0.993205 \(y = 72.63x + 5524.59\) |
| Kaempferol           | 10.9                 | 284.90 > 158.97, 117.10, 227.14 | 10               | 34, 40, 30           | 0.010                | 96.7–100.2 0.953357 \(y = 7419.93x + 19711.80\) |
| Hesperetin           | 10.96                | 301.02 > 108.01, 136.00, 163.99 | 20               | 36, 30, 24           | 0.009                | 95.5–98.9 0.940432 \(y = 3016.64x + 186550\) |
| Chlorogenic acid     | 5.52                 | 353.02 > 191.01, 179.09, 161.02 | 30               | 30, 28, 24           | 0.010                | 98.1–99.5 0.999024 \(y = 148.88x - 4047.60\) |
| Gallic acid          | 1.10                 | 168.95 > 125.02, 107.02, 97.02 | 20               | 25, 20, 14           | 0.012                | 94.3–99.4 0.937176 \(y = 1.4723x - 69.63\) |
| Chrysin              | 11.26                | 252.99 > 63.05, 107.05, 142.99 | 20               | 30, 25, 25           | 0.010                | 95.9–99.7 0.981600 \(y = 28.67x - 938.14\) |
Evaluation of the anticholinesterase activity

AChE and BChE inhibitory activities were measured by slightly modifying the spectrophotometric method. Briefly, sodium phosphate buffer (150 μL, 100 mM at pH 8.0), sample solution (10 μL) dissolved in ethanol at different concentrations, and AChE (5.32 × 10⁻³ U) or BChE (6.85 × 10⁻³ U) solution (20 μL) were mixed and incubated for 15 min at room temperature and then DTNB (10 μL, 0.5 mM) was added. Then, the reaction was initiated by the addition of acetylthiocholine iodide (10 μL, 0.71 mM) or butyrylthiocholine chloride (10 μL, 0.2 mM). The hydrolysis of these substrates was monitored spectrophotometrically at a wavelength of 412 nm with a 96-well microplate reader.

Statistical analysis

The results were reported as mean and standard deviation of the mean. Data were subjected to analysis of variance (ANOVA). The least significant difference test was applied to mean to the mean values using the STATISTICA for Windows release 5.0, and correlation coefficients (r) were computed to establish relationships between phenolic compounds and minerals of fruits.

Result and discussion

Accuracy, precision, linearity, sensitivity of evaluation of phenolic compounds

Linearity was determined for phenolic compounds in the concentration range of 0–100 mg kg⁻¹. Correlation coefficients (R²) of linear regression analysis from calibration curves displayed >0.93, between 0.937176 and 0.999939 (Table 1). The limit of detection for sensitivity was evaluated in the range of 0.005–0.020 mg kg⁻¹ and accuracy was determined by evaluating recovery values for each phenolic compound. The chromatography and mass spectrometry parameters values were shown in Table 1.

Phenolic compounds determination

The individual phenolic compound values for each cactus pear are presented in Table 2. One-way ANOVA of the data showed that the effects of cactus pear species were statistically significant.

| Phenolic compound | O. ficus-barbarica (mg kg⁻¹) | O. robusta (mg kg⁻¹) |
|-------------------|-----------------------------|---------------------|
| p-Hydroxy benzoic acid | 8.204 ± 0.012b | 9.352 ± 0.012a |
| Vanillin | 0.197 ± 0.009b | 0.417 ± 0.013a |
| Gentisic acid | 1.394 ± 0.010b | 2.309 ± 0.018a |
| Protocatechuic acid | 2.463 ± 0.011b | 2.549 ± 0.009a |
| p-Coumaric acid | 8.561 ± 0.010a | 1.491 ± 0.021b |
| Vanillic acid | 4.707 ± 0.010a | 2.009 ± 0.018b |
| Chrysin | 0.268 ± 0.009a | 0.181 ± 0.008b |
| Gallic acid | 1.801 ± 0.011a | 2.584 ± 0.010a |
| Caffeic acid | 5.414 ± 0.009a | 3.108 ± 0.010b |
| Ferulic acid | 26.931 ± 0.022b | 31.620 ± 0.018a |
| Homogentisic acid | 1.661 ± 0.015b | 2.741 ± 0.015a |
| Luteolin | 1.174 ± 0.014a | 0.361 ± 0.015b |
| Naringenin | 0.252 ± 0.009a | 0.203 ± 0.010b |
| Myricetin | 0.838 ± 0.010b | 1.850 ± 0.014a |
| Pyrogallol | 4.304 ± 0.014b | 4.501 ± 0.015a |
| Rutin | 0.235 ± 0.010b | 0.555 ± 0.012a |
| Quercetin | 0.650 ± 0.010a | 0.119 ± 0.013b |
| Pyrocatechol | 0.120 ± 0.007b | 0.337 ± 0.008a |
| 3,4-Dihydroxy benzaldehyde | 0.063 ± 0.005b | 0.098 ± 0.007a |
| Syringic acid | nd | 0.42 ± 0.009 |
| trans-Cinnamic acid | 0.14 ± 0.010 | nd |

