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Conventional and non-conventional extraction of functional compounds from jiotilla (*Escontria chiotilla*) fruits and evaluation of their antioxidant activity

Extracción convencional y no convencional de compuestos funcionales de frutos de jiotilla (*Escontria chiotilla*) y evaluación de su actividad antioxidante

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Abstract

There is a growing interest in obtaining bioactive compounds from underexplored plant organisms such as jiotilla (*Escontria chiotilla*), a species of the Cactaceae family whose fruit is a source of betalains and phenolic compounds. Therefore, it is relevant to establish an extraction methodology that allows a higher yield of bioactive compounds and greater functional activity to be obtained. In this study, the conventional extraction process was optimized to obtain betalains, phenolic compounds, and antioxidant activity from jiotilla extracts and results were compared with those obtained by ultrasound-assisted and ultrasound-microwave extraction. Optimum conditions for conventional extraction of betalains (90.56 ± 0.88 mg/100 g fresh weight (g_{fw})) and phenolic compounds (129.12 ± 14.21 mg/100 g_{fw}) were 37.5% v/v ethanol, an m:v ratio of 1:20, and 40 min maceration with shaking. Ultrasound-assisted extraction increased the phenolic compound content of the extract by 34.01% and its antioxidant activity by 25.68%, a positive correlation being found between these parameters. Ultrasound-microwave extraction did not improve extraction yields compared to the other two technologies. These results show that the extraction method affects the content of functional compounds and antioxidant activity and pinpoint that jiotilla fruits can be a viable alternative for extraction of betalains and phenolic compounds.

Keywords: Jiotilla, betalains, phenolic compounds, antioxidant activity, ultrasound.

Resumen

Existe un creciente interés en la obtención de compuestos bioactivos de especies poco exploradas como la jiotilla (*Escontria chiotilla*), una especie de la familia de las cactáceas cuyo fruto es fuente de betalaínas y compuestos fenólicos. Por ello resulta relevante establecer una metodología de extracción que permita obtener un mayor rendimiento de compuestos bioactivos y mayor actividad funcional. En este trabajo se optimizó el proceso de extracción convencional para la obtención de betalaínas, compuestos fenólicos y actividad antioxidante del extracto y se compararon los resultados con los obtenidos mediante extracción asistida por ultrasonido y por ultrasonido-microondas. Las condiciones óptimas para la extracción convencional de betalaínas (90.56 ± 0.88 mg/100 g peso fresco (g_{fw})) y compuestos fenólicos (129.12 ± 14.21 mg/100 g_{fw}) de jiotilla fueron etanol 37.5% v/v, una relación m:v de 1:20 y 40 min de maceración con agitación. La extracción asistida con ultrasonido incrementó en 34.01% el contenido de compuestos fenólicos y en 25.68% la capacidad antioxidante del extracto con una correlación positiva entre estos parámetros. La extracción asistida por ultrasonido-microondas no mejoró los rendimientos de extracción en comparación con las otras dos tecnologías. Estos resultados muestran que el método de extracción afecta el contenido de los compuestos funcionales y la capacidad antioxidante y establecen que los frutos de jiotilla son una alternativa viable para la obtención de betalaínas y compuestos fenólicos.

Palabras clave: Jiotilla, betalaínas, compuestos fenólicos, actividad antioxidante, ultrasonido.

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1 Introduction

Jiotilla (*Escontria chiotilla*) is an arborescent cactus growing wild in arid areas of the Mixteca Baja Oaxaqueña in Mexico (Soriano-Santos *et al*., 2007). This species is of cultural importance for the region and its fruits are consumed locally as a fresh product (Ruiz Huerta *et al*., 2015). Jiotilla fruits have red-purple skin and pulp and a sweet flavor, reaching an average weight of 17.90 ± 2.23 g at maturity stage, an average equatorial diameter of 3.21 ± 0.35 cm, and an average polar diameter of 3.03 ± 0.43 cm, with pulp and peel accounting for 61.13% and 38.9%, respectively (Ruiz Huerta *et al*., 2015). As in other fruits and vegetables, differences in physicochemical properties in jiotilla, such as pH, percentage of total soluble solids, and titratable acidity, have been reported in fruits harvested in different zones of the state of Oaxaca, like Mixteca Oaxaqueña and Valles Centrales (Soriano-Santos *et al*., 2007; Ruiz Huerta *et al*., 2015; Sandate-Flores *et al*., 2020).

Jiotilla fruits exhibit antioxidant activity due to their content of phenolic compounds and betalains (BET). Phenolic compounds are a heterogeneous group characterized by having one or more aromatic rings with one or more hydroxyl groups in their structure (Eseberri *et al*., 2022), that display a range of beneficial health effects such as antioxidant, anti-inflammatory, anticancer, obesity reduction, and antidiabetic properties (Gutiérrez-Grijalva *et al*., 2016). Some phenolic acids have been identified and quantified in clarified jiotilla juice, the most abundant being gallic acid (1.02 ± 0.01 mg/100 g fresh weight (g_{fw})) and caffeic acid (0.08 ± 0.00 mg/100 g_{fw}) (Sandate-Flores *et al*., 2020). BET are water-soluble nitrogen-containing pigments with antioxidant properties (Guerrero-Rubio *et al*., 2020). They fall into two different types: red-violet betacyanins (BC) and yellow betaxanthins (BX) (Carreón-Hidalgo *et al*., 2022). HPLC analysis of the methanolic extract of jiotilla has identified the main BET as the BX vulgaxanthin I, vulgaxanthin II, and indicaxanthin, and the BC betanin (Soriano-Santos *et al*., 2007). Besides the antioxidant activity, BET have been related to functional properties such as antitumor, anti-inflammatory, antilipidemic, antidiabetic, antiviral, and hepatoprotective properties (Carreón-Hidalgo *et al*., 2022).

