Ecosistemas y Recursos Agropecuarios

Ultrasound-assisted extraction (UAE) of phenolic compounds from *Brosimum alicastrum* fruit and their antioxidant capacity

Extracción asistida por ultrasonido (EAU) de compuestos fenólicos del fruto *Brosimum alicastrum* y su capacidad antioxidante

Gustavo Aguilar-Piloto[1], Andrea Carolina Negrón-Diaz[1], Víctor Manuel Moo-Huchin[1*], Manuel Octavio Ramírez-Sucre[2], Mariana Delgadillo-Díaz[3], Luis Fernando Cuevas-Glory[1], Enrique Sauri-Duch[1]

[1]Instituto Tecnológico de Mérida, km 5 Mérida-Progreso. CP. 97120. Mérida, Yucatán, México.
[2]Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C. Sede sureste, km 5.5 Carretera Sierra Papacal-Chuburna Puerto. CP. 97302. Mérida, Yucatán, México.
[3]Universidad Marista de Mérida, Periférico Nte Tablaje Catastral 13941. CP. 97300. Mérida, Yucatán, México.

[*]Corresponding author:
vmmoo@yahoo.com

Scientific note
Received: February 14, 2023
Accepted: November 27, 2023

How to cite: Aguilar-Piloto G, Negrón-Diaz AC, Moo-Huchin VM, Ramírez-Sucre MO, Delgadillo-Díaz M, Cuevas-Glory LF, Sauri-Duch E (2023) Ultrasound-assisted extraction (UAE) of phenolic compounds from *Brosimum alicastrum* fruit and their antioxidant capacity. Ecosistemas y Recursos Agropecuarios Núm. Esp. III: e3655. DOI: 10.19136/era.a10nIII.3655

ABSTRACT. The aim of this work was to compare the effect of solvents in the UAE on the content of total phenolic compounds (TPC) and *in vitro* antioxidant capacity DPPH and reducing power (RP) of the whole fruit *B. alicastrum*. The phenolic compounds of the freeze-dried fruit were extracted in an ultrasonic bath for 30 min at 30 ℃, 100% amplitude and 40 kHz frequency. The solvents used were: ethanol (Et-OH), methanol (Met-OH), 50% ethanol (50% Et-OH) and 50% methanol (50% Met-OH). The UAE of phenolic compounds of the fruit was improved by adding distilled water to the organic solvent. The highest TPC content and antioxidant capacity were obtained with 50% Met-OH. There was a high positive correlation between TPC and antioxidant capacity (DPPH and PR). It is suggested to use 50% Met-OH as solvent for UAE of phenolic compounds of fruit *B. alicastrum*.
Key words: Antioxidants, *B. alicastrum*, methanol, polyphenols, Ramon.

RESUMEN. El trabajo tuvo como objetivo comparar el efecto de disolventes en la EAU sobre el contenido de compuestos fenólicos totales (CFT) y capacidad antioxidante *in vitro* DPPH y poder reductor (PR) del fruto completo *B. alicastrum*. Los compuestos fenólicos del fruto liofilizado fueron extraídos en un baño de ultrasonidos durante 30 min a 30 ℃, 100% de amplitud y 40 kHz de frecuencia. Los disolventes utilizados fueron: etanol (Et-OH), metanol (Met-OH), 50% de etanol (50% Et-OH) y 50% de metanol (50% Met-OH). La EAU de compuestos fenólicos del fruto mejoró adicionando agua destilada al disolvente orgánico. El mayor contenido CFT y la mayor capacidad antioxidante se obtuvieron con 50% Met-OH. Hubo una alta correlación positiva entre CFT y la capacidad antioxidante (DPPH y PR). Se sugiere utilizar 50% Met-OH como disolvente para la EAU de compuestos fenólicos del fruto completo *B. alicastrum*.
Palabras clave: Antioxidantes, *B. alicastrum*, metanol, polifenoles, Ramón.

