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Item Type	Article
Authors	Sangram Bhosale, Sanyogita;Granato, Daniel;Mohammadi, Nima
Citation	Journal of Food Measurement and Characterization, 2025, 19, pp.2730–2738
Publisher	Springer
Download date	2026-03-08 04:23:11
Item License	https://creativecommons.org/licenses/by-nc-sa/4.0/
Link to Item	https://doi.org/10.34961/researchrepository-ul.29135471

Journal of Food Measurement and Characterization (2025) 19:2730–2738
https://doi.org/10.1007/s11694-025-03142-8

ORIGINAL PAPER

Purification and characterization of anthocyanin from Irish wild blackberry: impact on color, composition, and antioxidant capacity

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Received: 19 September 2024 / Accepted: 7 February 2025 / Published online: 22 February 2025
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Abstract

The extraction of anthocyanins from fruits and food waste using eco-friendly techniques has gained attention. This study, for the first time, used Amberlite XAD-7 resin to purify Irish wild blackberries, known for their high polyphenol and anthocyanin content. Ultrasound-assisted extraction (UAE) with 60% ethanol at 325 W for 7.5 min was employed. The crude extract (BCE) was purified with resin to remove impurities while retaining anthocyanins. The total phenolic content (TPC) was 9.35 ± 0.07 mg GAE/g for BCE and 8.82 ± 0.33 mg GAE/g for the purified extract (BPE). Total anthocyanin content (TAC) was 0.21 ± 0.01 mg CYE/g for BCE and 0.15 ± 0.01 mg CYE/g for BPE. HPLC identified cyanidin-3-glucoside (C3G) as the dominant anthocyanin. The C3G concentration was 5.50 ± 0.02 mg/g in BCE and 4.01 ± 0.01 mg/g in BPE at 520 nm. Antioxidant capacity assessed via CUPRAC, FRAP, and DPPH assays showed significant differences between BCE and BPE in CUPRAC (25.13 ± 1.09 mg AAE/g for BCE vs. 23.06 ± 0.26 mg AAE/g for BPE), with no significant differences in FRAP and DPPH assays. Amberlite XAD-7 resin effectively removed impurities while preserving antioxidant capacity, making BPE a potential ingredient for pharmaceutical and food applications due to its concentrated anthocyanin content, thereby introducing a novel approach in the field.

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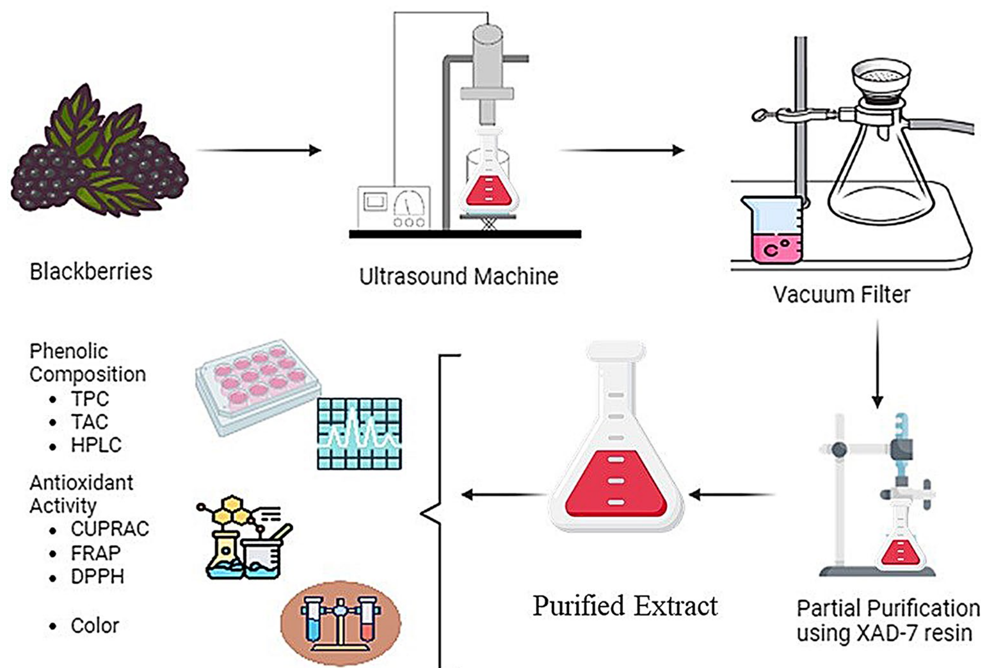
Graphical Abstract



Keywords Cupric ion reducing antioxidant capacity (CUPRAC) · Total anthocyanin content (TAC) · Total phenolic content (TPC) · Amberlite XAD-7 resin · Ferric reducing antioxidant power (FRAP)