The extraction of bioactive compounds is influenced by factors such as the type of matrix containing them, the nature of the bioactives, the type and nature of the solvent, the time used for the extraction, and the temperature (Albuquerque *et al*., 2020). To obtain bioactive compounds from plants, conventional and non-conventional extraction technologies have been employed. Conventional extraction methodologies including maceration and Soxhlet extraction are widely used due to their easy operation and instrumentation. However, they are considered inefficient because of their high solvent and energy consumption and long process times (Li *et al*., 2019), In contrast, non-conventional or assisted-extraction methodologies, like ultrasound and ultrasound-microwave, are more efficient due to lower consumption of solvent, shorter extraction times, and lower energy consumption (Akhtar *et al*., 2019).

The effect of using non-conventional technologies such as ultrasound and microwave-assisted extraction on other cacti fruits such as *Stenocereus stellatus*, *Hylocereus polyrhizus*, *Opuntia ficus-indica* L. Mill, *Opuntia engelmannii*, and *Stenocereus pruinosus* has been evaluated. Research shows that bioactive compound extraction (betalains and phenolic compounds) and extract antioxidant capacity improve with the application of these technologies, and correlate with short extraction times, low temperature (room or lower) for thermosensitive compounds and temperatures above 60 °C for thermosetting compounds, with ultrasound frequencies up to 50 kHz and microwave power up to 800 W (Cardoso-Ugarte *et al*., 2014; Ramli *et al*., 2014; Pérez-Loredo *et al*., 2017; García-Cayuela *et al*., 2019; Melgar *et al*., 2019). Moreover, it has been reported that the optimization of the factors depends on the matrix and the specific type of compound of interest (Ramli *et al*., 2014).

According to the previously mentioned background in other cacti fruits, it would be expected that, regarding the extraction of betalains and phenolic compounds, the optimization of the conventional method as well as the application of non-conventional extraction methods such as ultrasound or ultrasound-microwave assisted extraction would improve the extraction of functional compounds in terms of their thermostability, and that the antioxidant capacity of the extracts would depend on the functional compounds and their proportion.

Thus, the aim of this work was to carry out a comparison between optimized conventional extraction (CE) and ultrasound- (UAE) and ultrasound-microwave-assisted (UMAE) extraction for obtaining antioxidant-rich extracts, BET, and phenolic compounds from jiotilla fruits.

2 Materials and methods

2.1 Plant material

Escontria chiotilla fruits were collected in May 2018, in the municipality of Santiago, Chazumba, Oaxaca, Mexico (18° 16' 24" N; 97° 70' 93" W). Fruits were then disinfected and selected for the absence of physical defects and color uniformity, peeled by hand, and their pulp was ground and frozen at -70 °C until use. The moisture content was determined according to Bourhia *et al*. (2019); 2 g of sample was weighed and placed in an oven (3608 model, Lab-Line Instruments, Inc., Melrose Park, IL, USA) at 50 °C until reaching constant weight. Moisture was expressed as a percentage.

2.2 Extraction of phenolic compounds and BET

2.2.1 Optimization of CE

CE was carried out according to the methodology proposed by García-Cruz *et al*. (2013) with some modifications. Briefly, 1 g of frozen pulp was placed in a 125 mL Erlenmeyer flask, the required volume of solvent was added, and the mixture was placed in an orbital shaker at 280 rpm at 25 °C, according to the extraction conditions shown in Table 1. The resultant extracts were centrifuged at 12,096 × g for 10 min (Avanti J-30I, Beckman Coulter) at 4 °C. The supernatant phase was recovered, and the precipitate was submitted to a second extraction process using the same conditions. Finally, the supernatant phases obtained in both extractions were mixed and gauged to 25 or 50 mL depending on the mass:volume (m:v) ratio. All the extracts were stored at -70 °C until analysis.

The optimization of CE was based on the extraction recovery of BET, phenolic compounds, and antioxidant activity by ABTS and DPPH, using a central composite design (CDD) with three factors: extraction time (X_1), m:v ratio (X_2), and ethanol concentration (X_3). Three replicates of each experimental condition were used, and the results of each variable were fitted to a second-order model. The models obtained were used for a simultaneous optimization process in the experimental region tested with the software proposed by Max Kuhn (2016).

2.2.2 Ultrasound-assisted extraction (UAE)

One gram of frozen pulp was mixed with 10 mL of ethanol (37.5% v/v) and submitted to ultrasound using an ultrasonic probe processor (Vibra-Cell^{TM} VCX 130PB, Sonics, Newtown, CT, USA) at 20 kHz frequency and 80% amplitude for 3 min, reaching a final temperature of 47 °C. The resultant extracts were centrifuged at 12,096 × g for 10 min at 4 °C. The supernatant was recovered and gauged to 10 mL. The extract was frozen and stored at -70 °C until analysis.

