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COMPARISON OF PROXIMATE COMPOSITION, PHYTOCHEMICAL CONTENTS, ANTIOXIDANT CAPACITY AND POLYPHENOLS IN *BROSIMUM ALICASTRUM* LEAF, FRUIT AND SEED

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ABSTRACT

Ramon nut (*B. alicastrum*) is known to contain bioactive compounds and has been used for medicinal and food purposes for millennia. In the present study the objectives were to quantify and compare the proximal composition, phytochemical contents, *in vitro* antioxidant capacity and individual phenolic compounds in *B. alicastrum* leaf, fruit peel, seed and seed coat. Patterns were recognized using canonical discriminant analysis. After freeze-drying, proximate analyses were done. Phenolic compounds in the leaf, fruit peel, seed and seed coat of *B. alicastrum* were identified by HPLC-UV. Leaf had the highest ash, lipid and protein contents, seed coat had high crude fiber content and seeds high carbohydrates content. The highest (33.13 %) and lowest (4.77 %) yields of extraction (compounds) were obtained from the fruit peels and seed coat, respectively. Phytochemical screening revealed abundant terpenes and tannins in leaf tissue, and abundant sterols in seed coat. Total phenolic content (TPC) was highest ($P \leq 0.05$) in seeds (19.74 mg gallic acid equivalent GAE/g) and total flavonoids content (TFC) was highest ($P \leq 0.05$) in leaves (16.62 mg quercetin equivalent QE/g). The highest DPPH antioxidant capacity was identified in seed coat and the highest reducing power (RP) in leaf and fruit peel. The HPLC analysis identified caffeic acid as the most abundant phenolic compound in leaves, gallic acid as the most abundant in seeds, chlorogenic acid in seed coat and 3-hydroxytyrosol in fruit peel. Linear discriminant analysis (LDA) effectively differentiated the samples by tissue. Proximal chemical composition and bioactive compounds contents differed between *B. alicastrum* tissues, but they all contain bioactive compounds with antioxidant potential, highlighting their potential applications in food and non-food systems.

Key words: antioxidants; phytochemicals; plant-extracts; Ramón.

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INTRODUCTION

Plants are a seemingly inexhaustible source of materials containing active molecular entities with potential uses in the food, pharmaceutical and cosmetic industries (Michalak, 2022). *B. alicastrum* (Moraceae), known by numerous names including Ramon nut and *Ramón*, is a native Mesoamerican tree. It is distributed widely in the humid tropical forests of Mexico, including the Yucatan Peninsula. Commonly used in the region prior to European contact, it is still considered a multipurpose tree because all of its elements have medicinal and/or food uses (Ortiz *et al.*, 1995;García *et al.*, 2012). Ancient cultures such as the Maya used the seed as food, particularly when staples such as corn were insufficient to meet demand (Acuña-Gutiérrez *et al.*, 2019). One of its main applications in traditional medicine is use of the latex and leaves to prepare antitussive and diaphoretic infusions, or as remedies for asthma, bronchitis and tuberculosis, among other conditions; the bark is used in tonics to treat chest pain and asthma (Berg, 1972;Ortiz *et al.*, 1995). Cultivation and consumption of *B. alicastrum* have increased in recent years as its renown as a source of antioxidants and nutrients has spread.

Free radicals are known to cause oxidative damage in biomolecules such as proteins, lipids and nucleic acids, generating numerous cardiovascular diseases and cancer. Maintaining free radicals and antioxidants in balance within the body is therefore vital to remaining healthy (Parcheta *et al.*, 2021). Natural antioxidants from plants have been receiving increasing attention as ingredients in functional foods and as dietary supplements with potentially positive effects in human health (Parcheta *et al.*, 2021).

B. alicastrum has been cursorily researched, with most studies focusing on the seed as an unconventional addition to corn and wheat tortillas, bread, and hot drinks such as *atole* (a corn-based beverage) (Domínguez-Zárate *et al.*, 2019;Subiria-Cueto *et al.*, 2019;Moo-Huchin *et al.*, 2021;Rodríguez-Tadeo *et al.*, 2021). Indeed, some *B. alicastrum* seed products are commercially available (e.g. Maya Superfoods, Productos Ecológicos Vida Vida, S.C. de C.V. de R.L.). Other plant elements, such as the fruit peel and seed coat are commonly treated as by-products and have not been evaluated.

Initial studies characterizing the seeds of *B. alicastrum* have been carried out. Cáceres *et al.* (2012) identified coumarins and anthraquinones in dehydrated *B. alicastrum* fruit, reporting a TPC of 17.28 ± 0.66 µg GAE/mg dry extract with 20 % iron reduction in 315.26 ± 1.10 µg dry extract.

