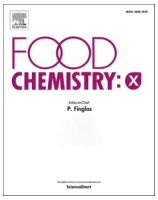
Food Chemistry: X 23 (2024) 101734

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Comprehensive structural analysis of anthocyanins in blue honeysuckle (*Lonicera caerulea* L.), bilberry (*Vaccinium uliginosum* L.), cranberry (*Vaccinium macrocarpon* Ait.), and antioxidant capacity comparison

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ARTICLE INFO

Keywords:
Blue honeysuckle
Bilberry
Cranberry
Anthocyanin
Antioxidant

ABSTRACT

The objectives of this research were to analyze anthocyanins in blue honeysuckle (*Lonicera caerulea* L.), bilberry (*Vaccinium vitis-idaea* L), and cranberry (*Vaccinium macrocarpon* Ait.), using HPLC-ESI-QTOF-MS^2, Fourteen, fifteen, and eight anthocyanins were identified in blue honeysuckle, bilberry, and cranberry, respectively. Cyanidin-3-glucoside (C3G) and peonidin-3-glucoside were detected in all three types of berries, with blue honeysuckle showing the highest C3G content at 5686.28 mg/100 g DW. Total phenolic content (TPC) and total flavonoid content (TFC), along with ABTS, DPPH, and FRAP assays, were measured. Blue honeysuckle exhibited the highest levels of TPC and TFC. The SOD, POD, and CAT activities in blue honeysuckle were 1761.17 U/g, 45,525.65 U/g, and 1043.24 U/g, respectively, which were significantly superior to those in bilberry and cranberry. The antioxidant mechanisms of these enzymes were investigated by molecular docking, C3G showed a higher affinity for POD, confirming the effectiveness of C3G as an antioxidant.

1. Introduction

Blue honeysuckle (*Lonicera caerulea* L.) is a small berry native to the northern hemisphere, and belongs to the Caprifoliaceae family. It is rich in phytochemicals such as polyphenols and flavonoids. The berries of blue honeysuckle are usually processed into beverages, jams, fruit wines, and snacks. These products are popular due to their high antioxidant properties (Guo et al., 2023; Negreanu-Pirjol et al., 2023). Blue honeysuckle polyphenols have potential biological activities, including antioxidant, antibacterial and anti-inflammatory effects. Anthocyanins are considered important compounds within the polyphenols of blue honeysuckle berries (Fan et al., 2023; Zhang et al., 2023). In addition, anthocyanins are considered the most important bioactive substances in bilberry (*Vaccinium vitis-idaea* L). Bilberry is a shrub that belongs to the Ericaceae family, characterized by blue, waxy peels. Harvesting bilberries is time-consuming due to their smaller size compared to blueberries, and most bilberries are wild rather than cultivated. (Fraisse et al., 2020; Medic et al., 2023). Cranberry (*Vaccinium macrocarpon* Ait.), is also in the Ericaceae family and has long been recognized as an important source of bioactive substances. The phenolic compounds in the secondary metabolites of cranberry include flavonoids, such as flavonols, anthocyanins, proanthocyanidins, and phenolic acids. (Kalin et al., 2015; Viskelis et al., 2009; Wang et al., 2017).

Antioxidants have a significant impact on human health as they inhibit adverse oxidative reactions in the body, thereby preventing oxidative stress associated with conditions such as high blood pressure, neurological diseases, and cancer. Antioxidant capacity can be assessed in various ways, commonly using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and the ferric ion reducing antioxidant potential (FRAP) assays (Rumpf