Introduction

Color is often regarded as a crucial quality characteristic of food products, significantly increasing customer satisfaction and promoting repeat purchases [1]. Many synthetic colorants have been extensively utilized as food additives to achieve the desired color. However, growing concerns about the safety of synthetic colorants have led to increased use of natural pigment extracts, such as anthocyanins [2]. Anthocyanins, a class of water-soluble flavonoid pigments, are responsible for the vibrant blue, purple, and red colors found in various fruits, vegetables, and flowers. In addition to their coloring properties, anthocyanins have been shown to offer a range of benefits, including antioxidant effects, cardioprotective properties, anti-inflammatory effects, antimicrobial activities, reduction of eyestrain, anticancer properties and protection against DNA damage [3–5]. As a result, interest in anthocyanins for their potential use as food colorants and dietary antioxidant supplements has been growing in recent years [2]. This strategy aligns with the “clean label” trend [6].

Blackberries, members of the *Rubus* genus in the Rosaceae family, are native to North America and have recently expanded their presence worldwide. They are renowned for their high levels of phytochemicals, which contribute significantly to their health benefits. These phytochemicals include a variety of phenolic compounds such as flavanols, ellagic acid, cyanidins, gallic acid, quercetin, ellagitannins, and tannins, all of which are responsible for the fruit’s potent antioxidant capacity [7]. The main anthocyanins in blackberries are cyanidin-3-rutinoside (C3R) and cyanidin-3-glucoside (C3G). C3G accounts for 75–84% of the total anthocyanins in various blackberry cultivars, while C3R is present only in trace amounts [8].

Anthocyanins are typically extracted from plants through a solid-liquid extraction using various solvents such as ethanol, methanol, acetone, or combinations. The traditional solvent extraction method is often time-consuming and inefficient. Additionally, prolonged thermal extraction can lead to the degradation of anthocyanins and a reduction in the antioxidant capacity of the extracts [8]. Hence, adopting green technologies and utilizing eco-friendly solvents are essential for minimizing the environmental footprint of the extraction process while enhancing its efficiency. To achieve this, the UAE approach is highly effective in extracting antioxidant components from blackberries [9]. Acoustic cavitation, which involves breaking the plant’s cellular structure and forming pores through ultrasonic vibrations, enhances the release of phenolic compounds and significantly improves extraction efficiency [10]. Once a

blackberry crude extract (BCE) is obtained, further purification is required to remove organic acids and simple sugars, resulting in a blackberry purified extract (BPE). The purified extract, derived from green technologies, is a valuable ingredient in food products and dietary supplements, enhancing formulations and supporting food manufacturing applications. The anthocyanin pigments from these methods are considered suitable for food use due to their GRAS (generally recognized as safe) status [11, 12].

Microporous resins, such as Amberlite XAD-7, are commonly used for purification steps. XAD-7 is particularly effective for adsorbing and desorbing bioactive compounds like anthocyanins, offering advantages such as porosity, chemical stability, and high surface area compared to other resins like XAD-2 and XAD-4. However, its larger pore size, while beneficial for adsorbing larger molecules, may limit its application for smaller bioactive compounds [13]. XAD-7's high adsorption selectivity and reusability make it a sustainable choice, as it can be regenerated and reused multiple times without significant loss of capacity [14]. Additionally, XAD-7 reduces the need for hazardous organic solvents [15], offering significant environmental benefits compared to traditional methods like liquid-liquid extraction [14, 16]. Consequently, the use of XAD-7 resin, combined with green extraction methods like UAE, contributes to a more efficient and environmentally friendly process [15].

To our knowledge, the use of UAE for extracting anthocyanins from blackberries and analyzing their antioxidant properties has been reported in many studies [10, 17, 18]. Despite this, none of these studies have addressed BCE and BPE's chemical composition and antioxidant capacity from Irish blackberries. Therefore, this study aimed to extract anthocyanins from Irish blackberries using UAE, purify the BCE, and evaluate the impact of purification on the chemical composition, antioxidant capacity, and color properties of the BPE. This approach could demonstrate its alignment with key United Nations Sustainable Development Goals, specifically SDG 9 (Industry, Innovation, and Infrastructure) and SDG 3 (Good Health and Well-being).

Materials and methods

Chemical and reagents

Gallic acid was purchased from Fluka (USA). Hydrochloric acid (HCl), Folin-Ciocalteu reagent, sodium carbonate, neocuproine (2,9-dimethyl-1,10-phenanthroline), potassium chloride (KCl), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium acetate, 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ascorbic acid, ammonium acetate, copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$), and Amberlite XAD-7 resin were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade solvents, including methanol, acetonitrile, and ethanol, were supplied by Merck (Burlington, USA) HPLC-grade standard of cyanidin-3-glucoside was obtained from Extrasynthese (Genay, France).