2.2.3 Ultrasound-microwave-assisted extraction (UMAE)

Four grams of frozen pulp was mixed with 40 mL ethanol (37.5% v/v) in a 500 mL round-bottom flask. The mixture was placed in the chamber of the CW-2000 A extraction/reaction equipment at a frequency of 2450 MHz and an ultrasonic transducer with a power of 50 W at a frequency of 40 kHz. Microwaves were applied at 600 W power for 5 min at 30 °C. The extract was then centrifuged at 12,096 × g for 10 min at 4 °C. The supernatant was recovered and gauged to 50 mL. The extract was frozen and stored at -70 °C until analysis.

2.3 Determination of BET content

BC and BX contents were determined by spectrophotometry according to Castellanos-Santiago and Yahia (2008). The content of BC or BX was calculated using Equation 1 and expressed as betanin or indicaxanthin equivalents, respectively.

$$\text{BC or BX}[mg/(100g_{fw})] = \frac{A \cdot DF \cdot MW \cdot Vd}{\varepsilon \cdot L \cdot Wd} \quad (1)$$

where: A is the absorbance at λ = 483 for BX and λ = 538 nm for BC, DF is the dilution factor, MW is the molecular weight of the molecule of reference (indicaxanthin = 308 g/mol or betanin = 550 g/mol), Vd is the final volume, ε is the molar extinction coefficient (indicaxanthin: 48,000 L/mol*cm or betanin: 60,000 L/mol*cm), Wd is the sample weight in g, and L is the optical path length (1 cm). BC and BX contents were expressed as mg betanin equivalents/100 g_{fw} and mg indicaxanthin equivalents/100 g_{fw}. Total BET content was obtained from the sum of BC and BX contents.

2.4 Determination of total phenolic compound content

The content of total phenolic compounds (TPC) was determined according to Singleton *et al*. (1999) with some modifications. Two hundred microliters of the extract was mixed with 1000 μL of the Folin-Ciocalteu solution at 10% v/v. After 1 min of incubation, 800 μL of a sodium carbonate solution at 7.5% w/v was added and mixed. The extract was left in the dark for 60 min and the absorbance was recorded at 765 nm. TPC was expressed as mg gallic acid equivalents (GAE)/100 g_{fw}. A standard curve was obtained using gallic acid and plotted between the concentrations of 25 and 100 μg/mL.

2.5 Determination of antioxidant activity

2.5.1 ABTS assay

$ABTS^{\bullet+}$ was used to evaluate the antioxidant activity following the methodology proposed by Re *et al*. (1999) with some modifications. The $ABTS^{\bullet+}$ radical was obtained from the reaction of 16.5 mg of potassium persulfate with 96.2 mg of ABTS for 16 h in the dark. Once the radical was formed, an aliquot was taken and diluted with distilled water until an absorbance of 0.70 at 734 nm was obtained. One hundred microliters of the extract was added to 1000 μL of the diluted $ABTS^{\bullet+}$ radical solution and allowed to react for 10 min at room temperature. Absorbance was then measured at 734 nm. Antioxidant activity was expressed as μmol Trolox equivalents/g_{fw}.

2.5.2 DPPH assay

Radical scavenging evaluation was determined by the DPPH assay following the method reported by Brand-Williams

et al. (1995) with the following modifications. A 0.6 mM solution of the DPPH radical was prepared. Subsequently, the absorbance of the solution was recorded at 515 nm and adjusted to 1.10. Then, 50 μL of extract and 950 μL of the adjusted DPPH solution were used for the assay. After 30 min in the dark, the change in absorbance was evaluated. Quantification was performed by interpolation on a Trolox standard curve. Results were expressed as μmol Trolox equivalents/g_{fw}.

2.6 Semi-synthesis and purification of BX

Semi-synthesis of BX was carried out following the methodology of Castellanos-Santiago and Yahia (2008) with some modifications. Vulgaxanthin I and indicaxanthin were synthesized with the aim to use them for identifying these BX in the extract of jiotilla pulp. Ten milligrams of betanin was mixed with 1 mL of ammonium hydroxide (3 N) and vortexed until complete dissolution, and the formation of betalamic acid was measured through the absorbance of the mixture at 420 nm for 45 min at room temperature. For the semi-synthesis of vulgaxanthin I, the resultant betalamic acid was mixed with glutamine at a molar ratio of 1:10, respectively. The absorbance was followed at 483 nm from 20 to 60 min. The reaction product was concentrated using a rotary evaporator (BÜCHI R-200, BÜCHI Heating Bath B-490, and BÜCHI Vacuum Pump V-100) at 30 °C and the solid residue was resuspended in 1 mL of water. BX was then purified on a glass column (1 cm inner diameter × 46 cm length) using 3 g of C18 reverse phase (7 cm height). The column was first activated by eluting with 5 column volumes of methanol, followed by 5 column volumes of acidified water with concentrated trifluoroacetic acid (pH 2). The reaction product, vulgaxanthin I, was added and eluted with 2 column volumes of methanol. At this step, the pH of the eluent was monitored to ensure it ranged between 6 and 7. The same procedure was used for semi-synthesizing indicaxanthin but using L-proline as the amino acid source.

