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Total phenolic, monomeric anthocyanin content and antioxidant activity of *Berberis commutata* Eichler fruits

Contenido fenólico total, antocianinas monoméricas y actividad antioxidante de los frutos de *Berberis commutata* Eichler

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ABSTRACT

Background: *Berberis commutata* Eichler is a berry that grows in the Peruvian Andes and has been consumed in the Andes of South America since ancient times. The edible fruits have an intense purple color and are rich in anthocyanins and phenolic compounds that are available from February until May each year. The color of the fruits is a soft purple dye for natural fibers, and many birds use them as food. **Objective:** This study quantified the total phenolic, monomeric anthocyanin content and antioxidant activity of *Berberis commutata* Eichler berries. **Methods:** The total phenolic content was determined by the Folin-Ciocalteu colorimetric assay. Monomeric anthocyanin content was determined by the method is pH differential, and the antioxidant activity was measured using the Brand-Williams method. **Results:** The total phenolic content was 7,490 ± 0.85 mg GAE/100g, and the monomeric anthocyanin content was 70 ± 0.03 mg/100g. The antioxidant activity of the berries showed a tendency to increase with *B. commutata* extract concentration; an EC50 of 0.91 mg/mL was calculated, indicating a high antioxidant power. **Conclusion:** Our results showed that *B. commutata E.* has both high total phenolic content and monomeric anthocyanins comparable to other superfruits and high antioxidant activity, which means that it is possible to use this berberis species as a functional food.

Keywords: *Berberis commutata* Eichler, DPPH Antioxidant, EC50, Total phenolic content, monomeric anthocyanins

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RESUMEN

Introducción: *Berberis commutata Eichler* es una baya que crece en los Andes peruanos. Los frutos comestibles tienen un intenso color púrpura rico en antocianinas y componentes fenólicos que están disponibles desde febrero hasta mayo de cada año. El color de sus frutos se utiliza como un suave colorante púrpura para las fibras naturales y muchas aves los utilizan como alimento. Sin embargo, desde la antigüedad los frutos de esta especie han sido consumidas en los Andes de Sudamérica. **Objetivo:** Este estudio cuantificó el contenido fenólico total, antocianinas monoméricas y la actividad antioxidante usando el método del radical DPPH de las bayas de *Berberis commutata Eichler.* **Método:** El contenido fenólico total se midió a través del ensayo colorimétrico de Folin-Ciocalteu, el contenido de antocianinas monoméricas se determinó mediante el método del pH diferencial y la actividad antioxidante se midió con el método de Brand-Williams. **Resultados:** El contenido fenólico total fue de 7,490 ± 0.85 mg GAE/100g y el contenido de antocianinas monoméricas fue de 70 ± 0.03 mg/100g. La actividad antioxidante de las bayas mostró una tendencia a aumentar con la concentración del extracto de *B. commutata*, se calculó un EC50 de 0.91 mg/mL que indica un alto poder antioxidante. **Conclusión:** Nuestros resultados mostraron que *B. commutata E.* tiene tanto un alto contenido fenólico total, así como antocianinas monoméricas comparables con otras superfrutas y una elevada actividad antioxidante, lo que significa que es posible utilizar esta especie de berberis como alimento funcional.

Palabras clave: *Berberis commutata Eichler*, antioxidante DPPH, EC50, contenido fenólico total, antocianinas monoméricas

INTRODUCTION

The genus Berberidaceae includes about 500 species worldwide, and almost 99 species are distributed in South America, of which 32 are found in Perú and 14 are endemic (1). They consist of spiny deciduous, evergreen shrubs, with yellow flowers and wood (3 – 6 mm long), with both six petals and sepals (usually with the same color) in alternating whorls (2) and small red or blue ripening berries (5 – 15 mm) (3).

There is a growing interest and demand for plants, especially fruits that are healthy, delicious, and grow in an unpolluted environment, mainly wild and preserving their naturalness (4). Wild fruits are very suitable sources for the food industry (5) because of their nutritional and medicinal value; these wild edible fruits are becoming increasingly valuable for people who want to consume natural foods with pharmacological properties (6). Numerous studies have reported the Berberis genus as a rich source of phytochemical compounds with antioxidant properties (7); this is the case of *B. vulgaris* L., which shows significant antioxidant activity and health benefits (8,9). Some other studies have confirmed that they are a well-known source of bioactive compounds mainly due to the presence of phenolic and anthocyanins (10,11). For this reason, these plants represent a promising alternative for producing food derivatives such as desserts, wines, and jams (12).