Blackberries extraction using UAE

Two kg of fully ripe wild blackberries were hand-harvested in August 2023 at Castletroy, Limerick, Ireland. After being sanitized for 15 min with a 100 mg/L NaClO solution, the berries were cleaned with water and kept at -20 °C until their analysis. First, 40 mL of an ethanol-water solution in a 60:40 (v/v) ratio was mixed with 2 g of crushed blackberry sample (Fig. 1). After that, the ultrasound method was carried out using a ultrasonic liquid processor (model CL-334, Thermo Fisher Scientific, Karlsruhe, Germany) and a titanium ultrasonic probe with a 20 kHz frequency and a tip diameter of 12.7 mm. The samples (8 independent batches) were subjected to ultrasonic treatment for 7.5 min at 325 W in a chilled water bath to avoid temperature increase, followed by centrifugation for 5 min at 5300 rpm at room temperature. The resulting extracts were combined and subsequently labelled as BCE. The extraction duration and ultrasonic power settings were based on optimized conditions from our previous study, which assessed the effects of 5, 7.5, and 10-min extraction times and power levels of 400, 325 and 250 W in UAE [19]. The ethanol-to-water ratio

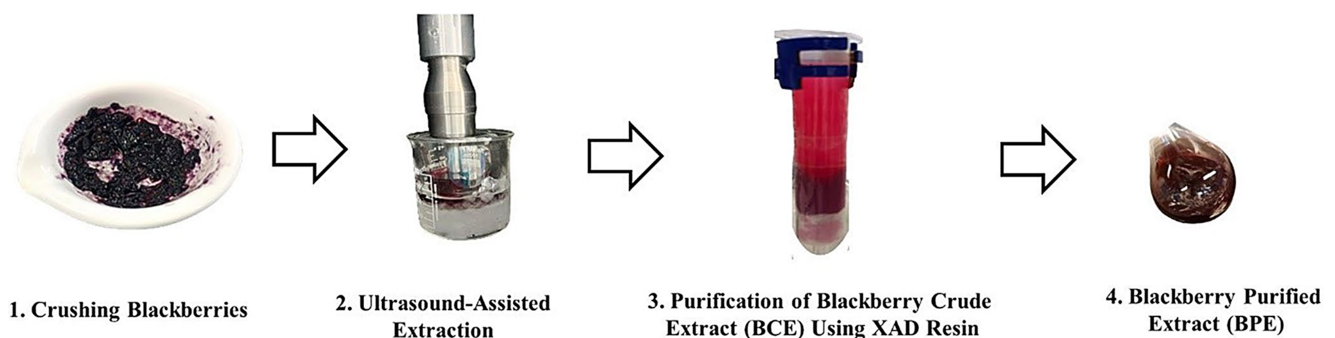


Fig. 1 Process flow for the extraction and purification of blackberry extract

was determined based on findings from previous studies [17, 19]. Using response surface methodology (RSM) from our prior research [19], we identified the optimal extraction parameters for blackberry samples: 325 W of power and a 7.5-min extraction time, with a solvent composed of 60% ethanol and 40% water.

Purification of the crude extract

The purification was performed by combining pre-activated Amberlite XAD-7 (10 g of resin in 100 mL ethanol for 1 h under magnetic stirring) with the BCE at a 1:5 (w/v) ratio, followed by stirring for 1 h protected from the light. Following the incorporation of Amberlite XAD-7, the solution was transferred to a 1 cm diameter, 20 cm long glass column. The column was first rinsed with 300 mL of acidified water (pH 2, adjusted with HCl) to remove polysaccharides, organic acids, and other impurities. An additional 300 mL of distilled water was used to clean the column to ensure the remaining extract was cleared. Finally, the anthocyanins were eluted with 250 mL of absolute ethanol and vacuum evaporated at 40 °C to remove the ethanol while maintaining the initial ethanol-to-water ratio of 60:40 v/v, as in BCE, to obtain BPE [15].

Chemical composition

Total phenolic content

The total phenolic content (TPC) of BCE and BPE was assessed using the Folin-Ciocalteu reagent. Briefly, 25 µL of each extract were mixed with 200 µL ultrapure water, 25 µL Folin–Ciocalteu reagent, and 25 µL of 10% sodium carbonate solution. The mixture was incubated for 1 h, and absorbance was measured at 725 nm using a microplate reader (Biotek Synergy H1, Agilent, Santa Clara, CA, USA). Results were expressed as mg of gallic acid equivalents per g of fresh fruit (mg GAE/g) [15].

