SUPPLEMENTARY MATERIAL

Physicochemical composition, phytochemical analysis and biological activity of ciricote (*Cordia dodecandra* A. D.C.) fruit from Yucatán

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Abstract

The physicochemical properties, proximate composition, minerals, total polyphenols, carotenoids, phenolic compounds, antioxidant, and antibacterial activities of ciricote (*Cordia dodecandra* A. DC.) tropical fruit were investigated. Minerals were quantified by using micro-Energy Dispersive X-Ray Fluorescence. Lutein and β-carotene were identified in ciricote fruit by using UPLC-PDA analysis. The highest values of the total polyphenols content and antioxidant activity were presented in ethanolic crude extracts obtaining by the ultrasonic-assisted method with freeze-dried fruit. The phenolic acids profile was identified and quantified by UPLC-PDA-ESI-MS. The main phenolic acids were caffeoyl hexoside, rufescenolide, quercetin 3-O-rutinoside, and rosmarinic acid. The ciricote extracts presented antibacterial activity against *Staphylococus aureus* (Gram+) and *Salmonella typhymurium* (Gram−). In conclusion, the ciricote (*Cordia dodecandra* A. DC.) tropical fruits could be very useful source of biological macromolecules, micro-elements, and phytochemical compounds for the food and pharmaceutical industry.

Keywords: Tropical fruit, micro-Energy Dispersive X-Ray Fluorescence, UPLC-PDA-ESI-MS, phenolic acids, *Staphylococus aureus*, *Salmonella typhymurium*

Experimental section

Materials and chemicals

Ciricote (*Cordia dodecandra* A. DC.) fruits were harvested in an orchard from Merida, Yucatan, Mexico. The taxonomic analysis of the ciricote plant was performed by the work group of the Plant Fisiology Laboratory, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, (CIATEJ), Mérida, Mexico. The identification of the plant was confirmed with the specimen deposited in the herbarium "Alfredo Barrera Marín" (voucher number: J. S. Flores **12576**) by José Salvador Flores Guido, Ph.D. at the Campus de Ciencias Biológicas y Agropecuarias, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Mexico. The pulp was extracted manually. About 2 kg of fresh fruit pulp was analyzed immediately, 2 kg of pulp was packed and kept in polyethylene bags at −20 °C, and 2 kg of sample was dried by freeze-drying method using a Freeze Dry System (FreeZone6, Labconco Corporation, Kansas City, Mo, USA) at 0.200 Mbar and −50 °C during 72 h. Finally, the freeze-dried pulp fruit was crushed using a mortar and stored at -20 °C until analysis. All chemicals used in this study were of analytical grade. Folin-Ciocalteau reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), sodium carbonate (Na_2CO_3), potassium persulfate ($K_2S_2O_8$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), concentrated sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), ethyl acetate, petroleum ether, methyl *tert*-butyl ether, methanol, and absolute ethanol were purchased from Sigma-Aldrich (Toluca, State of Mexico, Mexico). Standards of phenolic acids (gallic acid, rutin, caffeic acid, rosmarinic acid, trans-cinnamic acid, and ferulic acid) and carotenoids (lutein, α-carotene, and β-carotene) were obtained from Sigma-Aldrich (Toluca, State of Mexico, Mexico). Water used for all the experiments was double distilled and deionized.

Physicochemical characterization

Approximately, 50 g of fresh fruit was homogenized in a mixer to obtain fine pulp, that was used to determine the total soluble solids using a refractometer (Atago PAL-1, Tokyo, Japan) and data was expressed in °Brix. The titratable acidity of the ciricote juice was determined using the standard method (AOAC 2005) and the value was expressed as the

percentage of citric acid. Total soluble solids/titratable acidity ratio was to be used as a predicted reference of the maturity index. The pH of the fruit juice was measured with a pH meter (Oaklon, Singapur). The water activity of the samples was determined on LabSwift-aw equipment (Novasina AG, Lachen, Switzerland). Major (X) and minor (Y) axes of ciricote fruit were measured with a digital vernier caliper and the sphericity percentage (SP) was calculated from SP = (Y × 100)/X (Wanitchang et al. 2010). Weight (W) of each fruit (n=10) was measured by electronic balance (COBOS ATX-224, Shimadzu Corporation, Kyoto, Japan).