E. ISSN: 2007-901X

INTRODUCTION

The *B. alicastrum* (known as Ramón) is a tree of the deciduous forests of the Yucatan Peninsula, but can also be found along the Gulf of Mexico and extending through Central America; it was a tree used by the ancient Mayas in traditional medicine and as a food resource for animals and humans; however, over the years it has lost importance due to the oblivion of the millenary traditions of its use (Ramírez-Sánchez *et al*. 2017). There is currently a trend towards the revaluation of *B. alicastrum*, attributed to its abundance of fruit, nutritional value and antioxidant properties (Ozer 2017, Moo-Huchin *et al*. 2019, Sarmiento-Franco *et al*. 2022). The seed of the fruit is used for the commercial production of gluten-free flour and a coffee substitute that does not contain caffeine (Sosa-Zepeta y Ticante-Pérez 2022); however, there are only a few studies on the content of bioactive compounds and antioxidant activity of these products. Ozer (2017) performed a comparison of phenolic compound content and antioxidant activity between roasted (ground) seeds of *B. alicastrum* and commercial nuts. In such study, a Soxhlet extractor was used with aqueous ethanol (80% in distilled water, v/v) at 80 °C for 30 min to obtain the extract of the samples, where it was found that *B. alicastrum* showed higher content of phenolic compounds and antioxidant activity compared to walnut, peanut and almond. On the other hand, Moo-Huchin *et al*. (2019) investigated the extraction of phenolic compounds from unroasted *B. alicastrum* seed through orbital agitation (250 rpm) for 2 h at 25 °C, evaluating different solvents such as distilled water, methanol, ethanol, acetone, and mixtures of each organic solvent with distilled water (50%, v/v). These authors identified the ethanol-water mixture (50%, v/v) as the best solvent in the extraction process of phenolic compounds from the seed. In addition to the solid-liquid extraction method with Soxhlet equipment and orbital shaking reported by Moo-Huchin *et al*. (2019) and Ozer (2017) on *B. alicastrum* seeds; other non-conventional methods are also available such as UAE, which uses ultrasonic waves with frequencies between 20 kHz and 10 MHz that can propagate in an elastic medium such as liquids, producing acoustic cavitation, leading to the release of extractable components from the plant matrix, and this method has advantages over traditional maceration, such as high extraction yield of phenolic compounds, high reproducibility, low cost, easy implementation of the equipment and also UAE can extract bioactive compounds in less time (Selvamuthukumaran and Shi 2017, Carrillo-Lomelí *et al*. 2022). To date, there are no UAE reports of phenolic compounds from the whole fruit of *B. alicastrum* using different organic solvents or mixtures of organic solvents with water. From all the above and recognizing that extraction is a process that affects the analytical methodology in the study of phenolic compounds in a plant matrix, the present work aimed to evaluate the effect of various solvents in the UAE on the content of total phenolic compounds (TPC) and antioxidant capacity *in vitro* of the whole fruit (lyophilized) of *B. alicastrum*.

MATERIALS AND METHODS

Obtaining the lyophilized fruit

The *B. alicastrum* fruits were collected from 15 random trees (from the garden of the Instituto Tecnológico de Mérida, Yucatán, México) selected for their maturity indicated by the orange coloration of their peel with average values of color L*, a*, b*, hue angle (h°) and chromaticity (C*) of 62.12 $\pm$ 2.10, 14.10 $\pm$ 1.37, 60.77 $\pm$ 2.61, 76.80 $\pm$ 1.20 y 65.10 $\pm$ 4.5, respectively. About 30 kg of whole fruit were harvested in September 2021, where, fruit showing physical damage were excluded from the analyses. Fruits were washed with tap water and ground for subsequent lyophilization in an Edwards Modulyo EF4 1044 Freeze Dryer for 72 h. The lyophilized whole fruit was ground in a Nutribullet® food processor for 45 s, sieved with a Tyler No. 40 mesh (0.425 mm) and finally, the lyophilized powder was stored in airtight Ziploc® bags and frozen at -20 °C until the extraction of phenolic compounds.

Preparation of extracts

The lyophilized fruit was homogenized in extraction solvent (Et-OH, Met-OH, 50% Et-OH y 50%

E. ISSN: 2007-901X

Met-OH) at a 60:1 mL g^{-1} ratio. The mixtures were placed in the ultrasonic bath (CScientific CS-UB100) for 30 min at 30 ℃, 100% amplitude and 40 kHz frequency. At the end of the extraction, the samples were centrifuged for 10 min at 4,000 rpm at 25 ℃ to obtain the phenolic compound extract (supernatant). With the sediment (pellet), the extraction process was repeated (for more compounds) following the procedure previously described. The supernatants (extract) from the two extraction cycles were pooled, completing their volume to 20 mL with the same extraction solvent and stored at -20 ℃ under darkness until analysis. The selection of solvents and UAE parameters was made based on literature and preliminary tests performed in the Laboratory that are not described in this research.