Roasted and ground *B. alicastrum* seeds are reported to have a higher TPC content and antioxidant capacity than commercial nuts such as walnuts, almonds, and peanuts (Ozer, 2017). In a comparison of different extraction solvents with unroasted, ground seeds, an ethanol-water (1:1, v/v) extraction produced the highest gallic acid, chlorogenic acid and vanillic acid contents, as well as the highest antioxidant capacity (Moo-Huchin *et al.*, 2019). Quantification of antioxidant capacity in *B. alicastrum* leaf found it to have a DPPH EC50 value >300 µg/mL (Dzib-Guerra *et al.*, 2016). In another study, leaf powder was reported to have a 45.18 mg GAE/g TPC content and a 67.27 µmol Trolox/g DPPH antioxidant capacity (Gullian-Klanian and Terrats-Preciat, 2017).

Seed and leaf of *B. alicastrum* clearly have multiple uses in traditional medicine and food, and some are known to contain antioxidants. However, little data is currently available on the phytochemical and nutrient contents of its different tissues. The objectives of the present study were to quantify and compare the proximal chemical composition, phytochemical content, *in vitro* antioxidant capacity, and individual phenolics compounds in *B. alicastrum* leaf, fruit peel, seed and seed coat.

MATERIALS AND METHODS

Vegetal material. This work was carried out in the Food Development Laboratory of the Graduate and Research Department of the Technological Institute of Merida, Yucatan. In August 2021, leaves and fruit (orange peel color) were randomly collected from fifteen *B. alicastrum* trees in the gardens of the Merida Technological Institute (Instituto Tecnológico de Mérida –ITM) in the state of Yucatan, Mexico (21°00'44.8"N 89°37'23.5"W, at an elevation of 11 m a.s.l). Voucher specimen (JBR-99.111) are deposited in the herbarium of the Centro de Investigación Científica de Yucatán, A. C (CICY), in Yucatan, Mexico. The vegetal material (Figure 1) was washed in tap water to remove any particles, stored separately at −20 °C and freeze-dried (Labconco FreeZone 4.5 Freeze dryer Labconco, Kansas City, MO). The different elements were then ground separately (NutriBullet® 900 W, NutriBullet, Los Angeles, CA, USA) to produce a powder. This was screened through No. 40 mesh and the resulting fine powder placed in sealed polyethylene bags and stored at −20 °C until use.

Proximate chemical composition. The freeze-dried tissues were analyzed to quantify moisture (%), ash (%), protein (%), fiber (%) and lipids (%) content following established methods (AOAC, 1997). Moisture content was measured gravimetrically after drying in a convection oven (Felisa FE-291, México) at 105 °C. Ash was quantified by incineration in a muffle furnace (Felisa FE-340, México) at 550 °C. Crude protein content (N x 6.25) was calculated with the micro-Kjeldahl method. Crude fiber was estimated by acid/alkaline hydrolysis of insoluble residues. Lipids were measured gravimetrically after Soxhlet extraction. Carbohydrate content (%) was estimated by difference, using the levels of other nutrients (Can-Cauich *et al.*, 2021).

Extract preparation. Extraction of phytochemical compounds from the tissues was done by ultrasonic assisted extraction (UAE). The lyophilized samples (10 g) were placed in 100 mL of an ethanol:water mixture (1:1, v/v) in an ultrasonic bath (CScientific CS-UB100) for 30 min at 30 °C and 100 % power amplitude (Carrillo-Lomelí *et al.*, 2022). The resulting crude extracts were centrifuged (Eppendorf 5702-R, Germany) at 3,500 rpm for 10 min at 20 °C and the supernatant recovered. The pellet was run through a second extraction cycle, as above. The supernatants of both extractions were combined and final volume completed to 200 mL using the same extraction solvent. The extract was stored at –20 °C until analysis.

A 10 mL aliquot of each tissue extract was evaporated until dry in a vacuum rotary evaporator and extraction yield calculated using the formula:

$$\text{Extraction yield (\%)} = \frac{(\text{Dry extract weight in g})(\text{total extract volume in mL})}{(\text{Lyophilized tissue weight in g})(\text{evaporated aliquot in mL})} \times 100$$

Phytochemical analysis. Simple, rapid and selective chemical tests were run to qualitatively identify the presence of phytochemicals (tannins, terpenes, sterols, anthraquinones, coumarins, saponins, alkaloids, leucoanthocyanidins, cardiotonic glycosides, reducing sugars, and free amino acids) (Valencia *et al*., 2020;Akpor *et al*., 2021). Phytochemical presence was graded in four levels (García-Granados *et al*., 2019): +++ abundant presence; ++ moderate presence, + minimal presence and – absence.

TPC and TFC quantification. Total phenols and total flavonoids contents (TPC and TFC, respectively) were measured with the colorimetric method, using the Folin-Ciocalteu assay for TPC and aluminum chloride for TFC (Moo-Huchin *et al*., 2014). Absorbance was measured at 765 nm (TPC) and 415 nm (TFC) using a UV-Vis spectrophotometer (Agilent Cary 60, Australia). The TPC and TFC of *B. alicastrum* tissues were calculated from the calibration curve regression equation using the standards gallic acid (TPC) and quercetin (TFC). Mean values were reported as mg gallic acid equivalents (GAE)/g dry weight (DW) for TPC and mg quercetin equivalents (QE)/g DW for TFC.