Total monomeric anthocyanin content

Briefly, 20 µL of BCE or BPE were mixed with 180 µL of potassium chloride buffer (0.025 M, pH 1.0) or sodium acetate buffer (0.40 M, pH 4.5) in a 96-well microplate. Absorbance was measured at 520 nm and 700 nm using a microplate reader. Total monomeric anthocyanin content (TAC) was expressed as mg of cyanidin-3-glucoside equivalents per g of fresh fruit (mg CYE/g) [20]. For cyanidin-3-glucoside, the molecular weight is 449.2 g/mol and the molar absorption coefficient is 26,900.

$$A = (A_{520} - A_{700})pH_1 - (A_{520} - A_{700})pH_{4.5} \quad (1)$$

High-performance-liquid chromatography

High-performance liquid chromatography (HPLC) was used to determine the anthocyanin content, specifically C3G, in BCE and BPE. The analysis was performed using an Agilent 1200 Infinity Series HPLC system with a Telos LU C18 column (ID 5 µm, 150 × 4.6 mm), at a 30 µL injection volume and 35 °C column temperature. The mobile phase consisted of solvent A (water with 1% phosphoric acid) and solvent B (acetonitrile), with a 0.8 mL/min flow rate. A gradient elution was performed as follows: 92% solvent A at 0 min, 88% A at 3 min, 86% A at 25 min, 84% A at 28 min, and 92% A at 30 min. These optimal HPLC conditions were determined based on our previous study [19]. Detection occurred at 520 nm, and anthocyanin identification was based on retention times and UV spectra compared with standards [21]. Quantification used C3G-specific calibration curves, with linearity confirmed (R^2=0.999), and LOD and LOQ determined as 2.65 mg/L and 4.02 mg/L, respectively. Method reliability was validated by duplicate analyses of standard solutions. Additionally, various wavelengths (280, 320, 360, and 520 nm) were monitored to assess the effect of purification on impurity removal. Additionally, different wavelengths (280, 320, 360 nm, and 520 nm) were monitored by HPLC in both BCE and BPE to assess the impact of the partial purification step on removing impurities.

Color analysis

Color properties of each extract were measured at 420 nm (yellow pigments), 520 nm (red pigments), and 620 nm (blue pigments) using a UV-Vis spectrophotometer. The color intensity (CI) and the percentage proportions of YP, RP, and BP were determined based on Eqs. 3–6 [19]. For comparison purposes, a commercial red food color solution (Goodall's of Ireland, Valeo Foods, Dublin, Ireland; ingredients: Ponceau 4R E124, and Sunset Yellow E110 pigments) was prepared using 1 mL of the red pigment solution in 100 mL of distilled water.

$$CI = Abs_{420nm} + Abs_{520nm} + Abs_{620nm} \quad (2)$$

$$YP = 100\times \left[(Abs_{420nm}) / CI \right] \quad (3)$$

$$RP = 100\times \left[(Abs_{520nm}) / CI \right] \quad (4)$$

$$BP = 100\times \left[(Abs_{620nm}) / CI \right] \quad (5)$$

Chemical antioxidant assays

Cupric-ion reducing antioxidant capacity

In the CUPRAC assay, 50 µL of BCE, BPE, or distilled water (blank) was mixed with 500 µL of a solution containing neocuproine, ammonium acetate buffer, and copper chloride. After incubating for 30 min at room temperature, absorbance at 450 nm was measured. Results are expressed as mg of ascorbic acid equivalents per g of fresh fruit (mg AAE/g) [22].

Ferric-reducing antioxidant power

For the assay, 20 µL of BCE, BPE, or distilled water (blank) was added to a 96-well plate, followed by 180 µL of FRAP solution. After 30 min at room temperature, absorbance at 593 nm was measured using a microplate reader. Results are expressed as mg AAE/g of fresh fruit [15].

DPPH

A DPPH stock solution was made for the test by weighing 3.95 mg of DPPH and diluting it with methanol to make 100 mL of DPPH at 0.10 mmol/L. After mixing, the solution was transferred to an amber glass volumetric flask. For the analysis, 40 µL of either BCE, BPE, or distilled water (as a blank) was added, along with 260 µL of DPPH solution, to a 96-well plate. After 30 min at room temperature, the reaction mixture's absorbance was measured at 517 nm using a spectrophotometer, and results were expressed as mg AAE/g of fresh fruit (Eq. 6) [22].

$$\text{Scavenging activity (\%)} = \frac{A\,blank - (A\,sample - A\,control)}{A\,blank} \times 100 \tag{6}$$

Table 1 Chemical composition and antioxidant capacity analysis of blackberry crude extract (BCE) and blackberry purified extract (BPE)