TPC and antioxidant capacity *in vitro*

The TPC content of the lyophilized fruit was quantitatively determined according to the method described by Olvera-Aguirre *et al.* (2022). A calibration curve was obtained with the absorbance values resulting from the reaction of gallic acid at different concentrations with Folin-Ciocalteu's reagent. The results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} of lyophilized fruit.

The *in vitro* antioxidant capacity DPPH and the reducing power (RP) of the lyophilized fruit were evaluated by the methods reported by Olvera-Aguirre *et al.* (2022). Results were expressed as mM Trolox 100 g^{-1} of lyophilized fruit for DPPH and mg ascorbic acid equivalents (AAE) 100 g^{-1} of lyophilized fruit for RP.

Statistical analysis

The experimental design was completely randomized with four treatments and three replicates. The variables were analyzed with a one-way ANOVA analysis of variance, and when there were significant statistical differences between extraction solvents, Tukey's test was applied, testing probabilities less than 0.05 using the statistical package StatGraphics Centurion XVI.

RESULTS AND DISCUSSION

For the study of the TPC content of the whole Ramon fruit, the determination of a suitable extraction solvent for the maximized recovery of phenolic acids and flavonoids from the plant matrix is advisable (Hapsari *et al.* 2022). In the present work, a variation in the TPC content (ranging from 1 051.72 $\pm$ 54.52 to 1 936.12 $\pm$ 19.05 mg GAE 100 g^{-1} of lyophilized fruit) of the whole fruit of Ramon was found due to the type of solvent used in UAE ($p \leq 0.05$) (Figure 1). The addition of distilled water in ethanol and methanol improved TPC extraction by 63.9 and 32.07% for 50% Et-OH and 50% Met-OH, respectively. This is explained by the fact that water increases the polarity of the organic solvent, making it more similar to the polarity of the phenolic compounds in the fruit, leading to intensified molecular forces, thus increasing the solubility of polar carbohydrates and polyphenol glycosides (Mikucka *et al.* 2022). Pure methanol (which has higher polarity than ethanol) allowed higher TPC extraction ($p \leq 0.05$) (1 465.96 $\pm$ 118.62 mg GAE 100 g^{-1} lyophilized fruit) than pure ethanol (1 051.72 $\pm$ 54.53 mg GAE 100 g^{-1} lyophilized fruit), this result is in agreement with that reported by Mikucka *et al.* (2022). Although it has been reported that pure ethanol recovers flavonoids more efficiently than methanol; however, the solubility of oxygen in ethanol is higher than in methanol, hence, degradation of polyphenols in pure ethanol is possible (Carrasco-Quiroz *et al.* 2023); moreover, methanol is likely to have higher affinity for phenolic compounds interacting with plant matrix components. Therefore, determining the effect of a given solvent on polyphenol recovery remains a challenge due to the diversity of compounds bound to the plant matrix (Mikucka *et al.* 2022). When comparing the four solvents in UAE, 50% Met-OH proved to be the best solvent for extraction of TPC from fruit.

In this research, the DPPH and RP antioxidant capacity assays were used, due to the existence of different reaction mechanisms and assay conditions, in addition to their limitations. The solvents in UAE influenced the *in vitro* antioxidant capacity of lyophilized fruit using both DPPH and RP measurement assays