Proximate composition analysis

Proximate analysis of ciricote fruit was conducted as follows: moisture, ash, fat, and crude fiber contents were determined using standard methods outlined by (AOAC 2005). Nitrogen percentage in the ciricote fruit was determined by the Kjeldahl method using a VELP system (Scrubber SMS, Recirculating water aspirator JP, Heating Digester DKL, and Distillation unit UDK-129; VELP Scientifica, Italy). The protein was calculated by the conversion factor of 6.25 (AOAC 2005). All determinations were done in triplicate. The total carbohydrates by difference were calculated by subtracting the sum of the percentage of crude protein, crude fat, crude ash, crude fiber, and moisture.

Crude total carbohydrates (%) = 100 – [moisture (%) + crude fiber (%) + crude ash (%) + crude fat (%) + crude protein (%)]

Mineral analysis

Minerals were determined by a micro-Energy Dispersive X-Ray Fluorescence (μ-EDXRF) system (M4 Tornado^{TM}, Bruker, Germany) according to Cardoso et al. (2018) with slight modifications. In all of the measurements, an X-ray generator was operated at 50 kV and 200 μA. Detection of the fluorescence radiation was performed by an energy-dispersive silicon drift detector, XFlash^{TM}, with a 30 mm^2 sensitive area and an energy resolution of 142 eV for all elements. All determinations were done in triplicate.

Extraction and analysis of carotenoids

Carotenoids were extracted with the method described by Irías-Mata et al. (2018b) with slight modifications. Briefly, 1.0 g of the freeze-drying fruit pulp, with 50 mg calcium carbonate ($CaCO_3$), was extracted with 5 mL of the extraction solvent (methanol/ethyl acetate/petroleum ether, 1:1:1, *v/v*). The mixture was stirred under magnetic stirring for 1 min at room temperature and incubated for 30 s in an ultrasonic bath. The sample was centrifuged at 6,500 rpm for 15 min using a Centrific 225 centrifuge (Thermo Fisher Scientific, USA), and the supernatant was collected. The organic extract was evaporated to dryness under gentle nitrogen. Finally, the carotenoid extract was re-dissolved in 2 mL of a mixture of methyl *tert*-butyl ether (MTBE) and methanol (1:1, *v/v*) and membrane-filtered (Hydrophobic PTFE, 0.20 µm, Millex-FG, Merck Millipore, MA, USA) into amber glass vials.

Carotenoids identification and quantification were performed using an ACQUITY ULPC™ system (Waters, Milford, MA, USA) equipped with a PDA eλ detector. Chromatographic separation was achieved using an ACQUITY UPLC™ BEH C18 (2.1 × 100 mm i.d., 1.7 µm particle size) column (Waters, Ireland). Mobile phases were composed of methanol (eluent A) and methanol/ammonium acetate (1 M) (70:30; eluent B). The elution gradient was as follows: isocratic at 95% A and 5% B for 5 min. The total run time was 25 min at a flow rate of 0.2 mL/min and a column temperature of room temperature (22 °C). The injection volume was 2 µL. Carotenoids were monitored at 430 nm. Identification of carotenoids was accomplished by comparison of retention times and UV/Vis absorption of the standards (lutein, α-carotene, and β-carotene). UPLC-PDA quantification was achieved using linear calibration curves of lutein, α-carotene, and β-carotene from 20 to 100 ppm in methanol/MTBE (1:1, *v/v*). Results were expressed as µg g^{-1} dry weight (*d.w.*).

Colour measurements

CIE-L*a*b* colour values of ciricote fruits were determined using a MiniScan EZ 4500 L spectrophotometer (Hunter Associates Laboratory, Inc., Sunset Hills Road Reston, VA, USA). Color squares were produced by converting L*, a* and b* values to R, G, and B using http://colormine.org/convert/rgb-to-lab and Microsoft PowerPoint software (Zhang et al. 2019). The Hue angle, Chroma, and Saturation were also calculated using the following equations:

Hue angle = $\tan^{-1}$(b*/a*)