nd: Not detected.
(p < 0.05) in the content of all detected phenolics. In this study, all phenolic compounds were detected by UPLC–ESI–MS/MS. Nineteen phenolic compounds were detected in *O. ficus-barbarica* and *O. robusta*. However, trans-cinnamic acid only in *O. ficus-barbarica* (0.14 ± 0.010 mg kg⁻¹) and syringic acid only in *O. robusta* (0.42 ± 0.009 mg kg⁻¹) were determined. Additionally, trans-2-hydroxy cinnamic acid resveratrol, apigenin, kaempferol, epicatechin, hesperetin, chlorogenic acid, catechin gallate, catechin hydrate, genistein, and galanthamine were not detected in both *Opuntia* species.

Twelve (p-hydroxybenzoic acid, vanillin, gentisic acid, protocatechuic acid, gallic acid, ferulic acid, homogentisic acid, myricetin, pyrogallol, rutin, pyrocatechol, and 3,4-dihydroxy benzaldehyde) among the detected phenolic compounds contents were found in *O. robusta* higher than those of *O. ficus-barbarica*. However, p-coumaric acid, vanillic acid, chrysin, caffeic acid, luteolin, naringenin, and quercetin were detected in *O. ficus-barbarica* higher amount than *O. robusta*. As the other plant species have high phenolic content, the studied *Opuntia* species can be considered not only as rich sources of phenolics and flavonoids but as promising sources of natural antioxidants as well. The total of phenolic compounds amount was calculated as 69.237 and 66.385 mg kg⁻¹, respectively, in *O. ficus-barbarica* and *O. robusta*.

The results show that 3,4-dihydroxy benzaldehyde contents were the lowest phenolic compound which was found 0.063 ± 0.005 mg kg⁻¹ in *O. ficus-barbarica* and 0.098 ± 0.007 mg kg⁻¹ in *O. robusta*. Additionally, quercetin, chrysin, naringenin in *O. robusta* and pyrocatechol, naringenin, chrysin, rutin, vanillin in *O. ficus-barbarica* were detected in low amounts. As it is presented in Table 2, ferulic acid was the highest quantities, 31.620 ± 0.018 and 26.931 ± 0.022 mg kg⁻¹ in *O. robusta* and *O. ficus-barbarica*, in all phenolic contents, respectively. In both, *Opuntia* species have quite similar in the total of phenolic content; however, p-coumaric acid displayed exceptional results that the detected content in *O. ficus-barbarica* was sixfold higher than *O. robusta*.

**Mineral content determination**

The minerals determined in both species were analyzed using ICP–MS and the results were displayed in Table 3. For each *Opuntia* species, five macroelements (Na, Ca, K, P, Mg) and five microelements (Fe, Mn, Zn, Mo, Cu) were determined. Generally, the macroelements calcium and potassium were the most abundant in both *Opuntia* species. The effects of *Opuntia* species were statistically significant (p < 0.05) in the content of all minerals. The total mineral content was determined 907.829 mg 100 g⁻¹ for *O. ficus-barbarica* and 1067.882 mg 100 g⁻¹ for *O. robusta*. Also, total macroelements content was occurred 865.125 mg 100 g⁻¹ for *O. ficus-barbarica* and 1044.035 mg 100 g⁻¹ for *O. robusta*. On the other hand, *O. robusta* contained less total microelement content than that of *O. ficus-barbarica*. The total microelement content was 42.183 and 23.084 mg 100 g⁻¹ for *O. ficus-barbarica* and *O. robusta*, respectively.