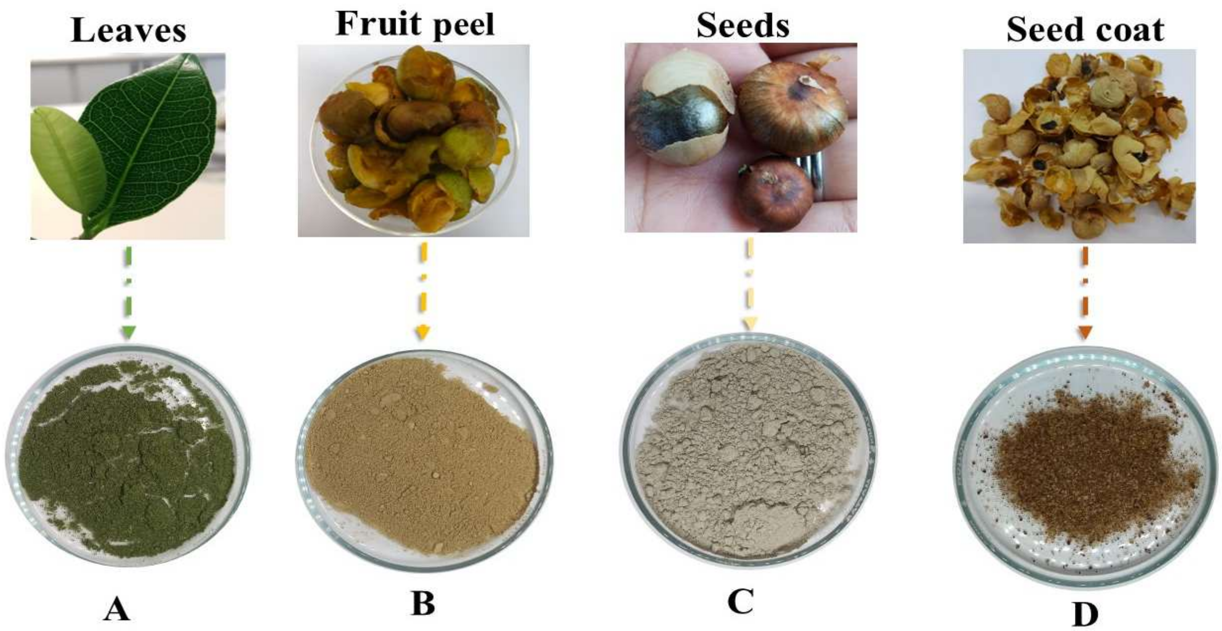

Figure 1. ***B. alicastrum*** **tissues used in analyses: A) Leaves; B) Fruit peel; C) Seeds and D) Seed coat.**

In vitro **antioxidant capacity.** Tissue antioxidant capacity was quantified with the DPPH (2,2-Diphenyl-1-picryl-hydrazyl) and reducing power (RP) assay (Can-Cauich *et al*., 2017). After the stipulated reaction time, sample absorbance was measured at 515 nm for DPPH and 700 nm for RP using a UV-Vis spectrophotometer (Agilent Cary 60, Australia). Trolox and ascorbic acid were used as standards for the calibration curve. Results were expressed as mM Trolox/100 g DW for DPPH and mg ascorbic acid AA/100 g DW for RP.

Phenolic analysis by high performance liquid chromatography (HPLC). The analysis of phenolic compounds of the studied leaf and fruit parts was carried out on a chromatographer (Thermo Scientific Dionex Ultimate 3000 HPLC) coupled with a UV-Vis multiwavelength detector using established column, mobile phase, elution gradient and flow rate characteristics (Carrillo-Lomelí *et al*., 2022). Phenolic compounds were quantitatively analyzed (at 280 nm) through preparation of the calibration curve of each authentic standard dissolved in HPLC grade methanol plus HPLC water (1:1, v/v). Each tissue's dry extract (20 mg) was dissolved in 3.0 mL of the same solvent used for the authentic standards. These were filtered through a polyvinylidene fluoride Millipore (PVDF, 0.45 µm Millex-HV) and 20 µL used for injection. Polyphenols in the samples were identified by comparing retention times with the standards, and peak areas were used to calculate concentrations using the Dionex Chromeleon® software. Average values were expressed as mg/100 g DW.

Statistical Analysis. Data were analyzed with one-way analysis of variance (ANOVA) under completely randomized design (CRD) with three replicates per tissue extract. Tukey test was applied to compare means between the tissues. Statistical significance was set at $P \leq 0.05$. All measurements were done in triplicate and results expressed as the mean ± standard deviation (SD). Analyses were done with the Statgraphics® Centurion XVI software.