Chroma = $\sqrt{(a*)^2 + (b*)^2}$

Saturation = Chroma/L*

Preparation of crude extracts

Ciricote fresh, frozen, and freeze-drying fruit pulps were used to obtain the extracts by conventional and ultrasonic-assisted methods using ethanol 50% and methanol 50% (*v/v*) as the solvents (Table S5, Supplementary material). Conventional extraction was realized by maceration of deseeded fruit sample (1.0 g) with 50 mL of 50% aqueous ethanol or 50% aqueous methanol. The samples were then homogenized using a magnetic stirrer for 60 min at room temperature (25 °C). The samples were centrifuged, and the supernatant was filtered using vacuum filtration. The filtrate was collected and adjusted to the initial volume. The samples were stored at −40 °C until the analysis. Extractions using an ultrasonic-assisted method were performed using a 20 kHz and 130 W GEX130PB ultrasonic device (Ultrasonic Processor, Sonics & Materials, Inc., Newtown, CT, USA) with a diameter probe of 13 mm. Fresh, frozen, and freeze-drying samples were accurately weighed (1.0 g) and immersed in 50 mL of each solvent. The samples were then sonicated for 10 min at 80% amplitude. A cold bath was used to maintain the temperature below 50 °C. After applying the ultrasound, the processed samples were centrifuged, and the supernatants were recovered off the same way that it was described before. The extracts were stored at −40 °C until the analysis.

Total phenolics content (TPC) determination

The TPC was determined with Folin-Ciocalteau reagent according to Patrón-Vázquez et al. (2019) with slight modifications. The TPC values were expressed as mg of gallic acid equivalent (GAE) per g of dry weight (*d. w.*) samples through a calibration curve of gallic acid from 50 to 700 ppm. Briefly, the extract (20 µL) was mixed with 250 µL of Folin-Ciocalteau 1N reagent, 1,250 µL of Na_2CO_3 (7.5% *w/v*), and 480 µL of distilled water. This mixture was vortexed and stored in darkness for 30 min. The absorbance at 760 nm was measured using a BioMate 3S UV–visible spectrophotometer (Thermo Fisher Scientific, Inc., Walthman, MA, USA).

Antioxidant capacity by DPPH and ABTS assays

The antioxidant activity of extracts was evaluated using the DPPH radical scavenging activity assay according to Shimada et al. (1992) with slight modifications. Briefly, 100 µL of sample extract was added to 2900 µL of 0.1 mM DPPH in methanol, then stored in darkness for 30 min at room temperature (25 °C). The absorbance of the reactions was measured at 515 nm using a UV-vis spectrophotometer (BioMate 3S, Thermo Fisher Scientific Inc., USA).

The antioxidant activity by the ABTS assay was evaluated according to Alonso-Carrillo et al. (2017).

Activity antioxidant was expressed in both DPPH and ABTS as microMol of Trolox equivalent (µM TE) per g of dry weight (*d.w.*) samples through a calibration curve of Trolox.

Identification and quantification of phenolics compounds

For identification and quantification of phenolic compounds by the UPLC-PDA-ESI-MS method were according to Ana et al. (2018) with slight modifications. The ciricote extracts were filtered through a hydrophobic membrane (PTFE, 0.20 µm; Millex-FG Samplicity Filter, Merck; Germany) and used for analysis. The analysis of phenolic compounds was carried out using an ACQUITY Ultra Performance Liquid Chromatography (UPLC) system (Waters Corporation, Milford, MA, USA) equipped with a quaternary solvent manager, sample manager, and Photodiode Array (PDA) eλ detector coupled to Xevo TQ-S micro Mass Spectrometer (MS) (Water; Milford, MA, USA) fitted with an electrospray ionization (ESI) source acting on negative mode. The ACQUITY UPLC^{TM} BEH C18 column (2.1 × 100 mm i.d., 1.7 µm; Waters, Ireland) was used. The injection volume was 2 µL, and the elution was completed in 31 min with a flow rate of 0.2 mL min^{-1}. Mobile phases were composed of formic acid in ultrapure water (0.1% *v/v*, eluent A) and formic acid in acetonitrile (0.1% *v/v*, eluent B). The elution gradients were used as follows: elution start with 100% A (from 0.0 to 2.0 min); reduction to 77% A (from 2.0 to 6.0 min); isocratic 77% A (from 6.0 to 17.5 min); increase to 100% A (from 17.5 to 18.0 min); reduction to 50% A (from 18.0 to 24.0 min); increase to 100% A (from 24.0 to 30.0 min); isocratic

100% A (from 30.0 to 31.0 min). The PDA spectra for phenolic acids were measured at 290 nm. The optimized MS parameters were as follows: source temperature of 150 °C, desolvation temperature of 500 °C, cone gas flow 150 L h^{-1}, desolvation gas flow 650 L h^{-1}, capillary voltage of 3.5 kV, cone voltage of 40 V, and collision energy of 3 V. The MS analysis was performed using a mass scanning from 50 to 700 *m/z*. MassLynkx 4.1 software (Waters, Milford, MA, USA) was used for data acquisition and processing. Quantification was made by injection of solutions of known concentrations ranging between 0.0 and 450 µmol L^{-1} of caffeic acid, rutin, and rosmarinic acid, as standards. The results of UPLC-PDA analyses were reported as µmol equivalent of analytical standard per g of dry weight (*d. w.*).