### Table 3. Mineral contents of *Opuntia ficus-barbarica* and *Opuntia robusta* fruits by ICP–MS.

| Abb.  | *O. ficus-barbarica* (mg 100 g⁻¹) | *O. robusta* (mg 100 g⁻¹) |
|-------|---------------------------------|--------------------------|
| K     | 240.347 ± 3.674b                | 281.869 ± 2.905a         |
| Ca    | 510.941 ± 4.702b                | 639.128 ± 1.457a         |
| Na    | 24.307 ± 1.428a                 | 14.096 ± 0.732b          |
| P     | 0.110 ± 0.004b                  | 0.138 ± 0.005a           |
| Mg    | 89.420 ± 1.155b                 | 108.804 ± 0.359a         |
| Fe    | 23.109 ± 0.304a                 | 6.981 ± 0.382b           |
| Mn    | 14.562 ± 0.517a                 | 9.458 ± 0.235b           |
| Zn    | 4.694 ± 0.099b                  | 6.783 ± 0.068a           |
| Mo    | 0.208 ± 0.012b                  | 0.380 ± 0.057a           |
| Cu    | 0.131 ± 0.013b                  | 0.245 ± 0.012a           |

1Symbols of the minerals.

Results expressed as mean ± standard deviation. Means in the same line followed by different letters are significantly different (p < 0.05).
The most abundant macroelement in *Opuntia* species was calcium, which was detected 639.128 ± 1.457 mg 100 g⁻¹ in *O. robusta* and 510.941 ± 4.702 mg 100 g⁻¹ in *O. ficus-barbarica*. Potassium and magnesium come in the second and third order in both *Opuntia* species. The content of potassium and magnesium was 240.347 ± 3.674 and 89.420 ± 1.155 mg 100 g⁻¹ in *O. ficus-barbarica*, on the other hand, 281.869 ± 2.905 and 108.804 ± 0.359 mg 100 g⁻¹ in *O. robusta*. Phosphorus was the mineral present in the lowest concentration, which was 0.110 ± 0.004 mg 100 g⁻¹ in *O. ficus-barbarica* and 0.138 ± 0.005 mg 100 g⁻¹ in *O. robusta*.

More differences were found in the iron content between *O. ficus-barbarica* and *O. robusta*. Among the microelements, iron was the most abundant mineral, 23.109 ± 0.304 mg 100 g⁻¹ in *O. ficus-barbarica*; on the other hand, manganese was found the most abundant mineral, 9.458 ± 0.235 mg 100 g⁻¹ in *O. robusta*. Nevertheless, the content of zinc, molybdenum; and copper was 4.694 ± 0.099, 0.208 ± 0.012, and 0.131 ± 0.013 mg 100 g⁻¹ in *O. ficus-barbarica*, and corresponding results were 6.783 ± 0.068, 0.380 ± 0.057, and 0.245 ± 0.012 mg 100 g⁻¹ in *O. robusta*.

Nutritional quality of daily food supply, especially with respect to essential nutrient minerals, such as magnesium, iron, and zinc, could be an important goal of leafy vegetable crops. These essential nutrient minerals can be fulfilled with the studied *Opuntia* species.

### Correlation coefficient between parameters

Pearson’s correlation coefficient (r) was obtained from correlation analysis and used to describe the correlations among 19 phenolic compounds in both *O. robusta* and *O. ficus-barbarica*. The correlation coefficients were determined among phenolic compounds for *O. robusta*. The highest correlation coefficient was significantly found between *p*-hydroxy benzoic acid and chrysin as 0.959. The other high correlation coefficients were calculated between vanillin and caffeic acid (0.868), naringenin and quercetin (0.867), caffeic acid and ferulic acid (0.858), and gentisic acid and chrysin (−0.843). *p*-Hydroxy benzoic acid was significantly and positively correlated with vanillic acid, chrysin, myricetin, and pyrocatechol and negatively correlated with gentisic acid.

Pyrocatechol content of *O. robusta* fruit was significantly and positively correlated with six of other phenolic compounds: *p*-hydroxy benzoic acid, gentisic acid, chrysin, naringenin, pyrogallol, quercetin, and 3,4-dihydroxy benzaldehyde. Myricetin was significantly correlated with vanillic acid (0.827), chrysin (0.785), gentisic acid (0.673), and *p*-hydroxybenzoic acid (0.663). Additionally, rutin was also was correlated with four of detected phenolics: ferulic acid, luteolin, naringenin, and caffeic acid. The coefficients of correlation were evaluated among minerals in *O. robusta* fruit. Zinc content of *O. robusta* was significantly correlated with all minerals except molybdenum. The highest correlation coefficient was observed between calcium and potassium (−0.921). One of highest correlation coefficients was found also with zinc and manganese (0.911).