RESULTS AND DISCUSSION

Proximate chemical composition. The chemical composition of plant tissues allows assessment of their nutritional potential. Composition differed (P≤0.05) between the lyophilized *B. alicastrum* tissues (Table 1). Average tissue moisture content ranged from 2.65 to 12.42 %, an adequate range for storage of powdered herbal materials (Razak *et al.*, 2020). Total ash content was highest in the leaves (14.43 %), followed by seed coat (6.79 %), fruit peel (6.12 %) and seeds (3.44 %). The leaves are a good source of minerals and provide nutritional value (Mgbemena *et al.*, 2022).

Lipids content was highest in the leaves (2.69 %), followed by fruit peel (1.75 %), seed coat (1.43 %) and seeds (1.16 %). Crude protein values ranged from 11.36 to 15.43 %, with leaves having the highest content. Seed coat had the highest crude fiber content (28.01 %) and seeds the highest carbohydrates content (72.40 %). Composition of the freeze-dried leaf and seeds is similar to previous reports (Subiria-Cueto *et al.*, 2019;Dzib Cauich *et al.*, 2021), but no data has been published on *B. alicastrum* seed coat and fruit peel composition. Seed protein (12.96±0.25 %) and lipids (1.16±0.08 %) contents were higher than previously reported for *B. alicastrum* seed meal (proteins, 11.74 ± 0.02 %; lipids, 0.58 ± 0.07 %) (Carter and Northcutt, 2023).The nutrient content of the analyzed *B. alicastrum* tissues is very similar to that of other powdered fruit and vegetable tissues (e.g. mango kernel, mango peel, pumpkin, mushroom, grape seed and pomegranate peel) currently in use as food fortifiers (Salehi, 2020), highlighting their potential use in food systems.

Table 1. Proximate chemical composition of *B. alicastrum* leaves, fruit peel, seeds and seed coat.

Tissues	Moisture (%)	Ash (%)	Lipids (%)	Crude protein (%)	Crude fiber (%)	Carbohydrates (%)
Leaves	2.65±0.05^{a}	14.43±0.51^{d}	2.69±0.05^{d}	15.43±0.50^{c}	13.93±0.91^{c}	50.5±2.01^{b}
Fruit peel	12.42±0.94^{d}	6.12±0.06^{b}	1.75±0.98^{c}	12.6±0.00^{b}	9.75±0.63^{b}	57.27±0.76^{c}
Seeds	4.86±0.09^{b}	3.44±0.00^{a}	1.16±0.08^{a}	12.96±0.25^{b}	4.83±0.05^{a}	72.40±0.40^{d}
Seed coat	9.91±0.05^{c}	6.79±0.04^{c}	1.43±0.00^{b}	11.36±0.75^{a}	28.01±0.06^{d}	42.47±0.94^{a}

Values are the mean ± SD. Different letter superscripts in the same column indicate significant difference (P≤0.05).

Extraction yield and phytochemical profile. Compound extraction is a key process in manufacturing herbal products, and affects both the quality and quantity of the bioactive ingredients extracted from plant tissues (Jha and Sit, 2021). Extraction yields varied (P≤0.05) between the evaluated tissues (Figure 2), with fruit peel having the highest (33.13 %) and seed coat the lowest (4.77 %). This variation may be related to the inherent properties of each tissue (morphology, structure and nutrient pool), which could affect solvent access to cellular components (Jha and Sit, 2021). In other words, higher yields indicate that a plant tissue's chemical compounds are highly soluble in the ethanol-water mixture (1:1, v/v), which is supported by the different chemical compositions of evaluated plant elements (Table 1).

The yields obtained in the present study for leaves and seeds are higher than in previous reports. In a study using ethanol:water (1:1 v/v) as a solvent, the extraction yield of powdered *B. alicastrum* was 11 % (Moo-Huchin *et al.*, 2019), lower than the 14.87 % observed in the present results. In a study using ethanol as solvent, yield from *B. alicastrum* leaves was 10 % (Dzib-Guerra *et al.*, 2016), almost half the 19.5 % observed in the present study. These differences can be attributed to the different extraction methods employed (e.g. maceration or an orbital shaker). Compared to conventional methods, the UAE method with ultrasonic bath used in the present study has advantages such as higher extraction yield and high reproducibility (Carrillo-Lomelí *et al.*, 2022).

Identifying the phytochemicals in a plant tissue is fundamental to assessing its biological properties. The *B. alicastrum* tissues analyzed here contained terpenes, sterols, tannins, coumarins, free amino acids and reducing sugars (Table 2). Leaf tissue had abundant terpenes content, followed by seed coat (moderate presence) and fruit peel (minimum presence), and seeds (absence). Unlike the other tissues, the seed coat had abundant sterols content. Tannins were abundant in leaf tissue and moderate in seed coat tissue. Coumarins were minimally present in fruit peel and seed coat. All the evaluated tissues had abundant free amino acids, while reducing sugar was moderately present in fruit peel and minimally present in leaf tissue. None of the evaluated tissues contained saponins, anthraquinones, alkaloids, cardiotonic glycosides or leucoanthocyanidins.