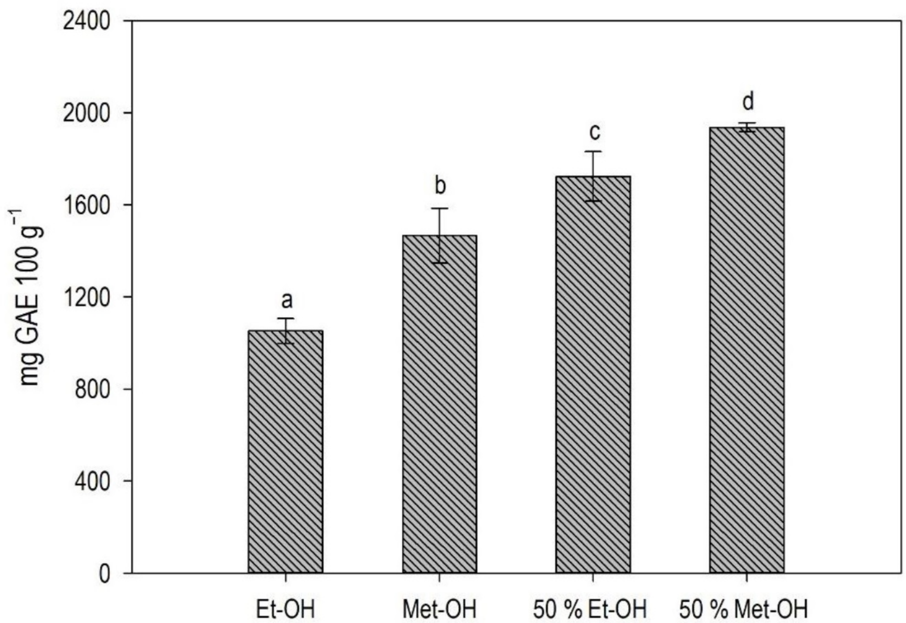

Figure 1. Effect of solvent-UAE on total phenolic compound (TPC) content of whole fruit of Ramon. GAE = gallic acid equivalent. Et-OH = pure ethanol; Met-OH = pure methanol; 50% Et-OH = 50% ethanol and 50% Met-OH = 50% methanol.

(Figure 2 and 3). The antioxidant capacity of the fruit evaluated with both assays was higher when distilled water was added to the organic solvent. In the DPPH assay, an increase ($p \leq 0.05$) in antioxidant capacity was found from 14.72 $\pm$ 0.56 (pure ethanol) to 38.51 $\pm$ 0.73 mM Trolox 100 g^{-1} (50% Et-OH) and form 34.20 $\pm$ 0.39 (pure methanol) to 56.98 $\pm$ 1.67 mM Trolox 100 g^{-1} (50% Met-OH). The antioxidant capacity RP ranged ($p \leq 0.05$) from 71.89 $\pm$ 1.52 (pure ethanol) to 123.59 $\pm$ 2.89 mg AAE 100 g^{-1} (50% Et-OH) and from 152.79 $\pm$ 2.39 (pure methanol) to 159.63 $\pm$ 1.85 mg AAE 100 g^{-1} (50% Met-OH). The higher antioxidant capacity (in both trials) of the fruit with the Met-OH solvent with respect to Et-OH (2 times higher) corresponded to the higher TPC content. Of all the solvents used in UAE, 50% Met-OH exhibited higher antioxidant capacity in both methods (corresponded with higher TPC), i.e., the phenolic compounds of the fruit extracted with 50% Met-OH had higher capacity to donate a hydrogen atom or an electron to neutralize the DPPH radical (it is converted into a reduced form); furthermore, these compounds had the highest capacity for reduction from a ferric (Fe^{3+}) to a ferrous (Fe^{2+}) form (Bibi Sadeer *et al*. 2020).

The values of TPC content and DPPH antioxidant capacity of the whole fruit are higher than those reported for Ramon seeds (Moo-Huchin *et al*. 2019). Pearson correlation analysis was performed to understand the interrelationship between polyphenols and *in vitro* antioxidant capacity (Table 1). In this study, the *in vitro* antioxidant capacity determined with the DPPH and RP assays was positively correlated with TPC with values of r = 0.96 y r = 0.81, respectively, which is considered a strong correlation between the variables according to the classification proposed by Taylor (1990). The high correlations indicate an important role of Ramon fruit polyphenols as reducing agents contributing to its antioxidant capacity. Sik *et al*. (2022) reported a similar trend in the correlation between TPC values and DPPH and FRAP antioxidant capacity values of six edible fruits from Hungary. Furthermore, Moo-Huchin *et al*. (2019) observed a high correlation between TPC and antioxidant capacity ABTS, DPPH and reducing power in Ramon seeds.