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https://doi.org/10.1016/j.fochx.2024.101734
Received 16 July 2024; Received in revised form 8 August 2024; Accepted 11 August 2024
Available online 13 August 2024

et al., 2023). SOD (Superoxide Dismutase), POD (Peroxidase), and CAT (Catalase) are critical antioxidant enzymes that protect cells from oxidative damage. SOD catalyzes the conversion of superoxide radicals (O_2^-) into less harmful substances. POD reduces hydrogen peroxide (H_2O_2) to water, thereby mitigating oxidative stress and cellular damage. Similarly, CAT catalyzes the decomposition of hydrogen peroxide into water and oxygen, further safeguarding cells from oxidative harm (Zeng et al., 2019). Blue honeysuckle, bilberry, and cranberry are rich in polyphenols with strong antioxidant capacities. These berries have been reported to contain anthocyanins, which regulate energy metabolism and have anti-inflammatory and antioxidant effects. Additionally, they have the potential to reduce the risk of various chronic diseases (Chen et al., 2020; Cheng et al., 2023; Xie et al., 2020). The extract of blue honeysuckle can significantly reduce the expression levels of pro-inflammatory factors in vivo and increase the activity of antioxidant enzymes. The main active substance in the extract is the anthocyanin cyanidin-3-glucoside (Cheng et al., 2023). Anthocyanins constitute the primary group of flavonoids in bilberries, offering high antioxidant capacity. They also serve as the main antioxidant compounds in cranberries (Jurikova et al., 2018; Wang et al., 2015). However, while blue honeysuckle, bilberry, and cranberry all contain anthocyanins and exhibit high antioxidant capacities, differences in their types of anthocyanins and their antioxidant capacities are seldom reported.

The objectives of this research were: (1) Quantitative and qualitative analysis of anthocyanins in blue honeysuckle, bilberry, and cranberry using HPLC-DAD-ESI-MS/MS; (2) Comparison of total phenol and total flavonoid contents in blue honeysuckle, bilberry, and cranberry; (3) Determination of DPPH, ABTS, and FRAP antioxidant capacities in blue honeysuckle, bilberry, and cranberry, and analysis of differences in antioxidant capacity among these berries; (4) Measurement of SOD, POD, and CAT enzyme activities in the three types of fruits, followed by molecular docking with cyanidin-3-glucoside (C3G); (5) Investigation of the correlation between total anthocyanins, total phenols, total flavonoids, and antioxidant capacity.

2. Materials and methods

2.1. Materials and chemicals

The blue honeysuckle berries “Lanjingling” were harvested at Xiangyang Agricultural Planting Station of Northeast Agricultural University, China. Wild bilberry and wild cranberry were provided by Irkutsk State Agrarian University, Russia. The three types of berries were individually packaged in plastic bags and stored at −20 °C until used for chemical analysis.

Acetonitrile, methanol, and formic acid were supplied by MACKLIN, China. Cyanidin-3- glucoside standard, (purity ≥98%) was purchased from Bomei, China. The water used was purified, and all reagents were of analytical reagent (AR) grade.

2.2. Extraction of phytochemicals from blue honeysuckle, bilberry, and cranberry

Place 30 g each of blue honeysuckle, bilberries, and cranberries on freeze-dryer trays, ensuring the berries don't touch each other to promote even drying. Freeze-dry them for 48 h using a small freeze-dryer (MITSUBISHI ELECTRIC GOT2000) set to −80 °C and a vacuum pressure of 10^{-3} mbar. Afterward, grind the freeze-dried berries into powder using a mortar. Weigh 1 g of each type of berry powder into separate 50 mL centrifuge tubes. Add 50 mL of hydrochloric methanol solution (10%, *v*/v) to each tube, shake well, and sonicate for 30 min. Subsequently, perform hydrolysis in boiling water for one hour. After hydrolysis, collect the supernatant and apply the crude extracts to a C18 cartridge. Elute the adsorbed anthocyanins with 15 mL of 0.01 M HCl, followed by elution with acidic methanol (0.1% HCl in methanol, v/v) until the eluate turns colorless. The collected eluate is designated as the anthocyanin fraction. Finally, filter the anthocyanin fraction using a 0.22 μm filter membrane before measurement.