Previous screening of an ethanol extract of dried *B. alicastrum* fruit identified the presence of chemical compounds such as coumarins, anthraquinones, flavonoids and tannins (Cáceres *et al.*, 2012). A study of a 70 % hydroalcoholic extract of fruit from *Brosimum gaudichaudii* (same genus as *B. alicastrum*) identified abundant or moderate flavonoids, steroids, saponins and cardiotonic glycosides (de Menezes Filho *et al.*, 2021).

The phytochemicals found in *B. alicastrum* tissues are groups of secondary metabolites with different mechanisms of action that contribute substantially to biological activity (e.g., hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, and anticancer, among others) in medicinal plants (Tran *et al.*, 2020).

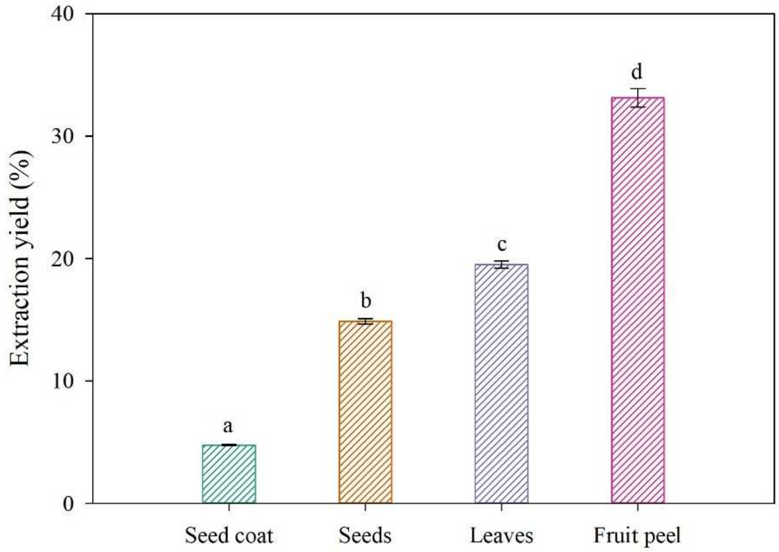

Figure 2. Extraction yield (%) from *B. alicastrum* leaves, fruit peel, seeds and seed coat using UAE-50% ethanol. Data are expressed as the mean ± SD of three replicates. Different letters above the bars indicate significant difference (P≤0.05).

Table 2. Phytochemical profile of hydroalcoholic extracts of *B. alicastrum* leaves, fruit peel, seeds and seed coat.

		Tissue			
Test	**Phytochemicals**	**Fruit peel**	**Seed coat**	**Leaves**	**Seeds**
Liebermann-Burchard	Terpenes	+	++	+++	-
Salkowski	Sterols	+	+++	-	+
Foam	Saponins	-	-	-	-
Iron chloride	Tannins	-	++	+++	-
Borntrager	Anthraquinones	-	-	-	-
Sodium hydroxide	Coumarins	+	+	-	-
Wagner Reaction	Alkaloids	-	-	-	-
Mayer Reaction	Alkaloids	-	-	-	-
Keller-Kiliani	Cardiotonic glucosides	-	-	-	-
Ninhydrin	Free amino acids	+++	+++	+++	+++
Fehling	Reducing sugars	++	-	+	-
Rosenheim	Leucoanthocyanidins	-	-	-	-

+++ = abundant amounts; ++ = moderate amounts; + = minimum amounts; – = absence.

TPC, TFC and antioxidant capacity. Both TPC and TFC values differed (P≤0.05) between the evaluated *B. alicastrum* tissues (Figure 3), and trended differently in each tissue. The highest TPC was in the seeds (19.74 mg GAE/g), followed by leaves (15.54 mg GAE/g), fruit peel (11.47 mg GAE/g) and seed coat (7.19 mg GAE/g). For TFC, the highest value was for leaves (16.62 mg QE/g), and the lowest for seeds (2.98 mg QE/g) and seed coat (2.83 mg QE/g). Comparison of these TPC and TFC values with the limited previous research is challenging since reported data is largely for the dry extract or dry powder of roasted seeds. One comparable study reported a TPC of 12.30 mg GAE/g and a TFC of 1.61 mg QE/g for raw *B. alicastrum* seed meal (Moo-Huchin *et al.*, 2019). These values are notably lower than in the present study and this difference can be attributed to the different extraction methods used in the two studies. Seed TPC was higher than reported elsewhere: 11.61 to 13.35 mg GAE/g dry weight (Losoya-Sifuentes *et al.*, 2023), and 1.54 mg GAE/g dry weight (Trujillo-Nava *et al.*, 2023).