Assays	BCE	BPE	*P*-value
TPC (mg GAE /g)	9.35 ± 0.07	8.82 ± 0.33	0.126
TAC (mg CYE/g)	0.21 ± 0.01	0.15 ± 0.01	< 0.05
CUPRAC (mg AAE/g)	25.13 ± 1.09	23.06 ± 0.26	< 0.05
FRAP (mg AAE/g)	7.88 ± 0.43	7.29 ± 0.49	0.550
DPPH (mg AAE/g)	4.74 ± 0.19	4.51 ± 0.36	0.447

Note: BCE = blackberry crude extract; BPE = blackberry purified extract; TPC = total phenolic content; TAC = total anthocyanin content; CUPRAC = cupric ion reducing antioxidant capacity; FRAP = ferric reducing antioxidant power; DPPH = free-radical scavenging activity; GAE = gallic acid equivalents; CYE = cyanidin-3-glucoside equivalents; AAE = ascorbic acid equivalents

Statistical analysis

All analyses were performed in quadruplicate, and results are presented as mean ± SD. Data were analyzed using IBM SPSS version 29.0.1.0, with a paired Student t-test to compare group differences ($P < 0.05$).

Results and discussion

Chemical composition

The TPC for BCE was 9.35 ± 0.07 mg GAE/g, and for BPE from Irish blackberries, it was 8.82 ± 0.33 mg GAE/g ($p > 0.05$). This indicates that the purification steps with the XAD-7 resin did not increase TPC (Table 1). These results agree with the TPC of purified and crude extracts of *Leonurus cardiaca*, a perennial plant, obtained using adsorption resins such as XAD-7 and SP207L [23]. They found that the TPC of the purified extract was 10,505 µg GAE/g, while the crude extract had a TPC of 14,647 µg GAE/g, indicating that purification with XAD-7 resin did not increase TPC [23]. In a similar study, partridgeberry bioactive compounds were extracted using solvent mixtures, including water, ethanol, methanol, and ethyl acetate. The solvents were used either with or without formic acid, followed by purification and fractionation on adsorbent resin (Sorbent SP-207-05) [24]. The TPC of partridgeberry was in the range of 21.4 to 25 µmol GAE/100 g FW. No significant difference was observed in TPC between extractions using methanol and water or ethanol and water, regardless of the presence of formic acid. The highest TPC, reported as 25 µmol GAE/100 g FW, was achieved with a mixture of water, ethyl acetate and formic acid. In contrast, the use of Sephadex resin for purification yielded different results. One study found that blackberry extract treated with Sephadex resin had 3.7 times higher phenolic content than the crude extract. The phenolic content of the crude extract was 87.25 mg GAE/g, while the purified extract contained 323.57 mg GAE/g [25]. Also, the phenolic content of blueberry extracts increased by 4.3 times using Sephadex resin; the purified extract had 425.07 mg GAE/g, compared to 99.48 mg GAE/g in the crude extract [25]. However, another study using a Sep-Pak C18 cartridge along with Sephadex LH-20 for the purification of bilberry extract found that the TPC of the purified bilberry extract was lower, at 11.0 µg GAE/L, compared to the crude extract, which had a TPC of 18.0 µg GAE/L [26].

Regarding TAC, the BCE had slightly higher anthocyanin content (0.21 ± 0.01 mg CYE/g) compared to BPE (0.15 ± 0.01 mg CYE/g) ($P < 0.05$). The slight decrease in TPC and TAC during purification with resin can be attributed to several factors. The resin's high adsorption capacity

may retain some compounds which are not fully recovered during desorption [27]. Additionally, selective adsorption and elution processes may fail to efficiently elute certain compounds, especially if elution conditions are not optimized [16]. Furthermore, the sensitivity of anthocyanins to pH and temperature during purification can lead to degradation, further reducing their content [28]. However, Researchers using Amberlite XAD-7 along with Sephadex LH-20 columns observed a significant increase in the total anthocyanin content (TAC) of methanolic extracts of Mexican blackberries [29]. The TAC in the crude extract ranged from 3.4 to 9.2 mg CYE/g dry weight (DW), whereas the purified extract ranged from 4.97 to 219.6 mg CYE/g DW [29]. Surprisingly, they found that TAC was not detectable after using Amberlite XAD-7 combined with Sephadex. It was also reported that TAC of black rice bran extracts obtained using a mixture of 60% citric acid and ethyl acetate increased significantly from 1.06 mg CYE/g in the crude extract to 4.44 mg CYE/g after partial purification with XAD-7 [30]. Similarly, researchers found that the crude extract from black peanut skin contained 5.01 mg CYE/g DW using ethyl acetate and 40% ethanol in water, acidified to pH 2.0 with HCl. After a partial purification step with Amberlite XAD-7 resins, the TAC purity of the purified extract increased to 25.40% [31]. Hence, both the resin type and solvent used are crucial factors in anthocyanin extraction. For example, researchers found that the purified extract of total anthocyanin content (TAC) from partridgeberry, obtained using water and acetone with adsorbent resin (Sorbent SP-207-05), had the lowest TAC recovery at 165.4 µmole CYE/100 g fresh weight (FW), compared to extracts using methanol, ethanol, and ethyl acetate. According to their study, the ethyl acetate-water-formic acid mixture (80:18:2) yielded the highest TAC at 165.4 µmole CYE/100 g FW [24].