2.7 Characterization of extracts by HPLC

2.7.1 Identification of BET

HPLC analysis was performed following the methodology proposed by García-Cruz *et al*. (2017) with some modifications. HPLC equipment (Shimadzu SPD-10A, SpectraLab Scientific Inc., Markham, ON, Canada) with a Zorbax Eclipse XDB column (4.6 mm × 250 mm, 5 μm) and a UV/Vis detector was used. The mobile phase conditions were acidified water (A, 0.1% v/v formic acid) and acidified acetonitrile (B, 0.1% v/v formic acid). The elution gradient consisted of 100% to 90% A from 0 to 25 min, 90% to 85% A from 25 to 34 min; 85% to 82% A from 34 to 43 min, and 82% to 50% A from 43 to 60 min, at a flow rate of 0.5 mL/min. Identification of the BET in extracts was done at 480 nm using the BX vulgaxanthin I, indicaxanthin, and isoindicaxanthin as standards, and at 535 nm for the detection of BC, using betanin and isobetanin as standards (Sigma-Aldrich Co., St. Louis, MO, USA).

2.7.2 Identification of phenolic compounds by HPLC

HPLC analysis was performed following the methodology proposed by Ramirez-Lopez and DeWitt (2014) with some modifications. HPLC equipment with a Zorbax Eclipse XDB column (4.6 mm × 250 mm, 5 μm) and a UV/Vis detector was used. The mobile phase conditions were the same as those used in section 2.7.1 with the following gradient elution: 92% to 85% A from 0 to 5 min, 85% to 40% A from 5 to 45 min; 40% A from 45 to 55 min, and 92% A from 55 to 60 min, at a flow rate of 0.7 mL/min. Identification of the phenolic compounds in the extracts was done at 280 and 370 nm, using kaempferol, quercetin, rutin, isorhamnetin, myricetin, naringenin, taxifolin, catechin, epicatechin, and the phenolic acids p-coumaric acid, ferulic acid, caffeic acid, 3,4-dihydroxybenzoic acid, and gallic acid as standards (Sigma-Aldrich Co., St. Louis, MO, USA).

2.8 Statistical analysis

All experiments were carried out in triplicate and the results are reported as the mean ± standard deviation. Data analysis for optimization was performed with the statistical program R (R Core Team, 2020) and the "desirability" package (Kuhn, 2016). Comparison of results by extraction method was performed in NCSS 12 statistical software (2018) (Kaysville, Utah, USA). Analysis of variance (ANOVA) set at $p < 0.05$ was performed.

3 Results and discussion

3.1 Optimization of CE

Table 1 shows the extracts' BX, BC, BET, and TPC content, and antioxidant activity determined by ABTS and DPPH assays. Table 2 shows the coefficients obtained from second-order model fitting for each one of the response variables. The adjusted coefficient of determination ranged from 0.30 to 0.74, with BC and BET having the highest values and ABTS the lowest, respectively.

According to the ANOVA (Supplementary Material 1), one or more first-order terms were significant ($p < 0.05$); m:v ratio displayed a significant effect ($p < 0.001$) for all the response variables. Quadratic terms only showed significant effects in some models, i.e., the X_3^2 term only had a significant effect ($p < 0.05$) on BC, BET, and DPPH antioxidant activity. The interaction effect occurred only between factors X_1 and X_3 (extraction time/m:v ratio) for BC and X_2 and X_3 (ethanol concentration/m:v ratio) for DPPH antioxidant activity. The fact that only some terms

were significant for BX (X_3 and X_3^2), BET (X_1, X_3, and X_1^2), ABTS (X_1, X_2, X_3, and X_1^2), and DPPH (X_1, X_3, X_2X_3, X_1^2, X_2^2, and X_3^2) models was reflected in the statistical lack of fit test (Table 2) (Bezerra *et al*., 2008).

Table 1. Response surface method (RSM) and results for BX, BC, BET, and TPC content, and antioxidant activity by ABTS and DPPH methods.

Treatment	X_1 time (min)	X_2 EtOH concentration (% v/v)	X_3 Ratio (m:v)	BX	BC	BET	TPC	Antioxidant Activity	
								ABTS	DPPH
1	40	60	1:20	51.91	41.05	92.96	124.27	6.95	5.01
2	30	45	1:16	48.8	37.14	85.94	106.89	5.9	4.79
3	13.2	45	1:16	48.55	38.82	87.36	103.13	6.22	4.23
4	30	45	1:06	44.08	32.87	76.95	95.49	5.95	3.82
5	20	30	1:20	52.97	42.85	95.82	120.6	7.42	4.82
6	30	70	1:16	49.75	38.37	88.13	119.29	5.01	5.43
7	40	30	1:10	45.59	35.56	81.15	103.58	5.7	3.92
8	46.8	45	1:16	49.89	39.47	89.36	130.25	8.83	4.83
9	40	30	1:20	52.09	41.64	93.73	125.03	7.11	5.03
10	30	45	1:16	48.1	38.67	86.77	115.96	6.5	5.03
11	30	20	1:16	48.99	38.48	87.48	104.97	7.79	4.24
12	30	45	1:24	50.04	39.61	89.65	116.49	6.75	5.76
13	20	30	1:10	43.98	33.14	77.11	100.62	5.3	3.48
14	20	60	1:20	51	39.18	90.18	122.11	7.4	3.69
15	40	60	1:10	40.6	35.36	75.96	108.88	5.58	4.24
16	20	60	1:10	42.28	31.17	73.45	100.8	5.47	3.77