Solvent type and polarity strongly influence phenolic acids and flavonoids extraction (Moo-Huchin *et al.*, 2019). Methanol and ethanol are commonly used to extract these compounds from plant tissue, but no single solvent has the ability to extract all the phenolic compounds found in tissues. This is why alcohol/water mixtures like that used in the present study are recommended to improve extraction.

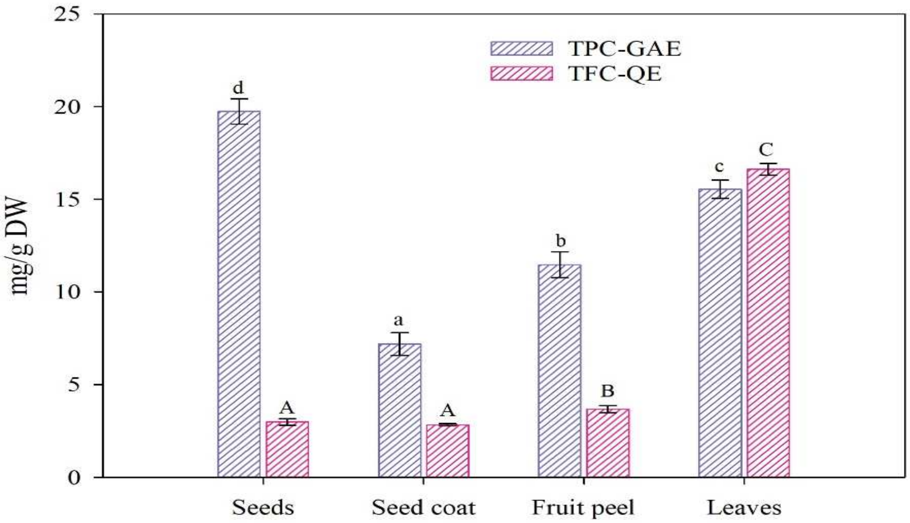

Figure 3. Total phenolics (TPC) and total flavonoids contents (TFC) extracted from *B. alicastrum* leaves, fruit peel, seeds and seed coat. Data are the mean ± SD of three replicates. Different letters above columns of the same color indicate significant difference (P≤0.05).

Antioxidant capacity (DPPH and RP) also differed (P≤0.05) between tissue types (Figure 4). The highest DPPH value (expressed in mM trolox/100 g DW) was in seed coat (47.88), followed by leaves (42.05), fruit peel (33.69) and seeds (16.7); that is, the bioactive compounds in the seed coat have a greater capacity to donate a hydrogen ion to convert DPPH free radicals into DPPH-H. This is notable because the seed coat did not have higher TPC and TFC contents than the other tissues, but its abundant to moderate terpenes, sterols, tannins and free amino acids contents contributed to its high DPPH. Seed DPPH values were higher than the 0.204 to 1.68 mM Trolox/100 g DW reported elsewhere for *B. alicastrum* seed meal (Losoya-Sifuentes *et al.*, 2023). Both the leaves and seed coat had values higher than the 38.51 mM Trolox/100 g DW reported for freeze-dried *B. alicastrum* fruit in a study using 50 % ethanol as extraction solvent (Aguilar-Piloto *et al.*, 2023).

RP was statistically highest in the leaves (181.22 mg AA/100 g), followed by the fruit peel (172.60 mg AA/100 g), seed coat (149.58 mg AA/100 g) and seeds (87.95 mg AA /100 g). This means that the bioactive compounds in the leaves and fruit peel had the highest reduction capacity from the ferric to the ferrous form. The differences observed in antioxidant capacity between tissues can be attributed to the presence of secondary metabolites (Table 2), and high variation in TPC, TFC and individual phenolic compounds (Figure 3 and Table 3, respectively). The RP values for leaves, fruit peel and seed coat were higher than the 123.59 mg AA/100 g reported for freeze-dried *B. alicastrum* fruit (Aguilar-Piloto *et al.*, 2023); however, seed RP in the present results was lower than in this study. Overall, the RP values for the studied tissues were lower than the 348.21 mg AA/100 g DW average reported for *B. alicastrum* seeds extracted with 50 % ethanol (Moo-Huchin *et al.*, 2019).

A study of meal made from toasted *B. alicastrum* seeds reported TPC and antioxidant values higher than in commercial nuts (Ozer, 2017). These and the present results confirm the antioxidant potential of *B. alicastrum* tissues.

HPLC analysis. Common in plants, phenolic compounds are secondary metabolites which can function as bioactive ingredients to prevent health conditions related to oxidative damage (Torres-Martínez *et al.*, 2021). HPLC was used to determine the phenolic compound profiles of *B. alicastrum* tissues.