2.3. Qualitative and quantitive analysis of anthocyanins using HPLC-ESI-QTOF-MS^2

HPLC-ESI-QTIF-MS^2 with diode-array detection (DAD) was performed using AB SCIEX equipment. A C18 column (Luna 5 μm, 250 mm × 4.6 mm, Phenomenex, CA, USA) was utilized to separate the different compounds in blue honeysuckle, bilberry, and cranberry. The column temperature was set at 25 °C, and the injection volume was 10 μL. The flow rate was maintained at 0.6 mL/min, and anthocyanins were detected at 520 nm. The eluents are water (60 mM formic acid solution, pH = 2.6) (B) and acetonitrile (5 mM Ammonium acetonitrile acetate solution, pH = 3.6) (A): 0–14% A (0–12.5 min), 14–16.5% A (12.5–17.5 min), 16.5–25% A (17.5–40 min), 80–50% A (40–55 min), 50–14% A (55–60 min), and washing with 86% B and 14% A for 20 min. The mass spectrometer operated in positive ion mode with a full scan mode of *m/z* 100–2000 (MS1) and m/z 50–2000 (MS2) specifically for anthocyanins. The ESI conditions included a capillary voltage of 5500 V and a temperature of 550 °C. (Xiao et al., 2023; Zhang et al., 2023).

2.4. Detection of total phenolic content (TPC), total flavone content (TFC), and total anthocyanins content (TAC)

The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were measured using the Epoch microplate spectrophotometer (Epoch 2, BioTek Instruments, VT, USA). For TPC determination, 20 μL of the sample, diluent, deionized water, and Folin-Ciocalteu reagent were sequentially added to a 96-well plate. The mixture was incubated in the dark for 5 min at room temperature, followed by the addition of 80 μL of sodium carbonate solution and a 2-h incubation under the same conditions. Absorbance was measured at 765 nm. For TFC determination, 30 μL of diluted extract was added to the 96-well plate, followed by 180 μL of ultra-pure water and 10 μL of 5% sodium nitrite solution. After a 6-min incubation at room temperature, 60 μL of 4% sodium hydroxide solution was added and incubated for 15 min. Absorbance was measured at 510 nm (Xiao et al., 2023; Zhang et al., 2023). Total Anthocyanin Content (TAC) is qualitatively and quantitatively determined as the sum of various anthocyanins using liquid mass spectrometry.

2.5. Determination of antioxidant capacity DPPH, ABTS, FRAP

The ABTS, DPPH, and FRAP assays were conducted following the methods outlined by Zhang and Xiao, with slight modifications, using a microplate spectrophotometer (Xiao et al., 2023; Zhang et al., 2023). For DPPH assay, a 25 mg/L DPPH solution was prepared. Five microliters of diluted sample and control were added to separate wells of a 96-well plate, followed by the addition of 195 μL of DPPH solution. The plate was then incubated in darkness at room temperature for 2 h, and absorbance was measured at 515 nm. In the ABTS assay, 38.4 mg of ABTS was dissolved in 10 mL of distilled water to prepare a 7 mM ABTS solution. Potassium persulfate solution was prepared by dissolving 0.662 g of potassium persulfate in 1 L of distilled water. The ABTS and K2S2O8 solutions were mixed and left in darkness for 12 h. After dilution with pure methanol, the absorbance of a 10 μL sample was measured at 734 nm after incubation with the solution for 10 min. For the FRAP assay, FRAP reagents were prepared by mixing 300 mM acetate buffer, TPTZ solution, and ferric chloride solution at a ratio of 10:1:1 (*v*/v/v). Ten microliters of sample, 30 μL of deionized water, and 150 μL of heated (37 °C) FRAP reagents were added to a 96-well plate. Absorbance was measured at 593 nm every minute for 30 min. These assays allow for the assessment of antioxidant capacities in the samples, providing valuable insights into their potential health benefits and applications.