X_1: Extraction time; X_2: Ethanol concentration; X_3: Sample weight:solvent volume ratio. BX (mg inidicaxanthin/100 g_{fw}), BC (mg betanin/100 g_{fw}), BET (mg betanin/100 g_{fw}), TPC (mg gallic acid equivalents/100 g_{fw}), antioxidant activity by ABTS and DPPH methods (μmol Trolox eq./g_{fw}).

Table 2. Mathematical models for conventional extraction of BET, BX, BC, TPC, DPPH and ABTS in jiotilla fruits.

Response variable	Model	R^2_{adj}
BX	$Y = 48.87 + 0.15X_1 - 0.65X_2 + 3.67X_3 - 0.19X_1X_2 + 0.01X_1X_3 + 0.57X_2X_3 - 0.04X_1^2 + 0.04X_2^2 - 1.12X_3^2$	0.61
BC	$Y = 38.09 + 0.68X_1 - 0.55X_2 + 3.26X_3 + 0.61X_1X_2 - 0.74X_1X_3 - 0.26X_2X_3 + 0.44X_1^2 + 0.08X_2^2 - 1.02X_3^2$	0.74
BET	$Y = 86.96 + 0.84X_1 - 1.19X_2 + 6.92X_3 + 0.42X_1X_3^2 - 0.73X_1X_3 + 0.3X_2X_3 + 0.4X_1^2 - 2.23X_3^2$	0.74
TPC	$Y = 110.81 + 4.67X_1 + 2.21X_2 + 8.99X_3 + 0.36X_1X_2 - 0.56X_1X_3 + 3.27X_1^2 + 0.97X_2^2 - 2.21X_3^2$	0.51
ABTS	$Y = 6.47 + 0.29X_1 - 0.34X_2 + 0.66X_3 - 0.06X_1X_2 - 0.16X_1X_3 - 0.03X_2X_3 + 0.39X_1^2 - 0.17X_2^2 - 0.19X_3^2$	0.39
DPPH	$Y = 5.06 + 0.27X_1 + 0.09X_2 + 0.49X_3 + 0.14X_1X_2 - 0.22X_2X_3 - 0.34X_1^2 - 0.19X_2^2 - 0.21X_3^2$	0.65

Table 3. Theoretical and experimental values of the simultaneous optimization process with desirability function for the variables BX, BC, BET, TPC and antioxidant activity by the ABTS and DPPH methods.

Response variable	Optimized values (theoretical)	Experimental values
BX [mg indicaxanthin/100 g_{fw}]	51.69	50.21 ± 0.44
BC [mg betanin/100 g_{fw}]	40.82	40.34 ± 0.69
BET [mg /100 g_{fw}]	92.52	90.97 ± 0.88
TPC [mg GAE/100 g_{fw}]	124.33	129.12 ± 14.21
ABTS [μmol Trolox eq./g_{fw}]	7.63	8.42 ± 0.74
DPPH [μmol Trolox eq./g_{fw}]	5.30	6.33 ± 0.42

With the second-order models for BX, BC, BET, TPC, ABTS, and DPPH, a simultaneous optimization process was carried out with the statistical software R (R Core Team, 2020) and the desirability package (Kuhn, 2016). Based on the model, the optimal conditions were an extraction time of 40 min, ethanol concentration of 37.5% v/v, and m:v ratio of 1:20. Although the lack of fit test was significant for some of the models, the experimental values were comparable to the theoretical predicted values (Table 3).

Due to the lack of reports on the optimization of

extraction conditions for jiotilla pulp extracts by CE, the results were compared with those reported in other cactus fruits. The optimum percentage of ethanol (37.5% v/v) obtained in this work for the extraction of BX, BC, BET, and TPC from jiotilla fruits is consistent with that reported by Sanchez-Gonzalez *et al*. (2013), who determined that the highest BC content of xoconostle fruits was obtained at 23.75 °C with 40% v/v ethanol. According to these researchers, solutions with a higher percentage of ethanol require an increase in temperature and extraction time to break hydrogen bonds by which BET remain attached to other components of the xoconostle (Sanchez-Gonzalez *et al*., 2013). On the other hand, the m:v ratio of 1:20 established in this study was similar to that reported by Nazeri and Zain (2018) for the extraction of phenolic compounds from pitaya peel; they reported that the solution becomes saturated with solute, decreasing the extraction efficiency when the m:v ratio is reduced.