Berberis commutata E. (*B. commutata* E.) is a wild berry that grows in the Mesoandean region, between 2,000 and 4,200 meters above sea level (MASL), especially in Perú. It is a spiny bush with sharp thorns, yellow flowers grouped in small bunches, and many dark purple berries 10 to 15 mm long and larger than other Berberis species berries. The berry's common names are huancachu, and t'ancar ch'eqche. The full-ripened fruits are available from February to May each year.

Wild edible berries have been consumed as nutritional supplements since ancient times (13) and are important sources of polyphenols (e.g., tannins, flavonoids, phenolic acids, and anthocyanins). Recent investigations on important nutraceutical polyphenolic compounds have attracted intense attention, as in the case of *Berberis vulgaris*, an important source of polyphenolic molecules (14).

The market for natural antioxidants is growing each year substantially due to their proven clinical benefits, which are effective against cardiovascular, cancer, neurodegenerative, and age-related oxidative problems (15-18).

Several studies have shown that phenolic compounds are responsible for the health benefits of consuming natural products. Anthocyanins are a subclass of phenolic phytochemicals. Many berries contain anthocyanins with potent antioxidant activity (19). The antioxidant activity of anthocyanins is well known, and the interest in vegetables with antioxidant properties has increased (20). Anthocyanins are bioactive components used as a nutraceutical and pharmaceutical ingredients. Anthocyanins extracted from plants have been used as food colorants; this is the case of E163, a commercial additive derived from grape skins, which is used as a food colorant in purple-colored jams and beverages. Synthetic food coloring has long been used for its lower cost and greater stability (21); however, synthetic food colorants have recently attracted public concern regarding their safety to human health. There is scientific evidence that synthetic colorants used as food additives may adversely affect children's health. For example, removing synthetic food dyes from the diets of children with attention-deficit/hyperactivity disorder could provide significant benefits (22). These findings have increased the search for natural food colorants, such as anthocyanin, as an interesting and potential alternative to synthetic food dyes (19).

In this study, our objectives were to quantify the total phenolic content and monomeric anthocyanins and to determine the antioxidant activity in the fruits of *B. commutata* E because we want to promote its use as a source of bioactive substances such as polyphenols, which have a positive impact on human health due to their nutraceutical potential, and as a food coloring agent due to its anthocyanin content, as a substitute for synthetic colorings, considering that this berry has been consumed for many years by the inhabitants of the South American Andes (23).

MATERIALS & METHODS

Plant material

Intact ripped fruits of *B. commutata* (500 g), free of cracks, cuts, or blemishes, were manually harvested from bushes in the Lares valley in Calca, Cusco. The fruits were dried for 2 months in a dark room at 8 – 10°C. M.Sc. Tupayachi Herrera an associated botanist to Vargas CUZ herbarium identified the plant, and a voucher was deposited in the herbarium of the Universidad Nacional de San Antonio Abad del Cusco (UNSAAC).

Characterization of *B. commutata* E. fruits

The weight was determined in grams and the size fruit was measured using a vernier caliper (in mm), and pH was determined in the fresh juice using a Jenway 3510 pH meter (Metrix Laboratorios, Mexico City, Mexico).

Phytochemical components extraction

Dried fruits of *B. commutata* separated from seeds (24) were placed in a blender with a solution of acetone/water (30:70 v/v) and then filtered. The cake residue was reextracted with the same mixture to obtain a diluted solution. All the filtrates were combined, transferred to a separating funnel, mixed with two volumes of chloroform, and kept at 4 ± 2° C overnight. The aqueous phase was obtained and concentrated to dryness using a rotary evaporator at 40° C under a vacuum.

Monomeric anthocyanins content (MAC)

Monomeric anthocyanin content (MAC) was measured using the pH differential method (25). The appropriate dilution factor was determined by dilution with potassium chloride buffer (pH 1.0) until the absorbance of the sample at the maximum wavelength was within the linear range of the spectrophotometer. The dilution factor was 0.5 and was obtained by dividing the final volume of the sample by the initial volume. Then, two dilutions of the acetone/water extract before drying (approximately 1 mL) were prepared, one using 0.025 M Potassium Chloride buffer (0.1 g KCl in 50 mL of deionized water and adjusted to 1.0 with HCl (18%)) and the other using 0.4 M sodium acetate buffer (2.72 g Sodium Acetate 3- hydrate in 50 mL of deionized water and adjusted to 4.5 with HCl (18%)), diluting each with the previously determined dilution factor. A UV/VIS PG Instrument T80+ Spectrophotometer (Woodway lane, Leicestershire, UK) was used for absorbance measurements at λ max (520 nm) and 700 nm, respectively, after 20 min equilibrium time. MAC was calculated as Cy-3-glu, using Eq. (1)

$$\mathrm{MAC}\left(\frac{mg}{mL}\right)=\left(\left[\left(A_{520}-A_{700}\right)_{pH1.0}-\left(A_{520}-A_{700}\right)_{pH4.5}\right]x\,MW\,x\,DF\right)x\left(\varepsilon\,x\,L\right)^{-1} \tag{1}$$