Antibacterial activity

The antibacterial activity of methanolic and ethanolic fruit extracts of *Cordia dodecandra* A. DC. was tested against pathogenic microorganisms (*Salmonella typhimurium* and *Staphylococcus aureus*) was determined according to Medina-Torres et al. (2019). The Minimum inhibitory concentration (MIC) determinations were performed by the microdilution method. MIC was defined as the lowest extract concentration that prevented this change and exhibited inhibition of bacterial growth.

Statistical analysis

All results were expressed as means ± standard deviation. One-way analysis of variance (ANOVA; $p < 0.05$) and Tukey's HSD test were carried out using Statgraphics Centurion XVI (Statistical Graphics Corp., Manugistics, Inc., Cambridge, MA, USA).

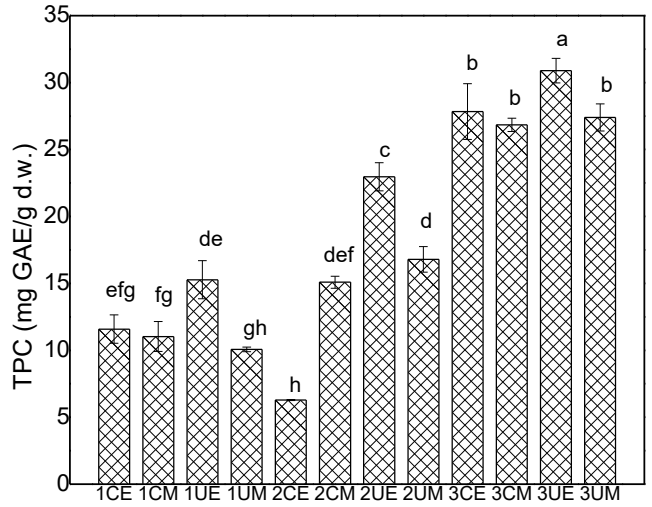
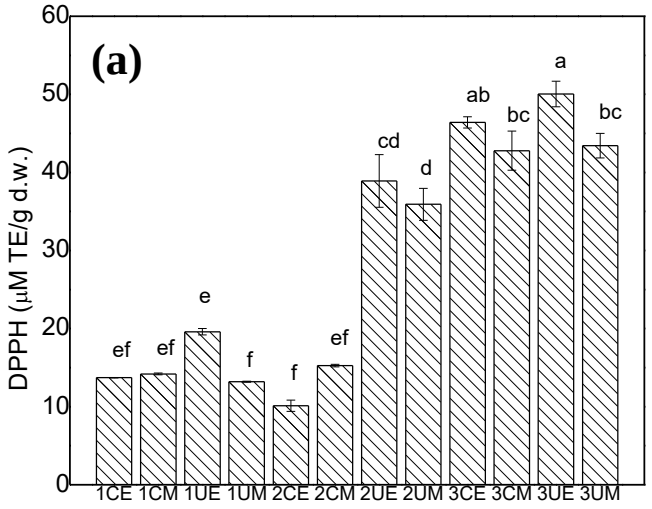


Figure S1. Total phenolic compounds content of the *Cordia dodecandra* A. DC. fruit pulp extracts. 1 = Fresh pulp; 2 = Frozen pulp; 3 = Freeze-dried pulp; C= Conventional extraction by maceration; U = Ultrasound-assisted extraction; E = 50% aqueous ethanol solution; M = 50% aqueous methanol solution. Different letters mean significant differences ($p \leq 0.05$).