E. ISSN: 2007-901X

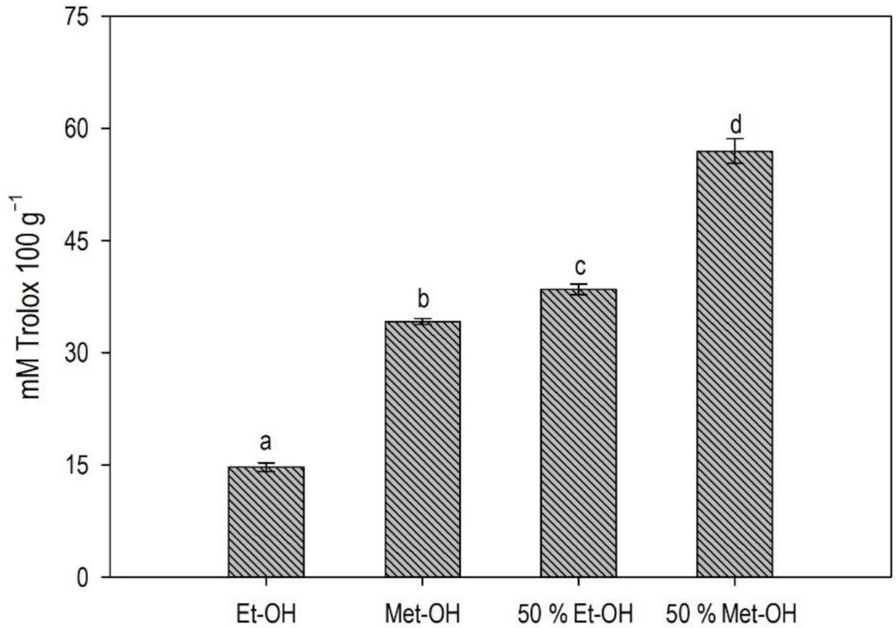

Figure 2. Effect of solvent-UAE on the DPPH antioxidant capacity of whole fruit of Ramon. Et-OH = pure ethanol; Met-OH= pure methanol; 50% Et-OH = 50% ethanol and 50% Met-OH= 50% methanol.

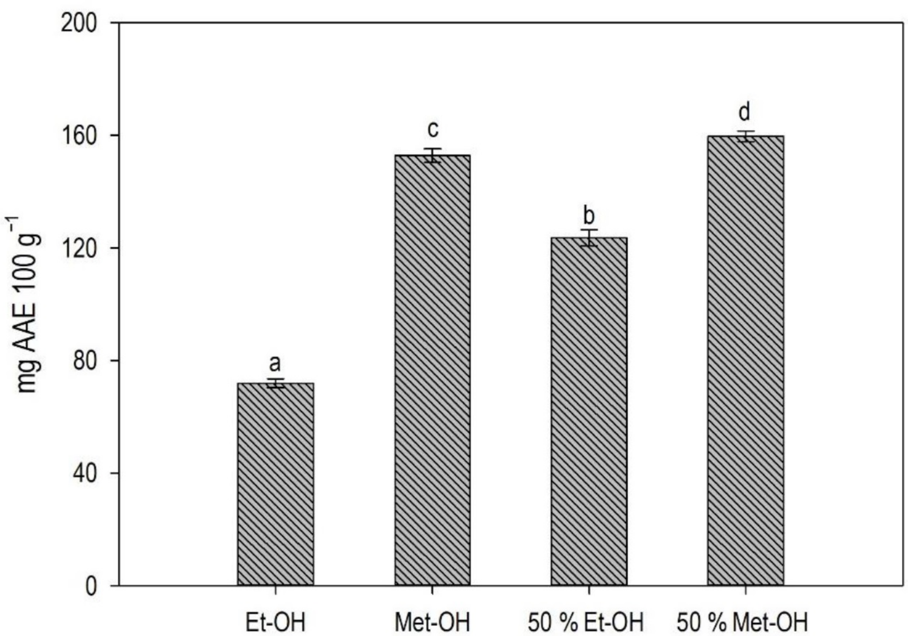

Figure 3. Effect of solvent-UAE on the antioxidant capacity RP of whole fruit of Ramon. AAE = ascorbic acid equivalent. RP = reducing power. Et-OH = pure ethanol; Met-OH = pure methanol; 50% Et-OH = 50% ethanol and 50% Met-OH = 50% methanol.

This research represents the first report of the UAE of polyphenols from the whole fruit (lyophilization) of the Ramon tree using different solvents (organic and aqueous). In this regard, it can be concluded that the type of solvent had a greater effect on the extraction of phenolic compounds from the whole fruit. The most effective solvent for extracting polyphenols from the whole fruit of Ramon was 50% Met-OH.

Table 1. Pearson's correlation between TPC and *in vitro* antioxidant capacity.

