Ultrasound-assisted extraction (UAE) of phenolic compounds from different *Brosimum alicastrum* tissues

Extracción asistida por ultrasonidos (EAU) de compuestos fenólicos de diferentes tejidos de *Brosimum alicastrum*

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ABSTRACT. The leaves and fruit of *Brosimum alicastrum*, a neotropical tree, contain many phenolic compounds, although it is still unclear which extraction techniques and protocols are most efficient. We analyzed the effect of solvent-to-tissue ratio (STR) and lyophilized tissue type (LT) in ultrasound-assisted extraction (UAE) of *B. alicastrum* leaf and seed elements on total phenolic compounds content (TPC) and extraction yield (EY). Phenolic compounds from lyophilized fruit peel, seed skin, seed and leaf were extracted with ethanol-distilled water (1:1, v/v) in an ultrasonic bath at three STRs (20, 40 and 60 mL g^{-1}) for 30 min, at 30 ℃ and 100% amplitude. The two factors (LT and STR) and their interaction affected UAE results. The highest STR value (60 mL g^{-1}) produced the highest TPC, while seed tissue had the highest TPC and fruit peel tissue the highest EY. Ultrasound-assisted extraction effectively extracts phenolic compounds from *B. alicastrum* using the evaluated conditions.
Key words: Antioxidants, bioactive extracts, fruit, by-product.

RESUMEN. Se evaluó el efecto de la proporción disolvente-tejido (PDT) y el tipo de tejido liofilizado (TL) en EAU sobre el contenido de compuestos fenólicos totales (CFT) y el rendimiento de extracción (RE) de *B. alicastrum*. Los compuestos fenólicos de la cáscara, piel de semilla, semilla y hoja liofilizado fueron extraídos con etanol-agua destilada (1:1, v/v) en un baño de ultrasonidos variando PDT entre 20 y 60 mL g^{-1} a 30 min, 30 ℃ y 100% de amplitud. Las variables de respuesta evaluadas fueron: CFT y RE. Los efectos principales (TL y PDT) y su interacción mostraron una influencia significativa en EAU de polifenoles. A mayor PDT se obtuvo el mayor contenido de CFT. Al comparar TL, la semilla y la cáscara del fruto exhibieron mayor valor de CFT y RE, respectivamente. A un valor PDT de 60 mL g^{-1} mejoró la EAU de compuestos fenólicos de los tejidos analizados.
Palabras clave: Antioxidantes, extractos bioactivos, fruto, subproducto.

INTRODUCTION

Brosimum alicastrum, commonly known as breadnut, ramon or Maya nut, is a tree distributed throughout the neotropics. Humans have found multiple uses for its vegetal elements. On the Yucatan Peninsula in southeast Mexico, the Maya use its leaves and bark in traditional medicine, and the seed of the fruit was a food resource for the ancient Maya (Moo-Huchin *et al*. 2019, Brechú-Franco *et al*. 2021). Its leaves are used as fodder for sheep, pigs and rabbits (Sarmiento-Franco *et al*. 2022). Some *B. alicastrum* products are marketed nationally and internationally, such as gluten-free flour made from the seed, roasted and ground seed as a coffee substitute, and tea from leaves. The fruit shell and seed skin are inedible by-products generated during processing which have no known use. The seeds are known to contain vitamins (A, C and B), minerals (Ca, K, Fe, etc.), fatty acids (linoleic acid and palmitic acid), nitrogen-free extract (starch and sugars) and essential amino acids (tryptophan, lysine, arginine and valine) (Carter and Northcutt 2023).

Only limited research has been done on the phenolic compounds content and antioxidant activity of different *B. alicastrum* tissues using non-conventional extraction methods. In one study, phenolic compounds extraction of roasted and ground *B. alicastrum* seed with a Soxhlet extractor using an ethanol-distilled water mixture (80:20, v/v) at 80 °C for 30 min produced total phenolics content (TPC) (2 467.0 $\pm$ 85.0 mg GAE 100 g^{-1} extract) and DPPH radical scavenging activity (79. 0 $\pm$ 2.7%) higher than in walnut, peanut and almond (Ozer 2017). Another study compared extraction of unroasted *B. alicastrum* seed flour with an orbital shaker at 25 °C for 2 h using an ethanol-distilled water mixture (1:1, v/v) as solvent to other extraction solvents such as distilled water, methanol, ethanol, acetone, methanol-water (1:1, v/v) and acetone-water (1:1, v/v) (Moo-Huchin *et al*. 2019); the ethanol-distilled water mixture produced the highest TPC content (1 230.12 $\pm$ 10. 61 mg GAE 100 g^{-1} dry weight, dw) and DPPH antioxidant activity (872.75 $\pm$ 26.95 μM Trolox 100 g^{-1} dw). One report found optimal conditions for ultrasound-assisted extraction (UAE) of phenolic compounds from *B. alicastrum* leaf powder to be 28 °C, 80% amplitude, 10 to 20 min UAE, and 80% aqueous methanol as extraction solvent; the resulting TPC was 45.18 mg GAE g^{-1} and DPPH antioxidant activity was 67.27 μM Trolox g^{-1} (Gullian-Klanian and Terrats-Preciat 2017).