2.6. Determination of SOD, POD, and CAT activities in the berries

Superoxide dismutase (SOD) activity assay kit, peroxidase (POD) activity assay kit, and catalase (CAT) activity assay kit were purchased from Beijing Solarbio Science & Technology Co., Ltd. A 0.1 g berry sample was homogenized with 1 mL of extraction buffer in an ice bath, centrifuged at 8000*g* at 4 °C for 10 min, and the supernatant was collected for measurement. According to the requirements of the kits, the reagents were added accordingly, SOD activity was measured at 450 nm, POD activity was measured at 470 nm, and CAT activity was measured at 240 nm. These assays allowed for the quantification of enzymatic activities in the berry samples, providing insights into their antioxidant capabilities and potential health benefits.

2.7. Molecular docking of C3G with antioxidant enzymes

The three-dimensional structures of SOD, POD, and CAT were obtained from the protein database. Ligands were drawn using ChemDraw (v.19.0). Subsequently, water molecules and ligands were removed using PyMOL software (PyMOL Molecular Graphics System, San Carlos, CA, USA). Molecular docking was conducted with AutoDock Tools (ADT, v.1.5.6), and the binding modes of the ligand-receptor interactions were visualized using PyMOL and Discovery Studio (Discovery Studio 2019 Client). (Sui et al., 2016).

2.8. Statistical analysis

The experimental results were analyzed using ANOVA, with statistical analysis conducted using SPSS 26.0 software. Data are presented as mean ± SD (standard deviation), where $p \leq 0.05$ was considered statistically significant. Principal Component Analysis (PCA) was carried out using Origin 2021.

3. Results and discussion

3.1. Comparative and quantitative analysis of anthocyanins in blue honeysuckle, bilberry, and cranberry

Anthocyanins in blue honeysuckle, bilberry, and cranberry were detected using HPLC-ESI-QTOF-MS^2. the chromatograms are presented (Fig. 1ABCD). The cyanidin-3-glucoside (C3G) was used as the reference standard for quantification of anthocyanins in blue honeysuckle, bilberry, and cranberry.

3.1.1. Qualitative analysis on anthocyanins of blue honeysuckle, bilberry, and cranberry

Anthocyanins in blue honeysuckle berries were detected and are presented in Fig. 1A and Table 1. Fourteen anthocyanins were identified in blue honeysuckle berries. Compound A1 was identified as cyanidin-3,5-diglucoside with an exact mass at *m/z* 611.1637 and product ions at *m/z* 449.1075 and 287.0541. Compound A2, with an exact mass of m/z 625.1770 and fragment ions at m/z 463.1233 and 301.0704, was identified as peonidin-3,5-diglucoside. Compounds A3, A5, A8, and A10 had a fragment at *m/z* 287, which is the same ionic fragment as cyanidin aglycon. The exact masses of A3, A5, A8, and A10 are 449.083, 595.1662, 419.0973, and 491.1186, respectively. They were identified as cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-xyloside, and cyanidin-3-(6″-acetyl)-glucoside, respectively (Fujita et al., 2020).