Assay	DPPH	RP
TPC	0.96*	0.81*

*Correlation was significant at the level of p $\leq$ 0.05. TPC = total phenolic compounds. RP = reducing power.

ACKNOWLEDGEMENTS

The first two authors would like to thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) for granting them a scholarship for their postgraduate studies.

LITERATURE CITED

Bibi Sadeer N, Montesano D, Albrizio S, Zengin G, Mahomoodally MF (2020) The versatility of antioxidant assays in food science and safety-Chemistry, applications, strengths, and limitations. Antioxidants 9: 709. DOI: 10.3390/antiox9080709.

Carrasco-Quiroz M, Alamo-Sanza Md, Martínez-Gil AM, Sánchez-Gómez R, Martínez-Martínez V, Nevares I (2023) Influence of Oxygen Management on Color and Phenolics of Red Wines. Molecules 28: 459. DOI: 10.3390/molecules28010459.

Carrillo-Lomelí DA, de Rodríguez DJ, Moo-Huchin VM, Ramón-Canul L, Rodríguez-García R, González-Morales S, Villarreal-Quintanilla JA, Peña-Ramos FM (2022) How does *Flourensia microphylla* extract affect polyphenolic composition, antioxidant capacity, and antifungal activity? Industrial Crops and Products 186: 115248. DOI: 10.1016/j.indcrop.2022.115248.

Hapsari S, Yohed I, Kristianita RA, Jadid N, Aparamarta HW, Gunawan S (2022) Phenolic and flavonoid compounds extraction from *Calophyllum inophyllum* leaves. Arabian Journal of Chemistry 15: 103666. DOI: 10.1016/j.arabjc.2021.103666.

Mikucka W, Zielinska M, Bulkowska K, Witonska I (2022) Recovery of polyphenols from distillery stillage by microwave-assisted, ultrasound-assisted and conventional solid-liquid extraction. Scientific Reports 12: 3232. DOI: 10.1038/s41598-022-07322-0.

Moo-Huchin V, Canto-Pinto J, Cuevas-Glory L, Sauri-Duch E, Pérez-Pacheco E, Betancur-Ancona D (2019) Effect of extraction solvent on the phenolic compounds content and antioxidant activity of Ramon nut (*Brosimum alicastrum*). Chemical Papers 73: 1647-1657.

Olvera-Aguirre G, Mendoza-Taco MM, Moo-Huchin VM, Lee-Rangel HA, Roque-Jiménez JA, Gómez-Vázquez A, Dzib-Cauich DA, Vargas-Bello-Pérez E, Chay-Canul AJ (2022) Effect of extraction type on bioactive compounds and antioxidant activity of *Moringa oleifera* Lam. leaves. Agriculture 12: 1462. DOI: 10.3390/agriculture12091462.

Ozer HK (2017) Phenolic compositions and antioxidant activities of Maya nut (*Brosimum alicastrum*): Comparison with commercial nuts. International Journal of Food Properties 20: 2772-2781.

Ramírez-Sánchez S, Ibáñez-Vázquez D, Gutiérrez-Peña M, Ortega-Fuentes MS, García-Ponce LL, Larqué-Saavedra A (2017) El ramón (*Brosimum alicastrum* Swartz) una alternativa para la seguridad alimentaria en México. Agro Productividad 10: 80-83.

Sarmiento-Franco L, Montfort-Grajales S, Sandoval-Castro C (2022) La semilla del árbol Ramón (*Brosimum alicastrum* Swartz): alternativa alimentaria energética para animales de producción y seres humanos. Bioagrociencias 15: 19-28.

E. ISSN: 2007-901X

Selvamuthukumaran M, Shi J (2017) Recent advances in extraction of antioxidants from plant by-products processing industries. Food Quality and Safety 1: 61-81.

Sik B, Ajtony Z, Lakatos E, Székelyhidi R (2022) The effects of extraction conditions on the antioxidant activities, total polyphenol and monomer anthocyanin contents of six edible fruits growing wild in Hungary. Heliyon 8: e12048. DOI: 10.1016/j.heliyon.2022.e12048.

Sosa-Zepeta O, Ticante-Pérez V (2022) Historia empresa OOX´BELIA. Revista Árbol Ramón-Óox 1: 20-21.

Taylor R (1990) Interpretation of the correlation coefficient: a basic review. Journal of diagnostic medical sonography 6: 35-39.