There is increasing interest in extracts rich in bioactive compounds with high antioxidant activity from *B. alicastrum* fruit and leaves. However, this requires a highly reproducible extraction method that provides high extraction yields in a short time, with low costs and easy development; an excellent option is UAE. The present study objective was to evaluate how the solvent-to-tissue ratio and the type of lyophilized tissue affects the efficiency of UAE in producing phenolic compounds from different tissues of *B. alicastrum* leaves and fruit.

MATERIALS AND METHODS

Plant tissue

Leaves and whole fruit of *B. alicastrum* were collected from trees located on the grounds of the Technological Institute of Merida (Instituto Tecnológico de Mérida) in Merida, Yucatan, Mexico (21° 00' N; 89° 37' W). Fruit was selected based on ripeness (orange skin color) and the absence of any physical damage. The fruit peel, seed, and seed covering were manually removed. All tissues were washed with tap water, cut into small pieces, separately placed in polyethylene bags and stored at -20 °C. Once frozen, each tissue was lyophilized (Edwards Modulyo EF4 1044) for 72 h. The lyophilized tissues were ground in a food processor (NutriBullet, Los Angeles, USA) at 40 s intervals to a No. 40 sieve particle size (0.420 mm). The powdered tissues were stored in sealable polyethylene bags at 4 °C until use (Moo-Huchin *et al*. 2019).

Extract preparation

Lyophilized plant tissue was homogenized in 10 mL aqueous ethanol (50% distilled water, v/v) at three different solvent-to-tissue ratios: 20, 40 and 60 mL g^{-1}. Extraction was done in an ultrasound bath (CScientific CS-UB100) for 30 min at 30 °C, 100%

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amplitude and 40 kHz frequency. After extraction, the mixtures were centrifuged at 4000 rpm for 10 min and the supernatant (antioxidants) removed and set aside. The resulting pellet was extracted again as above, and the supernatant removed. The supernatants from both extractions were combined and the total volume adjusted to 20 mL using the same extraction solvent. The extracts were stored at -20 ℃ in darkness until further use (Olvera-Aguirre *et al*. 2022).

Total Phenolic Compounds (TPC)

Measuring TPC in the *B. alicastrum* tissue extracts was done with the Folin-Ciocalteau (1 N) spectrophotometric method as described by Olvera-Aguirre *et al*. (2022). Wavelength and incubation were those indicated by Olvera-Aguirre *et al*. (2022), and the reference was aqueous ethanol (50% distilled water, v/v). Results were expressed as mg GAE g^{-1} dw, according to the following formula:

$$TPC(mgGAEg^{-1}) = \frac{[\frac{C}{1000}][V]}{W}$$

Where: C = mg GAE L^{-1} concentration based on calibration curve, V = total volume of extract (mL), and W = weight of the lyophilized sample (g).

Extraction yield (EY, %)

To quantify EY, a 5 mL aliquot of each extract was evaporated to dryness in a convection oven at 100 ℃ and EY calculated according to Olvera-Aguirre *et al*. (2022):

$$EY(\%) = \frac{dry\ extract\ weight\ (g)\ \times\ extract\ total\ volume(mL)}{powdered\ tissue\ weight\ (g)\ \times\ aliquot\ volume(mL)} \times 100$$

Statistical analysis

Three replicates were done of each experiment, and data were shown as the mean and standard error of means (SEM). A two-factor, 4 x 3 factorial design was used in which the first factor was plant tissue (shell, seed skin, seed and leaf) and the second factor was solvent-to-tissue ratio (20, 40 and 60 mL g^{-1}). Data were analyzed with a multifactorial analysis of variance (ANOVA), and a Tukey's test to identify significant differences ($p \leq 0.05$). All analyses were run with the Statgraphics Centurion XVI software (version XVI, Manugistic, Inc., Rockville, MD, USA).

RESULTS Y DISCUSSION

Both factors, lyophilized tissue type (LT) and solvent-to-tissue ratio (STR), and their interaction (LT x STR) influenced ($p \leq 0.05$) TPC and EY values (Table 1). The ethanol-water mixture (1:1 v/v) used here in the UAE of phenolic compounds was key to extraction results. Addition of distilled water in organic solvents (e.g., ethanol) generates a more polar medium, which facilitates extraction of phenolic compounds from plant matrices. In an ethanol-water system, ethanol breaks the hydrogen bonds and hydrophobic bonds existing between phenolic compounds and proteins, and phenolic compounds and cellulose (Kaderides *et al*. 2019). A 50% ethanol concentration (in distilled water) produces the highest extraction yields from plant tissues, making it the most appropriate for phenolic compounds extraction (Drevelegka and Goula 2020).