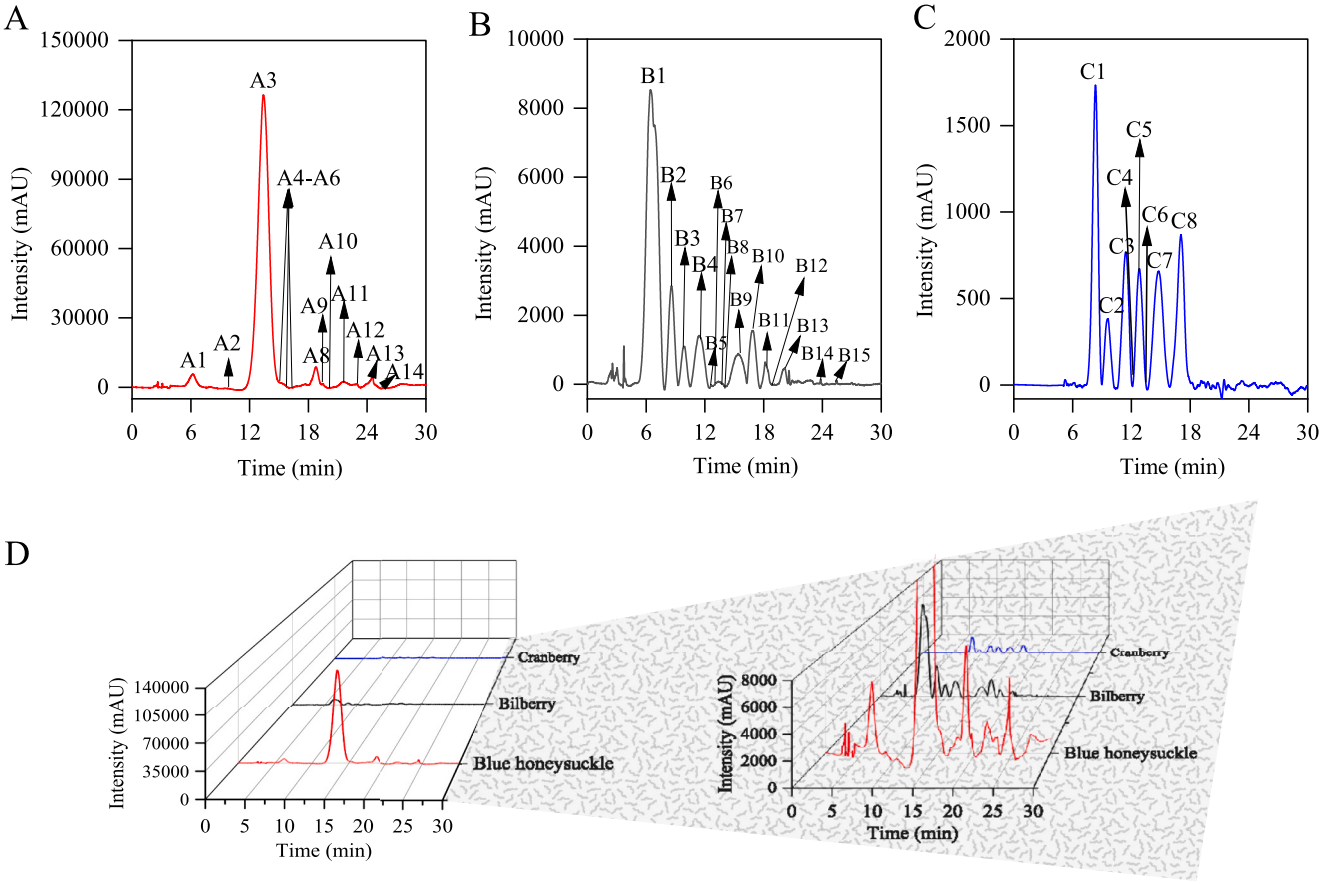


Fig. 1. HPLC chromatogram (UV520nm) of anthocyanins in blue honeysuckle (A), bilberry (B), and cranberry (C). Chromatographic contrast and magnification contrast of three kinds of berries (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Qualitative and quantitative analysis of anthocyanins in blue honeysuckle.