Calcium, phosphorus, iron, manganese, magnesium, zinc, and copper were significantly correlated with each other. Among these minerals, the highest coefficient of correlation was observed between iron and magnesium (−0.894). Sodium was significantly correlated with phosphorus, zinc, and copper. Molybdenum was only correlated with potassium and magnesium. Simple correlation coefficients between phenolic compounds and each mineral detected in *O. robusta* samples. There are no statistically significant (p = 0.05) relationship among *p*-coumaric acid, pyrogallol, quercetin, pyrocatechol, and 3,4-dihydroxy benzaldehyde with all determined mineral contents.

Luteolin showed significant positive correlations between calcium and magnesium and negative correlations between potassium, phosphorus, iron, manganese, and zinc. The highest correlation coefficient was observed between luteolin and manganese (−0.891). *p*-Hydroxy benzoic acid and naringenin were shown significant correlation with only iron. The other high and significant correlation coefficients were found between vanillic acid and magnesium and iron (−0.888 and 0.840), between luteolin and zinc (−0.880), and between myricetin and magnesium as −0.836. Gentisic acid, protocatechuic acid, chrysin, gallic acid, and rutin were significantly correlated with...
a few minerals such as manganese, magnesium, sodium. Overall, mostly moderate and weak correlation coefficients were observed between phenolic compounds and minerals in *O. robusta*.

Phenolic compounds of *O. ficus-barbarica* determined in this study were individually correlated with each other. In general, more significant correlation coefficients were found among phenolic compounds in *O. ficus-barbarica* than observed in *O. robusta*. The highest significant correlation coefficient was found between vanillin and myricetin \((r = 0.976, p = 0.05)\). Second and third high correlation coefficients were observed between vanillin and pyrocatechol, and between myricetin and pyrocatechol as \(-0.963\) and \(-0.926\), respectively. Also, high and significant correlation coefficient was calculated between vanillic acid and quercetin \((0.902)\). Vanillin also was negatively correlated with gallic acid and positively correlated with luteolin.

The other high correlation coefficients (above 0.800) were found between myricetin and luteolin and gallic acid, between naringenin and 3,4 dihydroxy benzaldehyde, between pyrogallol and gallic acid, and between gentisic acid and protocateghiuc acid. Rutin, naringenin, feulic acid, and gallic acid were significantly correlated with only one phenolic compound detected in this study.

The correlation coefficients among minerals in *O. ficus-barbarica* fruits are evaluated. Potassium, phosphorus, iron, magnesium, zinc, and molybdenum were significantly correlated with all minerals except copper, which was correlated only with calcium. The highest correlation coefficient was observed between zinc and molybdenum \((−0.902)\). Second and third high correlation coefficients were found between magnesium and phosphorus \((0.892)\), and between potassium and phosphorus \((−0.889)\) in detected minerals for *O. ficus-barbarica*. Also iron with phosphorus \((0.876)\), and magnesium with manganese \((−0.874)\) and potassium \((−0.872)\), showed high correlation coefficients.

Overall, strong correlations were observed among all minerals in *O. ficus-barbarica* fruit. The results of statistical analysis of all phenolic compounds and minerals of *O. ficus-barbarica* fruit are evaluated. Correlation coefficients \((r)\) exhibited significant differences at the 0.05 probability level, ranging from 0.601 to 0.853. The highest \(r\) value was found between gallic acid and potassium. Gallic acid showed the positively and negatively significant correlation coefficients with all minerals except sodium and copper. Also, pyrogallol showed high correlations with phosphorus \((−0.840)\) and manganese \((0.838)\).

There is no statistically significant relationship among *p*-coumaric acid, vanillic acid, ferulic acid, naringenin, and pyrocatechol with all detected mineral in *O. ficus-barbarica* fruits. In addition, *p*-hydroxy benzoic acid, vanillin, gentisic acid, chryisin, caffeic acid, luteolin, myricetin, and 3,4-dihydroxy benzaldehyde had no significant relationship with minerals except a few. In general, moderate correlations were observed between phenolic compounds and detected minerals in *O. ficus-barbarica* fruits.

**Antioxidant activity of *O. ficus-barbarica* and *O. robusta* fruits**

The antioxidant activity of *O. ficus-barbarica* and *O. robusta* fruit samples was measured in the extracts of hexane, ethyl acetate, methanol, and water. In terms of lipid peroxidation inhibition, the \(β\)-carotene-linoleic acid assay of hexane extracts of *O. ficus-barbarica* and *O. robusta* fruit samples \((IC_{50} = 9.56 ± 0.48 \text{ and } 8.03 ± 0.22 \mu \text{g mL}^{-1},\) respectively) revealed activity close to standards. The results were presented in Table 4. In the ABTS cation radical scavenging activity, ethyl acetate extracts of *O. ficus-barbarica* and *O. robusta* fruit samples showed moderate activity as \(SC_{50} = 23.47 ± 0.53\) and 21.75 ± 1.01 \(\mu \text{g mL}\), respectively, but still lower than standards. Both of the assays displayed less scavenging inhibition compared to standards in the extracts of ethyl acetate, methanol, and water.