In the present results, the most abundant phenolics were caffeic acid (50.18 mg/100 g) in leaves, gallic acid (10.72 mg/100 g) in seeds, chlorogenic acid (26.14 mg/100 g) in seed coat and 3-hydroxytyrosol (28.94 mg/ 100 g) in fruit peel (Table 3). These widely studied phenolics have proven high antioxidant activity since they inhibit free radical production, thus preventing oxidative damage to cells. 3-Hydroxytyrosol has also been associated with anti-inflammatory, antiproliferative and antifungal activity (Pasten *et al.*, 2019). 3-Hydroxytyrosol and vanillic acid were identified in the leaves, seeds and fruit peel, while gallic acid was found in the seeds, seed coat and fruit peel. Seed coat had 3.4 times more chlorogenic acid content than fruit peel, while leaves had 4.2 times more 4-hydroxybenzoic acid than seeds. Epicatechin was identified in seed coat and p-coumaric acid in leaves. In the leaves, caffeic acid levels were higher (P≤0.05) than in fruit peel and ellagic acid contents were higher than in seed coat. Seeds had a higher ferulic acid content than seed coat. Sinapic acid, 4-hydroxyphenethyl alcohol and *trans*-cinnamic acid were only identified in seeds. Most of the phenolic compounds identified in the present study have been reported in raw seed powder (Moo-Huchin *et al.*, 2019) and roasted seed powder (Ozer, 2017). Synergistic interactions between the bioactive compounds in *B. alicastrum* are highly probable and may contribute to its antioxidant capacity (Hye-Jung *et al.*, 2019).

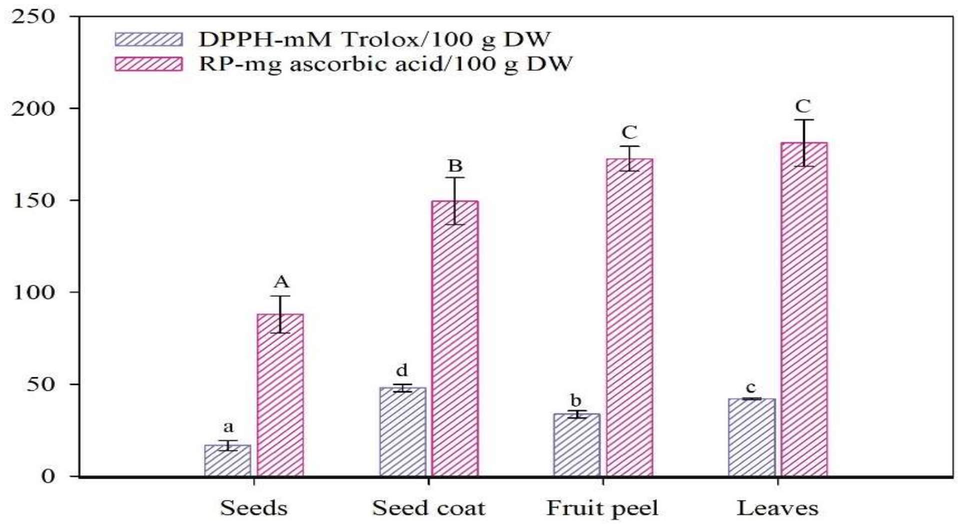

Figure 4. Antioxidant capacity (DPPH) and reducing power (RP) of *B. alicastrum* leaves, fruit peel, seeds and seed coat. Data are the mean ± SD of three replicates. Different letters above columns of the same color indicate significant difference (P≤0.05).

Discrimination analysis. The most important variables using stepwise discriminant analysis were extraction yield, and gallic acid, chlorogenic acid, 3-hydroxytyrosol, 4-hydroxybenzoic acid and caffeic acid contents. These variables effectively separated values between the different *B. alicastrum* tissues at significant levels (P≤0.05). Functions F1 and F2 were estimated and evaluated using the Wilks **λ** statistic (3.35×10^{-13} for F1 and 1.73×10^{-8} for F2) and the Chi-square test (χ^2) (517.02 for F1 and 321.71 for F2); both were statistically significant (P≤0.05) (Table 4). Function 1 explained 70.53 % of total variability and F2 25.17 %; together they explained 95.7 % of variance. The canonical correlation coefficients were high (0.999) for both functions, with low Wilks' **λ** values, which explains why the group's discriminant scores differ. Differences between the cluster centroids were highly significant (P=0.0000).

Table 3. Individual phenolic compound content in *B. alicastrum* leaves, fruit peel, seeds and seed coat.