Peak	Retention time (min)	Chemical formula	MW	$[M-H]^-$ (m/z)	Exact mass (m/z)	MS^2 (m/z)	Error (ppm)	Tentative assignment	λ_{max} (nm)	Content (mg/100 g DW)
Blue honeysuckle Anthocyanins										
A1	6.58	$C_{27}H_{31}O_{16}$	611	611.1620	611.1637	449.1075, 287.0541	−2.69	Cyanidin-3,5-diglucoside	511, 270	96.21 ± 1.94
A2	9.76	$C_{28}H_{33}O_{16}$	625	625.1770	625.1770	463.1233, 301.0704	−0.01	Peonidin-3,5-diglucoside	512	0.61 ± 0.02
A3	12.52	$C_{21}H_{21}O_{11}$	449	449.1078	449.083	287.0556	−1.01	Cyanidin-3-glucoside	516, 279	5686.28 ± 128.25
A4	13.57	$C_{22}H_{23}O_{11}$	463	463.1712	463.1712	301.0337	2.02	Peonidin-3-glucoside	519, 245	7.76 ± 0.18
A5	14.83	$C_{27}H_{31}O_{15}$	595	595.1666	595.1662	287.0554	0.65	Cyanidin-3-rutinoside	518, 277	5.33 ± 0.17
A6	16.49	$C_{21}H_{21}O_{10}$	433	433.1130	433.1133	271.0609	−0.74	Pelargonidin-3-glucoside	531, 278	5.93 ± 0.22
A7	19.28	$C_{28}H_{33}O_{15}$	609	609.1820	609.1819	301.0710	0.06	Peonidin-3-rutinoside	523, 276	0.52 ± 0.02
A8	19.98	$C_{20}H_{19}O_{10}$	419	419.0974	419.0973	287.0559	0.12	Cyanidin-3-xyloside	525, 278	70.33 ± 0.96
A9	20.14	$C_{23}H_{31}O_{14}$	531	531.2048	531.2047	369.1530	0.13	Peonidin-3-glucoside pyruvic derivative	521, 276	0.06 ± 0.01
A10	22.20	$C_{23}H_{23}O_{12}$	491	491.1187	491.1186	287.0552	0.06	Cyanidin-3-(6″-acetyl)-glucoside	521, 276	0.02 ± 0.01
A11	23.46	$C_{26}H_{29}O_{14}$	597	597.1254	597.1454	303.0506	0.04	Delphinidin-3-sambubioside	511, 252	0.02 ± 0.01
A12	24.08	$C_{26}H_{29}O_{15}$	581	581.1507	581.1506	287.0558, 449.1096	0.12	Cyanidin-3-sambubioside	549, 252	0.85 ± 0.03
A13	24.41	$C_{27}H_{31}O_{16}$	611	611.4102	611.1618	303.0494	−0.43	Delphinidin-3-rutinoside	547, 252	39.82 ± 1.46
A14	24.82	$C_{21}H_{21}O_{12}$	465	465.1527	465.1521	303.0508	1.13	Delphinidin-3-glucoside	526, 320	0.36 ± 0.02
Total anthocyanins content (TAC)										5914.09 ± 125.63
Bilberry anthocyanins										
B1	8.81	$C_{21}H_{21}O_{11}$	449	449.1081	449.1080	287.0548	0.24	Cyanidin-3-galactoside	523, 276	864.98 ± 18.73