The BC (40.34 ± 0.69 mg betanin/100 g_{fw}) and BX (50.21 ± 0.44 mg indicaxanthin/100 g_{fw}) contents in the optimized ethanolic extract (Table 3) were 76.30% and 77.93% higher, respectively, than those reported in a methanolic extract of fruits of the same species from Huajuapan de León, Oaxaca by Soriano-Santos *et al*. (2007). The moisture content of fruit in this study was 79.8 ± 0.01%; this was used to convert BC and BX fw to dry weight (dw). The amount of BC (248.56 ± 8.01 mg of betanin/100 g_{dw}) obtained in this study was similar to that reported in the aqueous extract obtained by shaking of the seedless pulp of jiotilla fruits from Santa María Zoquitlan, Oaxaca, Mexico by Sandate-Flores *et al*. (2020) who obtained values of 232 ± 23 mg/100 g_{dw} of BC while the amount of BX (199.70 ± 3.92 mg of indicaxanthin/100 g_{dw}) was 51% lower than that reported by the same authors. These differences in the content of BC and BX could be attributed to the climatic conditions of the different regions to which these fruits belong, to the fact that the pulp was free of seeds, and to the three consecutive extractions carried out by these authors. In addition, the contents of BC, BX, and BET (90.97 ± 0.88 mg/100 g_{fw}) were 73.24%, 39.79%, and 54.15% higher than those reported in aqueous extracts of *O. ficus-indica* fruits (Prakash Maran & Manikandan, 2012). TPC (129.12 ± 14.21 mg GAE/100 g_{fw}) in the optimized ethanolic extract (Table 3) was 23.15% higher than that reported in the aqueous extract of jiotilla fruits (Sandate-Flores *et al*., 2020).

The antioxidant activity obtained in the optimized extract was 8.42 ± 0.74 μmol Trolox equivalents/g_{fw} for ABTS and 6.33 ± 0.42 μmol Trolox equivalents/g_{fw} for DPPH (Table 3). These values are higher than those reported in white, yellow, and red prickly pears from the region of Sicily, Italy (Tenore *et al*., 2012).

In this study with jiotilla fruits, the correlation analysis performed showed positive correlations (ranging from 0.50 to 0.64) for BX, BC, BET, and TPC with antioxidant activity by ABTS and DPPH (Table 4). In fruits of Opuntia joconostle and *Stenocereus stellatus*, among others, positive correlations of the contents of phenolic compounds and BET with antioxidant activity have also been reported (Osorio-Esquivel *et al*., 2011; Pérez-Loredo, *et al*., 2017).

In general, antioxidant activity assays with ABTS showed higher values compared to those obtained with DPPH. Similar results have been reported and are attributed to the lower stability (higher reactivity) of the ABTS radical. It has also been reported that the DPPH radical reacts with polyphenols such as catechins and proanthocyanidins but not with phenolic acids and their glycosylated derivatives, while ABTS, due to its higher reactivity, has the ability to react with a greater number of reducing compounds (Mareček *et al*., 2017).

Table 4. Correlation values between the content of BX, BC, BET, TPC, and antioxidant activity by ABTS and DPPH methods, with conventional extraction (CE), ultrasound (UAE), and ultrasound-microwave assisted extraction (UMAE).

		BX	BC	BET	TPC
Conv.	ABTS	0.52*	0.58*	0.57*	0.59*
	DPPH	0.55*	0.64*	0.62*	0.50*
UAE	ABTS	0.56	0.28	0.46	0.98*
	DPPH	0.58	0.35	0.5	0.99*
UMAE	ABTS	-0.17	-0.13	-0.15	0.21
	DPPH	0.07	0.42	0.26	0.62*

*P value < 0.05

3.2 UAE and UMAE

UAE and UMAE were carried out using the optimal extraction conditions found for CE; Figure 1A shows the results obtained for BC, BX, BET, and TPC. A higher content of BX (50.21 ± 0.44 mg indicaxanthin/100 g_{fw}), BC (40.34 ± 0.69 mg betanin/100 g_{fw}), and BET (90.97 ± 0.88 mg/100 g_{fw}) was obtained for the optimized CE compared to UAE and UMAE, with no significant differences observed between the latter two. Pérez-Loredo *et al*. (2017) reported that microwave and ultrasound pretreatment with times up

to 60 min does not increase the BET content of *S. stellatus* fruit pulp. Similarly, Ramli *et al*. (2014) found that the BC content of *Hylocereus polyrhizus* fruits was higher when using CE compared to UAE. The higher content of the pigments obtained by CE could be due to the fact that BET are thermosensitive, so the low temperature (25 °C) in CE prevents their degradation.