In the case of DPPH radical scavenging activity, the DPPH assay of *Opuntia* samples displayed a good radical scavenging inhibition, similar to BHA and BHT standards, as half maximal inhibitory concentration \(SC_{50} = 69.32 ± 0.96\) and 67.57 ± 0.83 \(\mu \text{g mL}\) in ethyl acetate extracts of *O. ficus-barbarica* and *O. robusta* fruits, respectively. The extracts of ethyl acetate, methanol, and water exhibited limited activity, compared to BHA, BHT, and \(α\)-tocopherol standards. Thus, results of antioxidant activity of analysis of studied *Opuntia* fruits performed a good correlation between antioxidant activity and phenolic content. All activity data were displayed in Table 4.
Anticholinesterase activity of *O. ficus-barbarica* and *O. robusta* fruits

The BChE activity of the studied *O. ficus-barbarica* and *O. robusta* fruit samples exhibited higher activity, compared with galanthamine, as IC$_{50}$ = 51.05 ± 1.25 and 46.27 ± 1.57 μg mL$^{-1}$, respectively. On the other hand, all four extracts of *O. ficus-barbarica* and *O. robusta* fruit samples showed almost no activity against AChE (Table 4). To the best of our knowledge, there are some studies about total phenolic contents and minerals of flowers, cladodes, seeds, and juices of *O. ficus-indica* and a few other *Opuntia* spp. Despite an extensive literature search, information on the individual phenolic compounds and mineral contents of *O. robusta* and *O. ficus-barbarica*, which were detected using by UPLC–ESI–MS/MS and ICP/MS, respectively, are not available in the literature.

**Conclusion**

In this study, it is aimed to investigate the individual phenolic compounds, mineral content, and the antioxidant capacity of *O. robusta* and *O. ficus-barbarica* fruit samples. After the evaluation of the results, the studied *Opuntia* species displayed rich sources of phenolic profile, mineral content, and also promising sources natural antioxidant. Phenolic profile composition and mineral content varied among the species. Therefore, the chemical composition was used successfully to obtain information about the relationship among *Opuntia* species. It seems that *O. robusta* and *O. ficus-barbarica* fruits can supply a dietary intake of essential nutrients. This research shows the potential of fruits of *Opuntia* species. Those can be used as pulp and juices, which have an important source of natural antioxidants and nutraceuticals.

**Anticholinesterase activity of *O. ficus-barbarica* and *O. robusta* fruits**

The BChE activity of the studied *O. ficus-barbarica* and *O. robusta* fruits of ethyl acetate extract exhibited higher activity, compared with galanthamine, as IC$_{50}$ = 51.05 ± 1.25 and 46.27 ± 1.57 μg mL$^{-1}$, respectively. On the other hand, all four extracts of *O. ficus-barbarica* and *O. robusta* fruit samples showed almost no activity against AChE (Table 4). To the best of our knowledge, there are some studies about total phenolic contents and minerals of flowers, cladodes, seeds, and juices of *O. ficus-indica* and a few other *Opuntia* spp. Despite an extensive literature search, information on the individual phenolic compounds and mineral contents of *O. robusta* and *O. ficus-barbarica*, which were detected using by UPLC–ESI–MS/MS and ICP/MS, respectively, are not available in the literature.