Where: MW = molecular weight of Cyanidin-3-O-glycoside (449,2 g mol^{-1})

DF = dilution factor

ε = extinction coefficient (26 900 L (mol.cm)-1

L = path length (1 cm)

Total phenolic content (TPC)

Total phenolic content (TPC) was estimated by the Folin-Ciocalteu colorimetric assay. Briefly, 0.5 mL of extract (2 g/50 mL) was combined in a test tube with 0.5 mL of Folin Ciocalteu reagent and 0.5 mL of Na_2CO_3 10% (w/v). The mixture was kept in the dark for an hour. A standard curve was generated using gallic acid (10-50 mg/L) (Sigma Aldrich, St Louis, MO, USA). Results were calculated from the calibration curve and expressed as mg Gallic acid equivalent (GAE)/100 g of extract. Absorbance was measured at 760 nm (26).

DPPH radical-scavenging activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity was measured according to the method of Brand-Williams *et al.* (1995) (27). Briefly, 2mg of acetone/water dry extract was dissolved using 100 mL of methanol. 2.0 mL of methanolic DPPH solution was added to 1.0 mL of either methanolic solution of extract (sample) or methanol (control). Mixtures were vortexed and stored at room temperature in the dark. Absorbance was measured at 517 nm after 30 min. Ascorbic acid was used as a positive control. The % DPPH radical scavenging activity was estimated using Eq. (2).

$$\%DPPH\ radical\ scavenging\ activity = \{(A0 - A1)/A0\}x100 \quad (2)$$

Where: A0 = control absorbance, and A1= extract absorbance.

Then % of inhibition was plotted against concentration, and from the graph the EC50 (concentration required to obtain a 50% antioxidant effect) was calculated.

RESULTS AND DISCUSSION

Characterization of *B. commutata* E. fruits

The ripening fruits of *B. commutata* E. measured 10 ± 0.812 mm and weighed 0.318 ± 0.02 g. Compared to the ripening fruits of *B. boliviana* L., with an average size of 7.05 ± 0.355 mm and 0.103 ± 0.02 g (24), the berries of *B. commutata* E are larger and heavier. The fresh juice pH was 3.86. The acidic pH has a protective effect on the anthocyanin molecule, giving stability to its chemical structure.

B. commutata E. samples were compared by M.Sc. Tupayachi-Herrera with a specimen from the Vargas CUZ herbarium (UNSAAC–Cusco). According to the Cronquist classification system, in agreement with the Angiosperm Phylogeny Group APG III (2009), the taxonomic position is as follows: Division: Magnoliophyta (= Angiosperms), Class: Magnoliopsida (= Tricolpates – Eudicots), Subclass: Magnoliidae, Order: Ranunculales, Family: Berberidaceae, Genus: Berberis, Specie: *B. commutata* E (Figure 1).

Figure 1. Bush of *B. commutata* with ripe fruits (left), *B. commutata* fruits dried at 8 – 10°C for two months (right).

Phytochemical components extraction

The extraction process is critical and must be properly designed to obtain the desired substances in sufficient quality and quantity (28). The best extraction solvent is required to extract total phenolics, monomeric anthocyanins and to evaluate antioxidant activity.

The content extract/fruit was high for *B. commutata* E., and the percentage (53.92%) is within the yield extraction *Berberis croatica*, which ranged from 52.1% to 85.8% (26). Duymus *et al.* (2014) reported a yield extraction of 328 mg/g (mg of anthocyanins /g of dried fruit) of elderberry fruit (29), which is lower than the yield reported in this study (539.2 mg/g).