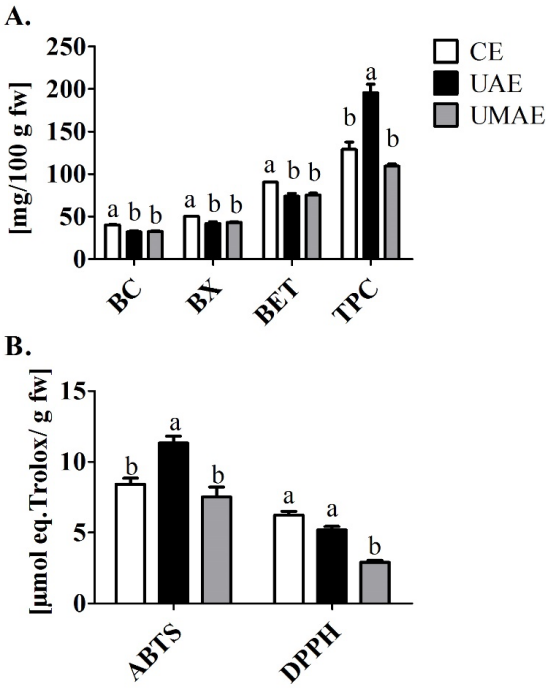

Figure 1. Comparison of functional compounds and antioxidant activity in *E. chiotilla* extracts obtained by conventional extraction (CE), ultrasound-assisted extraction (UAE), and microwave ultrasound-assisted extraction (UMAE). A. Content of BX (mg indicaxanthin/100 g_{fw}), BC (mg betanin/100 g_{fw}), BET, and TPC (mg gallic acid equivalent/100 g_{fw}) in jiotilla extracts. B. Evaluation of antioxidant activity by ABTS and DPPH methods (μmol Trolox eq./g_{fw}) in jiotilla extracts. fw: fresh weight. Different letters indicate significant differences between extraction methods according to Tukey's test (α = 0.05).

On the other hand, UAE had the highest TPC content (195.67 ± 17.28 mg GAE/100 g_{fw}), which was 51% higher than that obtained by CE and 78% higher than for UMAE (Figure 1A). These results are in line with previous reports where the use of ultrasound has improved the TPC yield (Gómez-Linton *et al*., 2019; Pérez-Loredo *et al*., 2017; Rocchetti *et al*., 2019). This increase is partially due to the cavitation phenomenon, which breaks up tissues and increases interaction of the solute with the solvent (Osorio-Tobón, 2020). Another factor that could improve the extraction of TPC is the increase in temperature during the process, which improves cell wall permeability and enhances solubility and diffusion of these compounds from the cell cytoplasm to the bulk of the solvent (Boudries *et al*., 2019).

To date, the acquisition of functional compounds and the antioxidant activity of jiotilla fruits by means of non-conventional technologies have not been reported; therefore, the comparison of the TPC content was carried out with studies reported for other cactus fruits. The TPC contents obtained in this work with UAE were 70-78% and 74-80% higher than those reported for the pulp of purple and yellow Spanish varieties of *O. ficus-indica*, respectively (García-Cayuela *et al*., 2019) and 36-68% higher than those reported also for UAE in other cactus fruits (*S. stellatus* red var. < *S. pruinosus* red var. < *S. pruinosus* orange var. < *S. stellatus* white var.) (García-Cruz *et al*., 2016).

Regarding the antioxidant activity, the extracts obtained by means of UAE exhibited the highest antioxidant activity by the ABTS method (11.33 ± 0.82 μmol Trolox eq./g_{fw}), while no significant difference was observed between the CE and UMAE extracts (Figure 1B). For the DPPH assay, no significant differences were observed between samples (p < 0.05), the activity being 6.33 ± 0.42 μmol Trolox eq./g_{fw} for CE, whereas for UAE it was 5.20 ± 0.39 μmol Trolox eq./g_{fw}. UMAE extracts showed lower antioxidant activity (2.92 ± 0.22 μmol Trolox eq./g_{fw}). This behavior could be due to the degradation caused by microwaves of some compounds with antioxidant activity such as organic malic, tartaric, and ascorbic acids (Ardestani *et al*., 2015; Dávila-Hernández *et al*., 2019), which were not quantified in this work. Correlation analysis of antioxidant activity with functional compounds in the UAE extract showed a positive correlation between TPC and antioxidant activity measured by ABTS and DPPH (0.98 and 0.99, respectively) (Table 4).

The ethanolic extract of jiotilla fruits showed a higher content of BC and BX than those reported in *O. ficus-indica* fruits, whose characterization and functional activity have been extensively studied. Moreover, the phenolic compound content of jiotilla fruits was higher than that reported for *O. ficus-indica*, *S. pruinosus*, and *S. stellatus* fruits.

3.3 Identification of BET

HPLC identification was performed by comparing the retention times (rt) of a commercial standard of betanin and laboratory semi-synthesized BX (vulgaxanthin I, indicaxanthin, and isoindicaxanthin; Supplementary Material 2). BC analysis of extracts showed three signals (Figure 2A) which correspond to betanin (rt: 33.84 min), isobetanin (rt: 36.65 min), and a third unidentified compound. The percentage area of peaks 1, 2, and 3 was 73.91%, 21.68%, and 4.39%, respectively. On the other hand, the analysis of BX at 480 nm (Figure 2B) showed six signals corresponding to vulgaxanthin I (rt: 21.25 min), isoindicaxanthin (rt: 30.72 min), and indicaxanthin (rt: 31.85 min). The fourth and fifth signals correspond to betanin and isobetanin, while the last remains as an unidentified

compound. Indicaxanthin was the major BX in the ethanolic extract of jiotilla with 63.07% of the total area, followed by vulgaxanthin I and isoindicaxanthin which accounted for 3.91% and 3.13% of the area, respectively. The remaining area corresponded to betanin (21.54%) and isobetanin (6.02%). The order of elution of the BX vulgaxanthin I and indicaxanthin corresponds to that reported previously for the