B2	9.69	$C_{22}H_{23}O_{11}$	479	479.1223	4,791,224	317.0652	−0.25	Petunidin-3-galactoside	519, 282	68.18 ± 1.50
B3	10.71	$C_{22}H_{23}O_{12}$	479	479.2440	479.2440	317.0643	−0.03	Petunidin-3-glucoside	521, 271	70.96 ± 1.83
B4	12.47	$C_{20}H_{19}O_{10}$	419	419.0974	419.0976	287.0542	−0.36	Cyanidin-3-arabinoside	493, 298	90.35 ± 0.80
B5	12.59	$C_{21}H_{21}O_{11}^{+}$	449	449.1080	449.1081	287.0549	−0.19	Cyanidin-3-glucoside	515, 271	72.34 ± 2.89
B6	12.82	$C_{22}H_{23}O_{11}$	463	463.1446	463.1473	301.0707	−5.65	Peonidin-3-galactoside	520, 296	9.32 ± 0.12
B7	13.03	$C_{21}H_{21}O_{11}$	449	449.1257	449.1256	317.0666	0.19	Petunidin-3-arabinoside	525, 271	5.53 ± 0.21
B8	13.13	$C_{22}H_{23}O_{11}$	463	463.1230	463.0505	301.0719, 286.0470	−0.01	Peonidin-3-glucoside	493, 271	8.46 ± 0.09
B9	15.82	$C_{23}H_{25}ClO_{12}$	493	493.1342	493.1337	331.0807	1.10	Malvidin-3-galactoside	538, 299	16.76 ± 0.30
B10	16.22	$C_{21}H_{21}O_{11}$	433	433.1129	433.1131	301.0703	−0.40	Peonidin-3-arabinoside	525, 296	76.45 ± 3.58
B11	17.10	$C_{23}H_{25}O_{12}$	493	493.1164	493.1169	331.0820	−0.98	Malvidin-3-glucoside	526, 296	0.14 ± 0.01
B12	17.55	$C_{21}H_{21}O_{11}$	463	463.2285	463.2288	331.0812	−0.59	Malvidin-3-arabinoside	538, 271	0.13 ± 0.01
B13	19.05	$C_{21}H_{21}O_{12}$	465	465.1029	465.1026	303.0487	0.61	Delphinidin-3-galactoside	539, 271	0.27 ± 0.05
B14	23.54	$C_{21}H_{21}O_{12}$	465	465.1723	465.1729	303.0504	−1.32	Delphinidin-3-glucoside	528, 242	2.16 ± 0.08
B15	25.05	$C_{20}H_{19}O_{11}$	435	435.0923	435.0924	303.0514	−0.19	Delphinidin-3-arabinoside	520, 271	0.01 ± 0.01
Total anthocyanins content (TAC)										1286.04 ± 18.48
Cranberry Anthocyanins										
C1	8.49	$C_{21}H_{21}O_{11}$	449	449.1082	449.1082	287.0545	0.19	Cyanidin-3-galactoside	516, 275	211.20 ± 4.14
C2	10.25	$C_{20}H_{19}O_{10}$	419	419.0975	419.0976	287.0561	−0.20	Cyanidin-3-arabinoside	545, 320	13.46 ± 0.50
C3	12.61	$C_{22}H_{23}O_{11}$	463	463.1237	463.1237	301.0709	−0.15	Peonidin-3-galactoside	515, 278	97.79 ± 0.74
C4	12.96	$C_{21}H_{21}O_{10}$	433	433.1125	433.1125	271.0605	−1.41	Pelargonidin-3-glucoside	515, 283	11.71 ± 0.02