|                  | Antioxidant activity | Anticholinesterase activity |
|------------------|----------------------|----------------------------|
|                  | β-Carotene-linoleic  |                           |
|                  | acid assay IC$_{50}$ | DPPH assay SC$_{50}$ | ABTS$^+$ assay SC$_{50}$ | AChE assay IC$_{50}$ | BChE assay IC$_{50}$ |
| Exports          | (μg mL$^{-1}$)       | (μg mL$^{-1}$)            | (μg mL$^{-1}$)            | (μg mL$^{-1}$)            | (μg mL$^{-1}$)            |
| *O. ficus-barbarica* | Hexane               | 9.56 ± 0.48              | 213.71 ± 2.57            | 56.82 ± 1.66             | 128.10 ± 0.98            | 110.32 ± 0.89          |
|                  | Ethyl acetate        | 12.21 ± 0.20             | 69.32 ± 0.96             | 23.47 ± 0.53             | 78.21 ± 1.23             | 51.05 ± 1.25           |
|                  | Methanol             | 39.99 ± 1.07             | 188.04 ± 3.41            | 39.04 ± 0.99             | 179.06 ± 1.33            | 163.30 ± 1.32          |
|                  | Water                | 87.32 ± 0.81             | 191.34 ± 2.72            | 125.40 ± 2.07            | 231.42 ± 3.00            | 303.07 ± 2.48          |
| *O. robusta*     | Hexane               | 8.03 ± 0.22              | 198.02 ± 2.35            | 55.30 ± 0.77             | 113.07 ± 1.86            | 109.45 ± 1.58          |
|                  | Ethyl acetate        | 13.43 ± 0.56             | 67.57 ± 0.83             | 21.75 ± 1.01             | 69.63 ± 2.34             | 46.27 ± 1.57           |
|                  | Methanol             | 47.04 ± 0.84             | 161.05 ± 2.93            | 42.07 ± 1.14             | 182.23 ± 2.50            | 198.50 ± 3.05          |
|                  | Water                | 67.07 ± 1.03             | 177.81 ± 1.65            | 119.27 ± 0.99            | 222.51 ± 1.21            | 289.91 ± 2.09          |
| Standards        | Galantamine          | NT                       | NT                       | NT                       | 10.12 ± 0.33             | 46.34 ± 1.45           |
|                  | BHA                  | 3.17 ± 0.10              | 66.86 ± 0.33             | 7.23 ± 0.10              | NT                       | NT                     |
|                  | BHT                  | 3.09 ± 0.17              | 67.05 ± 0.80             | 7.39 ± 0.12              | NT                       | NT                     |
|                  | α-Tocopherol         | 5.12 ± 0.09              | 10.67 ± 0.41             | 6.98 ± 0.10              | NT                       | NT                     |

IC$_{50}$: Inhibition concentration; SC$_{50}$: scavenging concentration. IC$_{50}$ and SC$_{50}$ values represent the means ± SD of three parallel measurements (p < 0.05).

BHA: Butylated hydroxyanisole; BHT: butylated hydroxytoluene.
NT: Not tested.

**Acknowledgments**

Author Şeyda Kivrak declares that she has no conflict of interest. Author İbrahim Kivrak declares that he has no conflict of interest. Author Erşan Karababa declares that he has no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
References