Average TPC values varied between the different *B. alicastrum* tissues (between 7.56 and 26.71 mg GAE g^{-1} dw), seeds having the highest and seed skin the lowest: seed, 26.71 mg GAE g^{-1}; leaf, 21.40 mg GAE g^{-1}; shell, 10.51 mg GAE g^{-1} and seed skin, 7.56 mg GAE g^{-1}. The seed TPC value was higher than the 11.61 $\pm$ 0.79 to 13.35 $\pm$ 0.80 mg GAE g^{-1} reported elsewhere for *B. alicastrum* seeds (Losoya-Sifuentes *et al*. 2023). Different TPC values have been reported for other *B. alicastrum* seed products. Dry extract of roasted seed has an average TPC content of 2 467 $\pm$ 85 mg GAE 100 g^{-1} (Ozer 2017), while seed flour has an average content of 1 230.12 $\pm$ 10.61 mg GAE 100 g^{-1} (Moo-Huchin *et al*. 2019). The present leaf TPC value (21.40 mg GAE g^{-1}) was higher than the 16.10 mg GAE g^{-1} previously reported (Montes-Pérez *et al*. 2019); a different study found a gallic acid content of 2.34 mg 100 g^{-1} in lyophilized leaf extract (González-González *et al*. 2019). Comparison of the present *B. alicastrum* fruit TPC values with those in the literature is challenging due to differences in tissue processing

Table 1. Total phenolics content and extraction yield from *B. alicastrum* tissues processed with ultrasound-assisted extraction, by solvent-to-tissue ratios and tissue type.

Variables	Lyophilized tissue type (LT)					Solvent-to-tissue ratio (v/LT, mL g^{-1}) (STR)				P-Value		
	Shell	Seed Skin	Seed	Leaf	SEM	20	40	60	SEM	LT	STR	LT x STR
*TPC (mg GAE** g^{-1} dw)	10.51^{b}	7.56^{a}	26.71^{d}	21.40^{c}	0.30	15.76^{a}	15.98^{a}	17.89^{b}	0.26	0.0000	0.0000	0.0000
EY (%)	38.09^{c}	10.06^{a}	11.92^{a}	19.94^{b}	0.68	20.52^{ab}	21.02^{b}	18.47^{a}	0.59	0.0000	0.0229	0.0001

Different lowercase letters in the same row within each factor indicate significant difference ($p \leq 0.05$). SEM = standard error of means. *TPC = Total phenolic compounds. EY = Extraction yield. **GAE = Gallic acid equivalent.

(roasting), extraction methods and results expression (dry extract weight, dry flour weight or extract volume).

Among the tissues analyzed here, the lyophilized shell had the highest EY (38.09%), while average EY for the leaf tissue was about two times higher than the seed and seed skin tissues (Table 1). This difference in EY values between tissues can be attributed to variation in morphology, structure and nutrient pool (glycoproteins and proteins) within *B. alicastrum* (Brechú-Franco *et al*. 2021). In the leaf tissue, EY (19.94%) was higher than the 10% reported elsewhere (Dzib-Guerra *et al*. 2016). Seed EY (11.92%) was comparable to the 11.0% previously reported by our team (Moo-Huchin *et al*. 2019), but higher than the 1 to 6% reported for Ficus religiosa seeds (Moraceae) (Pinipay *et al*. 2022).

The STR indicates the amount of solvent (mL) used in extraction of solute compounds (g). In the present results, the highest TPC value (17.89 mg GAE g^{-1} dw) and lowest EY value (18.47%) were produced at the 60 mL g^{-1} STR. Solvent viscosity and density are known to vary with increasing STR, which leads to higher EY values up to a certain point (maximum), after which they decrease as STR values increase (Kumar *et al*. 2021). The high extraction efficiency observed in the present results is probably due to the fact that a higher STR reduces the density of the ethanol-water mixture, increasing ultrasound wave propagation velocity, consequently raising energy transfer and improving phenolic compound extraction efficiency from plant matrices (Dzah *et al*. 2020). As solvent content increases in an extraction system, the effective ultrasonic intensity available for extraction reduces the number of extractables (Rao *et al*. 2021); this explains the lower EY at 60 mL g^{-1} (18.47%) relative to 40 mL g^{-1} (21.02%). No difference in EY was observed between 20 mL g^{-1} (20.52%) and 40 mL g^{-1} (21.02%). This coincides with a study stating that EY does not vary between 20 and 40 mL g^{-1} because certain compounds in the form of glycosides are highly soluble in a hydroalcoholic solution (Silva *et al*. 2007). This would explain the absence of variability in EY at high STR values since the number of extractables would remain the same.

The present study is the first time that TPC content and EY have been reported for *B. alicastrum* fruit by-products (shell and seed skin). Use of the 60 mL g^{-1} STR in UAE of the lyophilized *B. alicastrum* tissues (leaf, seed, shell and seed skin) produced the highest TPC content. Future research can focus on optimizing the UAE process to produce *B. alicastrum* extracts with high antioxidant activity.

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