The concentration of C3G, as measured by HPLC, was 5.50 ± 0.02 mg/g for the BCE, while BPE had a lower C3G concentration of 4.01 ± 0.01 mg/g at 520 nm. These results are consistent with those of researchers who found that the crude extract of cherry contained a higher quantity of C3G [32]. However, some C3G was likely lost during purification due to filtering, selective separation, and potential degradation. Also, The C3G content in both extracts was slightly higher than previously reported values for wild blackberries, which ranged from 1.30 to 1.97 mg/g in Türkiye [33] and 335.6 mg/100 g in Korea [34].

The impact of the partial purification step on removing impurities at various wavelengths (280, 320, and 360 nm) in BCE and BPE, as analyzed using HPLC, is shown in Fig. 2. The results indicated significant improvements in BPE, while BCE at different wavelengths displayed multiple extra peaks, likely due to residual impurities such as sugars, inorganic components, and amino acids [32]. This suggests that the purification procedure enhances the clarity, concentration, and identification of anthocyanins and phenolic compounds [35]. Amberlite XAD-7HP resin plays a crucial role by partially adsorbing and removing contaminants from BCE. It was reported that this step can improve the stability and quality of the anthocyanins in the product (X. Zhao et al., 2020). Similarly, researchers found that using the resin enhanced the clarity of chromatographic peaks, enabling more precise identification and quantification of individual

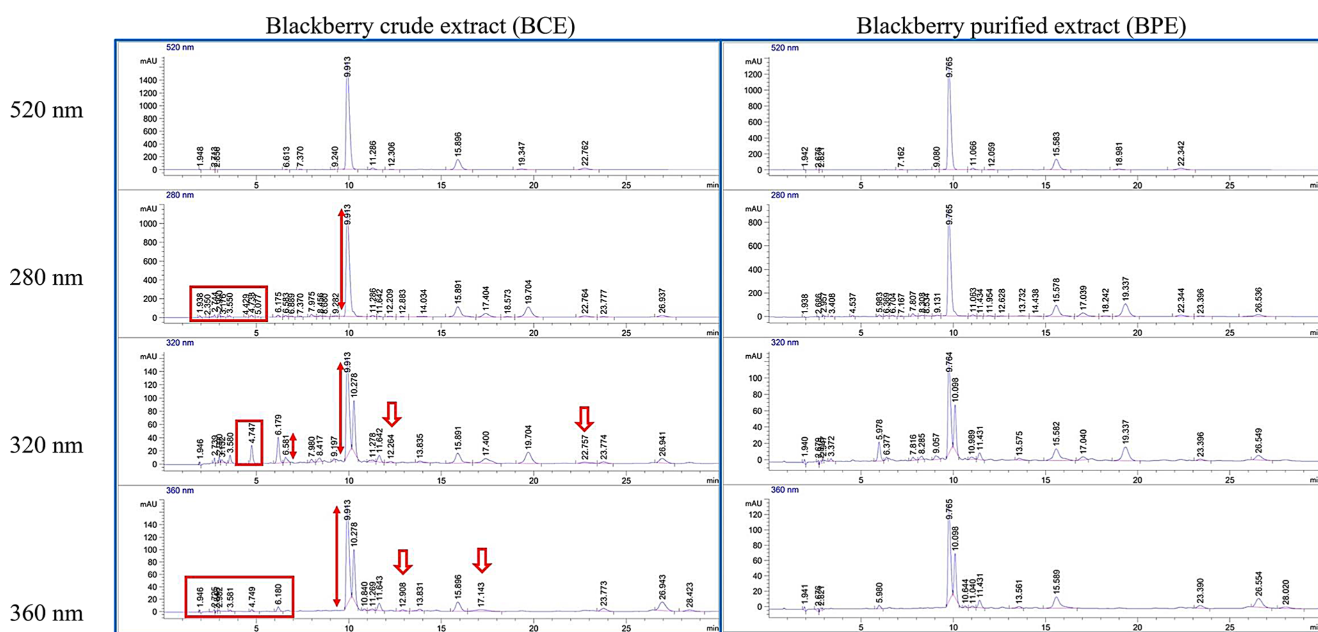


Fig. 2 HPLC spectrums of blackberry crude extract (BCE) and blackberry purified extract (BPE) at wavelengths 520 nm, 280 nm, 320 nm, and 360 nm