Phenolic Compound (mg/100 g DW)	Tissues			
	Leaves	Seeds	Seed coat	Fruit peel
Chlorogenic acid	nd	Nd	26.14 ± 0.02^{b}	7.5 ± 0.42^{a}
Gallic acid	nd	10.72 ± 0.24^{b}	0.35 ± 0.04^{a}	0.79 ± 0.03^{a}
3-Hydroxytyrosol	0.51 ± 0.00^{a}	7.93 ± 0.00^{b}	nd	28.94 ± 0.35^{c}
4-Hydroxybenzoic acid	3.32 ± 0.00^{b}	0.79 ± 0.00^{a}	nd	nd
Vanillic acid	9.8 ± 0.00^{c}	1.31 ± 0.00^{a}	nd	1.70 ± 0.06^{b}
Caffeic acid	50.18 ± 0.38^{b}	Nd	nd	1.29 ± 0.00^{a}
Ellagic acid	3.02 ± 0.78^{b}	Nd	0.65 ± 0.00^{a}	nd
Ferulic acid	nd	0.7 ± 0.00^{b}	0.13 ± 0.00^{a}	nd
Epicatechin	nd	Nd	0.42±0.03	nd
4-Hydroxyphenethyl alcohol	nd	2.92±0.14	nd	nd
p-Coumaric acid	0.83±0.07	Nd	nd	nd
Synapic acid	nd	0.15±0.00	nd	nd
p-Coumaric acid	0.83±0.07	Nd	nd	nd

Values are mean ± SD. Different superscript letters in the same row indicate significant difference (P≤0.05) in a Tukey test. nd: not detected.

Table 4. Discriminant canonical functions for the studied *B. alicastrum* tissues.

Function	Eigenvalues	Relative percentage	Canonical Correlation	Wilks λ	χ^2	p-value
1	51554.3	70.53	0.999	3.35×10^{-13}	517.02	0.0000
2	18393.7	25.17	0.999	1.73×10^{-8}	321.71	0.0000

Of the two linear Fisher discriminant functions, F1 represented the best linear discriminant combination since it explained most of the total differentiation between the evaluated tissues (Table 5). Based on the standardized discriminant function coefficients, chlorogenic acid was the most significant component for group differentiation in F1, while caffeic acid and 4-hydroxybenzoic acid were the most significant in F2.

Cross-validation using the discriminant linear function correctly (100 %) classified and assigned the samples to their respective tissue groups (Figure 5).

Table 5. Standardized canonical coefficients for the tissue discriminant functions.

Variable	Function 1	Function 2
Yield	0.88699	-0.72852
Gallic acid	0.16938	-0.03059
Chlorogenic acid	-1.52089	0.34216
3-Hydroxytyrosol	1.23041	-0.29272
4-Hydroxybenzoic acid	0.12803	1.71418
Caffeic acid	0.55200	1.68201

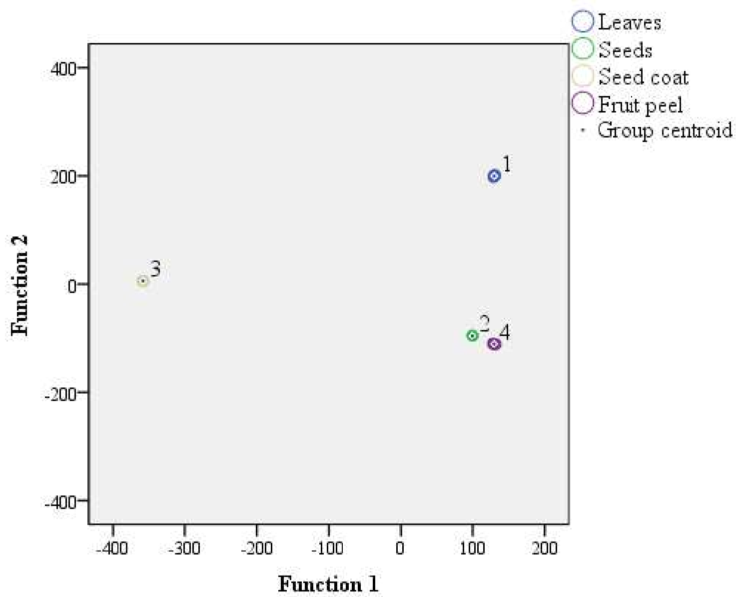

Figure 5. Canonical representation of *B. alicastrum* leaf, fruit peel, seed and seed coat tissues.

Conclusion. The studied *B. alicastrum* tissues (leaf, fruit peel, seed and seed coat) are rich in protein, fiber and carbohydrates with potential applications in formulating foods for human consumption. The present findings highlight their bioactive and antioxidant potential; in this sense, the leaves are the most promising tissue. This is the first report of *B. alicastrum* seed coat and fruit peel as important sources of natural antioxidants. The identified phenolic compounds are bioactive, with health protective functions when consumed in a normal diet. Therefore, *B. alicastrum* leaves (high caffeic acid content), seeds (high gallic acid content), seed coat (high chlorogenic acid content), and fruit peel (high 3-hydroxytyrosol content) could be promising ingredients in plant-based diets. The present results are a useful characterization and comparison of bioactive compounds in *B. alicastrum* tissues, as well as a valuable contribution in the search for natural tissues with potential antioxidant applications in food and non-food systems. These promising findings will contribute to adding market value to *B. alicastrum* products in various industries.

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