1. Karababa, E.; Coskuner, Y.; Aksay, S. Some Physical Fruit Properties of Cactus Pear (Opuntia Spp) that Grow Wild in the Eastern Mediterranean Region of Turkey. Journal of the Professional Association for Cactus 2004, 6, 1–8.
2. Pinos-Rodriguez, J. M.; Velázquez, J. C.; González, S. S.; Aguirre, J. R.; García, J. C.; Álvarez, G.; Jasso, Y. Effects of Cladode Age on Biomass Yield and Nutritional Value of Intensively Produced Spineless Cactus for Ruminants. South African Journal of Animal Science 2010, 40(3), 245–250.
3. Abdel-Hameed, E. S. S.; Nagaty, M. A.; Salman, M. S.; Bazaïd, S. A. Phytochemicals, Nutritional and Antioxidant Properties of Two Prickly Pear Cactus Cultivars (Opuntia Ficus Indica Mill.) Growing in Taif, KSA. Food Chemistry 2014, 160, 31–38. DOI: 10.1016/j.foodchem.2014.03.060.
4. Laura, Z.; Caliceti, C. F.; Sega, V. D. Dietary Phenolic Acids Act as Effective Antioxidants in Membrane Models and in Cultured Cells, Exhibiting Proapoptotic Effects in Leukemia Cells. Oxidative Medicine and Cellular Longevity 2012, Article ID 839298, 1–12.
5. Naveen, S.; Antioxidant Potential of Some Common Plant Sources. International Journal of Pharmaceutical Research and Development 2011, 3(1), 154–174.
6. Prasad, N.; Yang, B.; Kong, K. W.; Khoo, H. E.; Sun, J.; Azlan, A.; Ismail, A.; Bin Romli, Z. Phytochemicals and Antioxidant Capacity from Nypa Fruticans Wurmb. Fruiti. Evid. Based Complement. Alternative medicine 2013, Article ID 154606, 1–9.
7. Gordon, A.; Cruz, A.P.G.; Cabral, L.M.C.; de Freitas, S.C.; Taxi, C.M.A.D.; Donangelo, C.M.; de Andrade Mattietto, R.; Friedrich, M.; da Matta, V.M.; Marx F. Chemical Characterization and Evaluation of Antioxidant Properties of Acai Fruits (Euterpe Oleracea Mart.) During Ripening. Food Chemistry 2012, 133, 256–263. DOI: 10.1016/j.foodchem.2011.11.150.
8. Manojlovic, T. N.; Vasiljevic, P. J.; Maskovic, P. Z.; Juszkovic, M.; Bogdanovic-Dusanovic, G. Chemical Composition, Antioxidant, and Antimicrobial Activities of Lichen Umbilicaria Cylindrical (L.) Delise (Umbilicariaceae). Evidence-Based Complementary 2012, Article ID 452431. Alternat Med, 1–8.
9. Tohidi, B.; Rahimmalek, M.; Arzani, A. Essential Oil Composition, Total Phenolic, Flavonoid Contents, and Antioxidant Activity of Thymus Species Collected from Different Regions of Iran. Food Chemistry 2017, 220, 153–161. DOI: 10.1016/j.foodchem.2016.09.203.
10. Albano, C.; Negro, C.; Tommasi, N.; Gerardi, C.; Mita, Miceli, G. A.; De Bellis, L.; Blando, F. Betalains, Phenols and Antioxidant Capacity in Cactus Pear [Opuntia Ficus-Indica (L.) Mill.] Fruits from Apulia (South Italy) Genotypes. Antioxidants 2015, 4, 269–280. DOI: 10.3390/antiox4020269.
11. Cezudo-Bastante, M. J.; Chaalal, M.; Louailche, H.; Parrado, J.; Heredia, F. J. Betalain Profile, Phenolic Content, and Color Characterization of Different Parts and Varieties of Opuntia ficus-indica.J. Journal of agricultural and food chemistry 2014, 62, 8491–8499.
12. Mabrouki, L.; Zougari, B.; Bendhifi, M.; Borgi, M. A. Evaluation of Antioxidant Capacity, Phenol and Flavonoid Contents of Opuntia Streptacantha and Opuntia Ficus Indica Fruits Pulp. Nature & Technology 2015, 13, 02–08.
13. Butera, D.; Tesoriere, L.; Di Gaudio, F.; Bongiorno, A.; Allegra, M.; Pintaudi, A. M.; Kohlen, R.; Livrea, M. A. Antioxidant Activities of Sicilian Prickly Pear (Opuntia Ficus Indica) Fruit Extracts and Reducing Properties of Its Betalains; Betanin and Indican. The Journal of Agricultural and Food Chemistry 2002, 50, 6895–6901
14. Ishtiaque, S.; Naz, S.; Siddiqi, R.; Abdullah, S. U.; Khan, K.; Ahmed, J.; Badaruddin, M. Antioxidant Activities and Total Phenolics Contents from Extracts of Terminalia Catappa, Carissa Carandas, and Opuntia Ficus Indica Fruits. Recent Innovations in Chemical Engineering 2014, 7, 106–112.
15. Middleton, E., Jr.; Kandaswami, C.; Theoharides, T. C. The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease and Cancer. Pharmacological reviews 2000, 52, 673–751.
16. Abd El-Razek, F. H.; Hassan, A. A. Nutritional Value and Hypoglycemic Effect of Prickly Cactus Pear (Opuntia Ficus-Indica) Fruit Juice in Alloxan-Induced Diabetic Rats, Aust. Australian Journal of basic and applied sciences 2011, 5(10), 356–377.
17. Dua, A.; Gupta, S. K.; Mittal, A.; Mahajan, R. A. Study of Antioxidant Properties and Antioxidant Compounds of Cumin (Cuminum Cuminum). International Journal of Pharmaceutical & Biological Archive 2012, 3(5), 1110–1116.
18. Yeddes, N.; Chérif, J. K.; Guyot, S.; Sotin, H.; Ayadi, M. T. Comparative Study of Antioxidant Power, Polyphenols, Flavonoids and Betacyanins of the Peel and Pulp of Three Tunisian Opuntia Forms. Antioxidants 2013, 2(1), 37–51. DOI: 10.3390/antiox2020037.
19. Bernardino-Nicanor, A.; Hinojosa-Hernández, E. N.; Juárez-Goiz, J. M. S.; Montañez-Soto, J. L.; Ramírez-Ortiz, M. E.; Gonzalez-Cruz, L. Quality of Opuntia Robusta and Its Use in Development of Mayonnaise-Like Product. The Journal of Food Science and Technology 2015, 52(1), 343–350. DOI: 10.1007/s13197-013-0989-8.
20. Scalbert, A.; Manach, C.; Morand, C.; Remesy, C.; Jimenez, L. Dietary Polyphenols and the Prevention of Diseases. Critical Reviews in Food Science 2005, 45, 287–306. DOI: 10.1080/1040869059096.
21. Zhang, X. K.; Jin, X.; Lai, F. Y.; Lin, Q. S.; Jiang, J. G. Chemical Analysis and Antioxidant Activities in Vitro of Polysaccharide Extracted from Opuntia Ficus Indica Mill. Cultivated in China. Carbohydrate Research 2010, 82, 722–727.