Table 2 Comparison of color intensity (C.I) and pigment composition in blackberry crude extract (BCE) and blackberry purified extract (BPE) with a commercial red color pigment solution

Sample	Color intensity (C.I)	Yellow Pigment%	Red Pigment %	Blue Pigment %
BCE	1.72 ± 0.02[c]	42.21 ± 0.09[a]	44.56 ± 0.05[c]	13.27 ± 0.07[a]
BPE	1.93 ± 0.06[b]	35.74 ± 0.25[b]	54.05 ± 0.45[b]	10.20 ± 0.20[b]
Commercial red color pigment solution	5.72 ± 0.02[a]	30.16 ± 0.18[c]	69.11 ± 0.19[a]	0.73 ± 0.003[c]

anthocyanins [36]. Additionally, it was reported that the concentration of C3G in bilberry extract remained relatively stable in the purified extract compared to that in the crude extract [26]. Previous studies highlighted that applying resin on a large scale for anthocyanin purification requires careful consideration of certain factors. Key issues include non-selective interactions leading to co-adsorption of contaminants and reduced extract purity [37]. Additionally, anthocyanins may behave variably depending on the matrix and pH, causing inconsistent results and potential artefactual shifts in composition [38]. Optimizing parameters such as flow rates, solvent concentrations, and contact times is essential to maintain efficiency and product quality [39].

The color assay results showed significant differences in pigment content and CI at 420 nm, 520 nm, and 620 nm between BCE, BPE, and commercial samples (Table 2). The pigment content of BCE was 42.21 ± 0.09% yellow, 44.56 ± 0.05% red, and 13.27 ± 0.07% blue, resulting in a CI of 1.72 ± 0.02. The CI rose to 1.93 ± 0.06 after purification, and there was a noticeable change in the distribution of the pigments: BP slightly dropped to 10.20 ± 0.20%, RP climbed to 54.05 ± 0.45%, and YP declined to 35.74 ± 0.25%. By contrast, the commercial CI was much greater at 5.72 ± 0.02, composed of pigments that were 30.16 ± 0.18% yellow, 69.11 ± 0.19% red, and 0.73 ± 0.003% blue. These results suggested that purification increased the amount of RP while decreasing that of YP and BP. Similarly, researchers reported that the color characteristics of Andean blackberry (*Rubus glaucus Benth*) showed an average CI ranging from 2.70 to 8.40, with a mean of 5.90 [40]. This indicated a relatively high CI, necessary for the wine's aesthetic appeal. RP dominated the color composition, followed by YP and BP. Their study identified several anthocyanins, including cyanidin 3-rutinoside-5-glucoside, C3G, cyanidin 3-xylorutinoside, and C3R, which were primarily responsible for the wine's rich red color [40].

It is noted that after purification, the shift in pigment ratios and CI of anthocyanins results from the adsorption and desorption processes, which concentrate the anthocyanins while removing impurities such as sugars, organic acids, and proteins [16, 41, 42]. This purification results in a more refined extract, boosting CI and altering pigment ratios. Structural changes may also occur, like removing glycosidic moieties [43], impacting color characteristics and potentially causing bathochromic shifts [44]. Previous research showed that the stability of anthocyanins varied between crude and purified extracts, as stabilizing compounds present in crude extracts (such as phenols, organic acids, and sugars) contributed to greater stability [45]. Purified anthocyanins degraded more quickly than those in crude extracts, primarily due to the absence of inter- and intramolecular co-pigmentation interactions [46, 47]. However, impurities can significantly affect the physiological activity and overall quality of anthocyanins [41]. Therefore, purifying BCE for use in food formulations and pharmaceuticals is critical to obtaining anthocyanins with strong physiological activity and high quality.

Antioxidant capacity

The antioxidant capacity of anthocyanins, such as C3G, is linked to their ability to reduce pro-inflammatory responses, decrease reactive oxygen species (ROS) generation in tissues and plasma, promote endothelial function, normalize circulating plasma lipid levels, and boost nitric oxide (NO) production [3]. The antioxidant activities of BCE and BPE are shown in Table 1. As observed, the CUPRAC assay showed lower results for BPE (23.06 ± 0.26 mg AAE/g) and higher results for BCE (25.13 ± 1.09 mg AAE/g) ($p < 0.05$). Similarly, the study by researchers demonstrated the antioxidant capacity of CUPRAC for crude and purified anthocyanin extracts from *Carissa carandas*, with the extract exhibiting an EC_{50} value of 223.3 µg/mL. In comparison, the crude extract showed a lower EC_{50} value of 207.5 µg/mL [48]. Hence, the purified extract displayed lower antioxidant capacity than the crude extract. This may be attributed to other substances in the crude extract, such as amino acids, fiber, salts, and sugars, which could enhance its antioxidant potential. Additionally, the CUPRAC assay revealed varying antioxidant capacities between crude and purified extracts. Since flavonoids and phenolic compounds are key antioxidants contributing to the reducing power of crude extracts, they often result in higher CUPRAC values. In contrast, purified extracts may show varying CUPRAC values depending on the concentration of specific antioxidants; some extracts may have higher capacities if potent antioxidants are concentrated, while others may have lower values if less effective molecules are present [49].