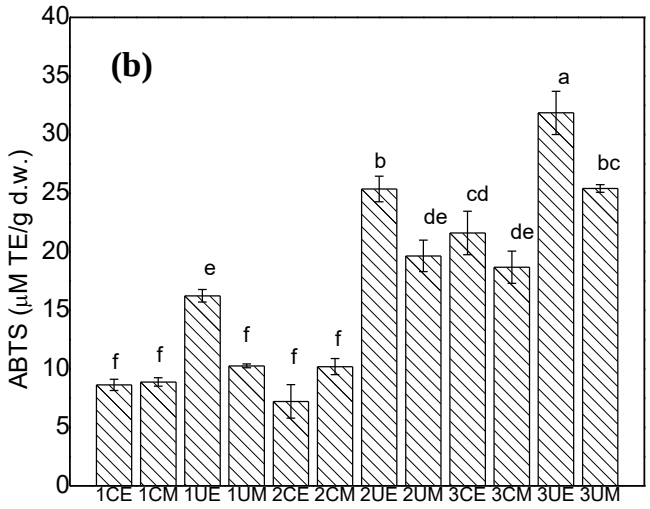


Figure S2. Antioxidant activity of the ciricote fruit pulp extracts by DPPH (a) and ABTS (b) assays. 1 = Fresh pulp; 2 = Frozen pulp; 3 = Freeze-dried pulp; C= Conventional extraction by maceration; U = Ultrasound-assisted extraction; E = 50% aqueous ethanol solution; M = 50% aqueous methanol solution. Different letters mean significant differences ($p \leq 0.05$).

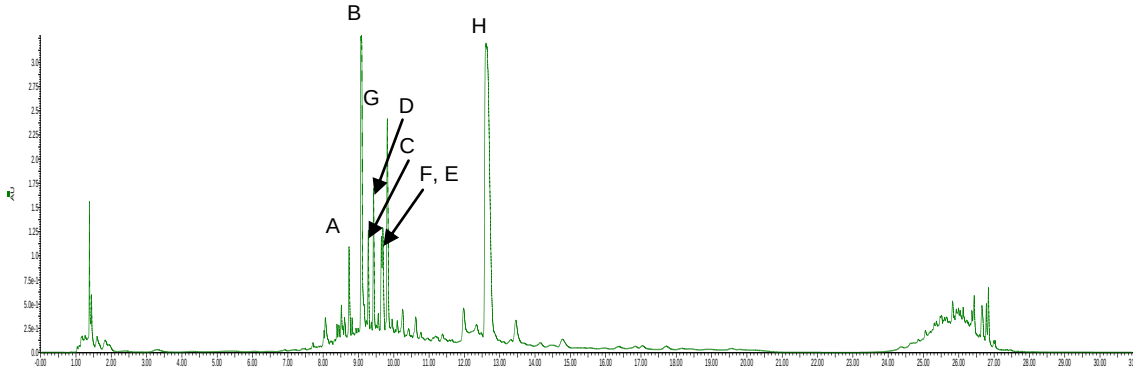


Figure S3. Chromatogram of phenolic compounds profile of ciricote (*Cordia dodecandra* A. DC.) fruit pulp.

Table S1. Physicochemical characterization and proximate composition of Cordia dodecandra A. DC. fruit pulp.

		Values and composition
Physicochemical properties	Sphericity (%)	91.67 ± 5.20^{a}
	Weight (g)	36.40 ± 5.97^{a}
	Total soluble solids (°Brix)	12.10 ± 1.00^{a}
	Titratable acidity (% citric acid)	0.52 ± 0.05^{a}
	Maturity index	22.95 ± 1.89^{a}
	pH	5.40 ± 0.03^{a}
	Water activity (A_w)	0.97 ± 0.00^{a}
Proximate composition	Crude total carbohydrates (%)	76.96 ± 0.43^{b}
	Crude fiber (%)	8.08 ± 0.77^{b}
	Crude protein (%)	7.28 ± 0.84^{b}
	Crude ash (%)	5.06 ± 0.20^{b}
	Crude fat (%)	2.62 ± 0.24^{b}
Minerals (mg/kg)	Potassium (K)	$58{,}926.3 \pm 833.6^{A}$
	Calcium (Ca)	$11{,}302.2 \pm 59.2^{A}$
	Sodium (Na)	$4{,}980.1 \pm 280.8^{A}$
	Magnesium (Mg)	$1{,}556.6 \pm 238.7^{A}$