Total phenolic content (TPC) and monomeric anthocyanin content (MAC)

Our results on the TPC were 7,490 ± 0.85 mg GAE/100g dry fruits and differ greatly from some related species. The possible reason for these huge differences could be due to some factors including genetic and agronomic or environmental that play important roles in phenolic content, other specific factors could be the berry variety, seasonal variation, and soil condition (30,31). Ardestani *et al.* (2013), reported a TPC of 48,000 and 23,000 mg/100g dry fruits of ethanolic and aqueous extracts of *Berberis integerrima*, respectively, and a TPC of 28,000 and 19,500 mg/ 100g dry fruits of ethanolic and aqueous extracts of Berberis vulgaris, respectively (32). In another study, Duymuş *et al*. (2014) found a TPC of 8,206 ± 167 mg/100g (dry weight) of 70% acetone extract from elderberry fruits (29).

Also, we have an elevated TPC in *B. commutata* E. compared to other well-known berries like Blueberrie Variety O´Neils (1,058.28±16.51 mg GAE/100 g dry fruit) (33); *Vaccinium uliginosum* (ranged from 308.52 ± 29.21 to 618.25 ± 46.48 mg GAE /100g fresh fruit) (34), *Chokeberry* (603 ± 14.9 mg GAE/100g fresh fruit) (35), blueberry (299.60 mg GAE/100 g fresh fruit) and raspberry (322.36 mg GAE/100 g fresh fruit) (36).

The MAC of *B. commutata* E. (70 ± 0.03 mg/100g) was different from that of *B. boliviana* (7,130 mg/100 g dry weight) (24) and low compared to some other anthocyanin-rich sources such as elderberry (651.1 ± 26 mg/100g of dry weight) (29), blueberry (*Vaccinum myrtillus*) (300 - 320 mg/100g of fresh fruit), elderberry (*Sambucus nigra*) (450 mg / 100g of fresh fruit) (37). Feng *et al.* (38) reported the anthocyanin content in wild berries ranging from 10 to 1,058 mg/100 g of fresh fruit.

DPPH radical-scavenging activity

Juríkova *et al.* (39) reported a good correlation between the antioxidant activity of vegetables and fruits and the number of their phenolic compounds. In our study, an EC50 of 0.91 mg/mL was found, this low EC50 value indicates a high antioxidant power, and it is close to other superfruits such as blackberry (1.40 mg/mL), raspberry (0.80 mg/mL) and blueberry (0.70 mg/mL) in fresh fruits (40).

The phenolic concentration in berries varies with plant genotype (cultivar), weather conditions, time of harvest, cultivation, and ripeness (41). The phenolic profile also varies among berries (42). Polyphenolic compounds play an important role in plants due to their scavenging capacity. A strong correlation between phenolic content and antioxidant activity has been reported (39,43).

Recently, the food industry has been interested in finding phenolic compounds with high antioxidant activity, especially fruits, vegetables, and herbs, due to their significant benefits for human health (44,45). Additionally, polyphenolic compounds in fruits and vegetables are naturally occurring and are more widely accepted by consumers (46). There is a worldwide interest in researching and discovering these polyphenolic compounds to identify new sources of antioxidant molecules for human health.

This is the first time that total phenolic content and anthocyanins of *B. commutata* E. were determined and seemed to be an important source of this component with high antioxidant activity.

Functional food is a term that defines foods with natural bioactive compounds for human nutrition because of their health benefits and disease prevention (47); therefore, enriching foods with natural bioactive compounds such as phenolics and anthocyanins is a strategy to produce functional foods with high antioxidant activity.

Wang *et al*. (2018) (48) consider that *Vaccinium* and Rubus genus are plants rich in anthocyanins, highlighting blackberry, blueberry, red raspberry, cranberry, and black raspberry; however, there are some genus such as berberis that could provide important sources rich in anthocyanins that can be extracted efficiently and at low cost.

CONCLUSION

In the present study, the total phenolic content, monomeric anthocyanin content, and antioxidant activity of the *B. commutata* E. fruit extracts were investigated for the first time. The higher total phenolic content in *B. commutata* E. fruits compared to other anthocyanin-rich berries was demonstrated. In contrast, the monomeric anthocyanin content was lower than other anthocyanin-rich sources. The extract was found to have antioxidant activity, determined by DPPH assay, and there is a tendency of higher antioxidant activity with increasing extract concentration of *B. commutata* E. Finally, the low EC50 value indicates a high antioxidant power and is similar to other superfruits. The consumption of berries has recently increased as fresh fruits and extracts in products such as yogurts, jams, beverages, and dietary supplements, so it is imperative to investigate new sources. Since the Berberis genus is an unexplored source of wild berries, especially in South America, it is necessary to consider further investigations to establish its use as a nutraceutical and food colorant.

CONFLICT OF INTEREST

The author reports no conflict of interest.

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