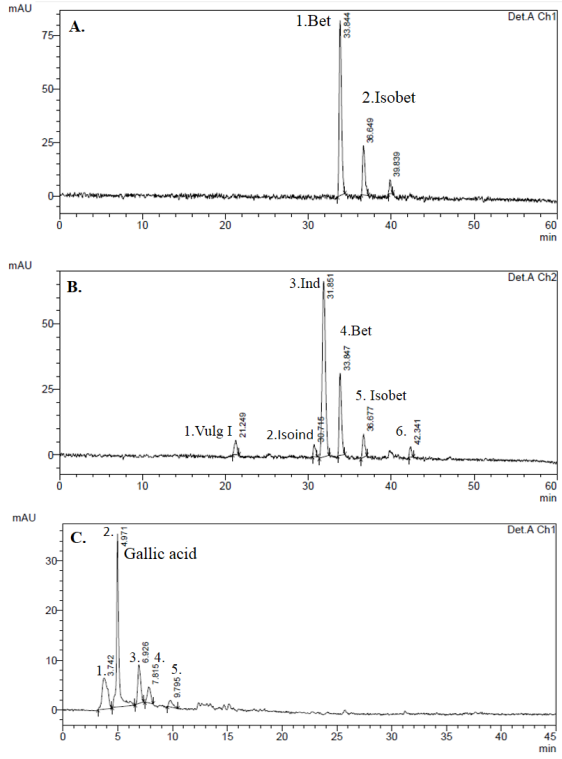

Figure 2. HPLC betalain profile of *E. chiotilla* extract [1 mg/mL] Abs at A. λ = 535 nm and B. λ = 480 nm. Bet: betanin, Isobet: isobetanin, Vulg I: vulgaxanthin I, Isoind: isoindicaxanthin, Ind: indicaxanthin. C. HPLC phenolic compound profile of *E. chiotilla* extract [1 mg/mL] at λ = 280 nm.

ethanolic extract of jiotilla by Soriano-Santos *et al*. (2007). Isoindicaxanthin has also been identified in methanolic extracts of *S. pruinosus* and *S. stellatus* (García-Cruz *et al*., 2017). Indicaxanthin has been reported as the most abundant and common BX in fruits of different cacti such as *O. ficus-indica* (Cejudo-Bastante *et al*., 2014), *O. dillenii* (Ker Gawl) Haw (Betancourt *et al*., 2017), *S. stellatus*, and *S. pruinosus* (García-Cruz *et al*., 2017).

3.4 Identification of phenolic compounds

The HPLC analysis for phenolic compounds in the extracts showed the presence of five signals. The major peak was identified as gallic acid (rt: 4.97 min) accounting for 51.53% of the area. The remaining peaks could not be identified and would require further metabolomics studies to be characterized. Peaks could probably correspond to compounds such as glycosylated phenolics, coumarins, saponins, alkaloids, organic acids, and soluble sugars that have been reported for other cacti fruits (Ramírez-Rodríguez *et al*., 2020). Sandate-Flores *et al*. (2020) reported similar results in jiotilla fruits from the Central Valleys region of Oaxaca. In their study, the content of gallic acid was determined to be the highest, at 1.02 ± 0.01 mg/100 g_{fw}. The other compound reported was caffeic acid, at 0.08 ± 0.00 mg/100 g_{fw}. This difference of approximately 12 times more gallic acid and such a low concentration of caffeic acid could explain why caffeic acid could not be detected in this study and under the applied conditions. Other explanations are that caffeic acid was probably not in free form or that the environmental conditions under which the jiotilla fruits were grown in this study varied the composition of functional compounds. Crop environmental conditions have been reported to affect the composition of functional compounds in fruits (Villa-Hernández *et al*., 2017; Gondim de Albuquerque *et al*., 2021).

Conclusions

In order to improve the extraction of phenolic compounds and BET from vegetable sources, optimization methodologies must be developed to identify the factors affecting the process, since extraction is influenced by the types of compounds and the matrix containing them, among other factors. In this work, CE was optimized with respect to the content of BET and phenolic compounds, and *in vitro* antioxidant activity. The optimal conditions were an extraction time of 40 min, ethanol concentration of 37.5% v/v, and m:v ratio of 1:20. The BC betanin and isobetanin, and BX vulgaxanthin I, isoindicaxanthin, and indicaxanthin were identified in the extract. The extraction method affected the content of BET and phenolic compounds as well as antioxidant capacity. Thus, CE allows a higher amount of BET to be obtained while ultrasound improves the extraction of phenolic compounds. Furthermore, a positive correlation was found in CE between antioxidant activity (ABTS and DPPH) and BET and phenolic compounds, while antioxidant activity in UAE correlated only with TPC, possibly due to their higher concentration in this extract. The differences observed in compound extraction could be partially due to the effects of each method on the plant matrix and, consequently, on the accessibility of the solvent to the compounds, as well as on the thermostability of the BET and phenolic compounds. These results indicate that the extraction method used will depend on the compounds of interest in jiotilla fruit. Therefore, UAE facilitates the extraction of phenolic compounds while CE promotes BET extraction.

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Nomenclature

BX	betaxanthins
BC	betacyanins
BET	betalains
TPC	total phenolic compounds
HPLC	high performance liquid chromatography
ABTS	2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate)
DPPH	2,2-diphenyl-1-picrylhydrazyl
g_{fw}	grams fresh weight

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