22. Osorio-Esquivel, O.; Ortiz-Moreno, A.; Álvarez, V. B.; Dorantes-Álvarez, L.; Giusti, M. M. Phenolics, Betacyanins and Antioxidant Activity in Opuntia Joconostle Fruits. Food Research International 2011, 44, 2160–2168. DOI: 10.1016/j.foodres.2011.02.011.

23. Pimienta-Barrios, E.; Méndez-Morán, L.; Ramírez-Hernández, B. C.; Alba-García, G. J. E.; Domínguez- Arias, R. M. Efecto De La Ingestión Del Fruto De Xoconostle (Opuntia Joconostle Web.) Sobre La Glucosa Y Lípidos Séricos. Agrociencia 2008, 42, 645–653.

24. Moussa-Ayoub, T. E.; El-Samahy, S. K.; Rohn, S.; Kroh, L. W. Flavonols, Betacyanins Content and Antioxidant Activity of Cactus Opuntia Macrorhiza Fruits. Food Research International 2011, 44, 2160–2168. DOI: 10.1016/j.foodres.2011.02.011.

25. Crozier, A.; Jaganath, I. B.; Clifford, M. N. Dietary Phenolics: Chemistry, Bioavailability and Effects on Health. Natural Product Reports 2009, 26, 1001–1043. DOI: 10.1039/b802662a.

26. Martínez, C. R. L.; Mateos, R. G.; Vázquez, C. G.; Castellanos, J. S. Antioxidant Components and Nutritional Quality of 15 Genotypes of Xoconostle (Opuntia Spp.). Journal of the Professional Association for Cactus 2015, 17, 33–49.

27. Guevara-Figueroa, T.; Jiménez-Islas, H.; Reyes-Escogido, M. L.; Mortensen, A. G.; Laursen, B. B.; Lin, L. W.; Leon-Rodriguez, A. D.; Fomsgaard, I. S.; Rosa, A. P. Proximate Composition, Phenolic Acids, and Flavonoids Characterization of Commercial and Wild Nopal (Opuntia Spp.). The Journal of Food Composition and Analysis 2010, 23, 525–532. DOI: 10.1016/j.jfca.2009.12.003.

28. Cha, M. N.; Jun, H. I.; Lee, W. J.; Kim, M. J.; Kim, M. K.; Kim, Y. S. Chemical Composition and Antioxidant Activity of Korean Cactus (Opuntia Humifusa) Fruit. The Food Science and Biotechnology 2013, 22(2), 523–529. DOI: 10.1007/s10068-013-0110-0.

29. Kivrak, I.; Kivrak, Ş. Assessment of Phenolic Profile of Turkish Honeys. International Journal of Food Properties 2016, 20(4), 864–876. DOI: 10.1080/10942912.2016.1188307.

30. Kivrak, I.; Kivrak, Ş. Antioxidant Properties, Phenolic Profile and Nutritional Value for Sorbus Umbellata Fruits from Turkey. Austin journal of nutrition and food sciences 2014, 2(8), 1043, 1–6.

31. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D. H. Comparison of ABTS, DPPH, FRAP, and ORAC Assays for Estimating Antioxidant Activity from Guava Fruit Extracts. The Journal of Food Composition and Analysis 2006, 19, 669–675. DOI: 10.1016/j.jfca.2006.01.003.

32. Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherston, R. M. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. Biochemical Pharmacology 1961, 7, 88–95. DOI: 10.1016/0006-2952(61)90145-9.

33. Arzani, A.; Zeinali, H.; Razmjo, K. Iron and Magnesium Concentrations of Mint Accessions (Mentha Spp.). Plant Physiology and Biochemistry 2007, 45, 323–329. DOI: 10.1016/j.plaphy.2007.03.023.