	Chlorine (Cl)	1,392.6 ± 51.2[A]
	Sulfur (S)	1,286.2 ± 356.9[A]
	Phosphorus (P)	811.0 ± 62.0[A]
	Iron (Fe)	58.4 ± 0.5[B]
	Zinc (Zn)	43.7 ± 0.2[B]
	Manganese (Mn)	26.9 ± 3.2[B]
	Copper (Cu)	19.4 ± 1.9[B]
Colour parameters	L*	70.64 ± 5.55
	a*	16.39 ± 3.27
	b*	47.23 ± 2.87
	R	233.96 ± 2.85
	G	170.39 ± 12.96
	B	79.71 ± 5.37
	Hue angle (°)	70.82 ± 4.97
	Chroma	50.12 ± 3.16
	Saturation	0.71 ± 0.05

Data are mean ± standard deviation (SD). [a] Values of physicochemical properties of ciricote fruit. [b] Proximate composition of *Cordia dodecandra* A. DC. fruit pulp (% dry weight). [A] Macroelements. [B] Microelements.

Table S2. Retention time (Rt), wavelengths of maximum absorption in the visible region (λmax), mass spectral data (MS2) and identification of the phenolic compounds profile present in the crude extract of Cordia dodecandra A. DC. fruit.

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^-$ (m/z)	MS^2 (m/z)	Identification
A	8.760	205, 261, 315			
B	9.172	199, 285, 322	341	179 (100), 135 (60), 341 (10)	Caffeoyl hexoside
C	9.262	205, 264, 310	311	311 (100)	
D	9.422	220, 287, 329			
E	9.650	220,287, 336	357	357 (100)	Rufescenolide
F	9.692	226, 287, 338	355	355 (100)	
G	9.874	199, 254, 342	609	609 (100)	Quercetin 3-O-rutinoside
H	12.764	205, 287, 328	359	359 (100), 161 (10), 197 (5)	Rosmarinic acid

Table S3. Phenolic compounds profile quantification (μM/g dry weight) in the crude extracts of Cordia dodecandra A. DC. fruit (mean ± SD).

	Caffeoyl hexoside*	**Quercetin 3-*O*-rutinoside****	**Rufescenolide*****	**Rosmarinic acid****
3CM	2.80 ± 0.04^{b}	0.98 ± 0.13^{b}	0.59 ± 0.04^{ab}	17.09 ± 0.46^{ab}
3CE	2.66 ± 0.02^{b}	1.00 ± 0.12^{b}	0.68 ± 0.01^{ab}	15.60 ± 1.11^{b}
3UM	3.33 ± 0.08^{a}	1.25 ± 0.03^{ab}	0.55 ± 0.05^{b}	18.50 ± 0.63^{a}
3UE	3.42 ± 0.12^{a}	1.46 ± 0.13^{a}	0.73 ± 0.10^{a}	18.97 ± 0.55^{a}

*, **, *** = equivalents of caffeic acid, rutin, and rosmarinic acid, respectively. 3 = Freeze-dried pulp; C= Conventional extraction by maceration; U = Ultrasound-assisted extraction;

E = 50% aqueous ethanol solution; M = 50% aqueous methanol solution. In each column and for the different extraction procedures, different letters mean significant differences ($p \leq 0.05$).

Table S4. Minimum inhibitory concentrations (μg/mL) of ciricote fruit extracts.

	Gram (−)	Gram (+)
	Salmonella typhymurium	*Staphylococcus aureus*
3CM	12.08	3.02
3CE	12.73	3.18
3UM	10.21	5.10
3UE	11.46	2.87

Table S5. Sample codes for the ciricote (*Cordia dodecandra* A. DC.) fruit extracts.

Codes	Pulp	Extraction method	Solvent
1CE	Fresh	Conventional	Ethanol
1CM	Fresh	Conventional	Methanol
1UE	Fresh	Ultrasound-assisted	Ethanol
1UM	Fresh	Ultrasound-assisted	Methanol
2CE	Frozen	Conventional	Ethanol
2CM	Frozen	Conventional	Methanol
2UE	Frozen	Ultrasound-assisted	Ethanol

2UM	Frozen	Ultrasound-assisted	Methanol
3CE	Freeze-dried	Conventional	Ethanol
3CM	Freeze-dried	Conventional	Methanol
3UE	Freeze-dried	Ultrasound-assisted	Ethanol
3UM	Freeze-dried	Ultrasound-assisted	Methanol

1 = Fresh pulp; 2 = Frozen pulp; 3 = Freeze-dried pulp; C= Conventional extraction by maceration; U = Ultrasound-assisted extraction; E = 50% aqueous ethanol solution; M = 50% aqueous methanol solution.