FRAP assay results for BCE and BPE demonstrate a significant difference in their antioxidant activities. The FRAP

value for BCE is 7.88 ± 0.43 mg AAE/g, while BPE shows a slightly lower value of 7.29 ± 0.49 mg AAE/g. These findings align with previous research on the FRAP experiment results, indicating differing antioxidant capacities for crude and purified extracts [49]. Crude extracts typically exhibit higher FRAP values due to a complex mixture of antioxidants, such as various phenolic compounds and other bioactive substances, which collectively enhance their reducing capacity [49]. In contrast, purified extracts may show lower or varying FRAP values depending on the specific antioxidants retained during purification. The purification may either concentrate potent antioxidants, resulting in higher values or remove some effective compounds, leading to lower values. In contrast, researchers found that the fractionated purified extracts of sweet potato, obtained from different solvent extractions (petroleum ether, ethyl acetate, and aqueous) following an initial ethanol extraction, demonstrated notably higher antioxidant capacity in the FRAP assay compared to the crude extract [50]. In another study, the FRAP value for 1 mg/mL of the fractionated purified extracts obtained from AB-8 resin was 1436 µM $FeSO_4$, 4.59 times higher than that of the crude extract [50].

Regarding DPPH, the result for BCE was 4.74 ± 0.19 mg AAE/g, and BPE showed a value of 4.51 ± 0.36 mg AAE/g ($p > 0.05$). This indicates that the purification step did not significantly affect the antioxidant capacity of the extracts. Another study on blueberries revealed that the blueberry anthocyanin-rich extract (BRE) demonstrated a strong antioxidant capacity with an EC_{50} value of 0.51 mg/mL [51]. In contrast, the blueberry anthocyanin crude extract showed a moderate antioxidant capacity with an EC_{50} value of 2.35 mg/mL. Also, the blueberry fruit supernatant exhibited the lowest antioxidant capacity, with an EC_{50} value of 4.80 mg/mL. Also, in another study, the DPPH assay results for myrtle berries extracts showed that the ethanol extract had a Trolox equivalent antioxidant capacity (TEAC) of 41.4 mmol/L. In contrast, the ethyl acetate extract had a TEAC of 17.7 mmol/L. Although the TPC of the ethanol extract was notably higher than that of the ethyl acetate extract, their antioxidant activities in the FRAP and DPPH assays did not show similarly significant differences [52].

The lower antioxidant capacity of BPE compared to the crude extract BCE in the CUPRAC assay, despite no significant differences observed in the FRAP and DPPH assays, can be attributed to these assays' distinct characteristics and mechanisms. While both CUPRAC and FRAP are electron transfer (ET)-based assays, CUPRAC uses copper(II)-neocuproine as the oxidizing agent, whereas FRAP uses ferric ions [22, 49]. Additionally, CUPRAC operates at a physiological pH, making it suitable for hydrophilic and lipophilic antioxidants, while FRAP works at an acidic pH [53]. CUPRAC is also more sensitive to thiol-type antioxidants, which FRAP does not effectively measure [53]. In contrast, DPPH, another ET-based assay, involves the reduction of the DPPH radical and can be influenced by solvent and pH conditions [22], making it more flexible than CUPRAC or FRAP in terms of pH requirements. While DPPH is more straightforward and faster, CUPRAC offers greater versatility and sensitivity to a broader range of antioxidants, including thiol-types, making it a more comprehensive method than DPPH.

Conclusion

This study examined the chemical composition, color, and antioxidant capacities of BCE and BPE obtained using XAD resin. BPE showed sustained antioxidant capacity in FRAP and DPPH assays. In contrast, the CUPRAC assay showed that BCE and BPE had different antioxidant activities. This study demonstrated that green extraction with ethanol, combined with ultrasound technology and partial purification using resin, can significantly preserve the functionality of C3G, making it more appropriate for incorporation into food formulations and pharmaceutical applications. Future research should focus on improving resin purification methods and exploring various resins to enhance the purification step. Developing high-value products that harness the health benefits of wild fruits, such as blackberries, offers significant potential. These techniques address the growing global demand for natural and effective antioxidant sources.

Funding Open Access funding provided by the IReL Consortium

Ethics declarations

Competing interests The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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