(*continued on next page*)

Table 1 (*continued*)

Peak	Retention time (min)	Chemical formula	MW	$[M-H]^-$ (*m/z*)	Exact mass (*m/z*)	MS^2 (*m/z*)	Error (ppm)	Tentative assignment	λ_{max} (nm)	Content (mg/100 g DW)
C5	13.61	$C_{21}H_{21}O_{11}$	449	449.1082	449.1082	287.0579	0.06	Cyanidin-3-glucoside	516,280	23.65 ± 0.42
C6	14.54	$C_{22}H_{23}O_{11}$	463	463.1238	463.1238	301.0712, 286.0477	−0.09	Peonidin-3-glucoside	521, 325	3.56 ± 0.05
C7	15.98	$C_{22}H_{23}O_{11}$	433	433.2838	433.2839	301.0712	−0.17	Peonidin-3-arabinoside	520, 271	24.02 ± 0.25
C8	17.89	$C_{28}H_{33}O_{16}$	625	625.1762	625.1760	463.1308, 301.0698	0.41	Peonidin-3,5-dihexoside	510, 277	82.09 ± 1.10
Total anthocyanins content (TAC)										467.48 ± 2.51

Data are expressed as mean ± standard deviation (n = 3).

Extraction of *m/z* 463 yielded a fragment ion at m/z 301, identified as peonidin-3-glucoside and labeled as A4. Compound A6 had a molecular ion at *m/z* 433 and produced a fragment at *m/z* 271.0609, corresponding to pelargonidin, and was tentatively identified as pelargonidin-3-glucoside. Compound A7 had a molecular ion at m/z 609 and produced a fragment at m/z 301.0710, corresponding to peonidin, and was identified as peonidin-3-rutinoside. Compound A9 had a molecular ion at *m/z* 531 and produced a fragment at m/z 369, corresponding to peonidin, and was identified as peonidin-3-glucoside pyruvic derivative (Guo et al., 2023; Zhang et al., 2023). Compounds A11, A13, and A14 produced the same fragment at *m/z* 303, corresponding to delphinidin aglycon, and were identified as delphinidin-3-sambubioside, delphinidin-3-rutinoside, and delphinidin-3-glucoside, respectively. Compound A12 was identified as cyanidin-3-sambubioside with an exact mass of *m/z* 581.1506 and product ions at *m/z* 287.0558 and 449.1096 (Gorzelany et al., 2023; Ruiz et al., 2013).

The anthocyanins in bilberry were detected and are presented in Fig. 1B and Table 1. Fifteen anthocyanins were identified in bilberry. Compound B1 (*m/z* 449), B4 (m/z 419), and B5 (m/z 449) had a fragment ion at m/z 287. B1 and B5 share the same molecular mass of 449, but due to different retention times, they were identified as cyanidin-3-galactoside and cyanidin-3-glucoside, respectively. B4 was identified as cyanidin-3-arabinoside. Compounds B2 and B3, both with a molecular ion of 479 and producing a fragment at *m/z* 317, were identified as petunidin-3-galactoside and petunidin-3-glucoside, respectively, based on their differing peak times (Benvenuti et al., 2018; Chen et al., 2020). Compounds B6 (*m/z* 463), B8 (*m/z* 463), and B10 (m/z 433) yielded an ion at m/z 301, corresponding to peonidin, and were identified as peonidin-3-galactoside, peonidin-3-glucoside, and peonidin-3-arabinoside, respectively, based on their peak times (Benvenuti et al., 2018; Paun et al., 2010). B7 was identified as petunidin-3-arabinoside with a fragment ion at *m/z* 317. Compounds B9 (m/z 493), B11 (m/z 493), and B12 (m/z 463) had a fragment ion at m/z 331, corresponding to malvidin, and were identified as malvidin-3-galactoside, malvidin-3-glucoside, and malvidin-3-arabinoside, respectively (Chai et al., 2021; Paun et al., 2010). Compounds B13 (m/z 465), B14 (m/z 465), and B15 (m/z 435) had a fragment ion at m/z 303, corresponding to delphinidin, and were tentatively assigned as delphinidin-3-galactoside, delphinidin-3-glucoside, and delphinidin-3-arabinoside (Colak et al., 2016).

The anthocyanins in cranberry were detected and are presented in Fig. 1C and Table 1. Eight anthocyanins were identified in cranberry. Compounds C1 (m/z 449), C2 (m/z 419), and C5 (m/z 449) had a fragment ion at m/z 287, corresponding to cyanidin, and were tentatively assigned as cyanidin-3-galactoside, cyanidin-3-arabinoside, and cyanidin-3-glucoside, respectively. Compounds C3 (m/z 463), C6 (m/z 463), C7 (m/z 433), and C8 (m/z 625) all presented a fragment ion at m/z 301. C6 produced an additional fragment at m/z 286, and C8 produced additional fragment ions at m/z 463. C6 was identified as peonidin-3-glucoside, and C8 was identified as peonidin-3,5-dihexoside. C3 and C7 were identified as peonidin-3-galactoside and peonidin-3-arabinoside, respectively. Compound C4 had a molecular ion at m/z 433 and a fragment ion at m/z 271, corresponding to pelargonidin, and was identified as pelargonidin-3-glucoside (Karaaslan & Yaman, 2016; Xie et al., 2020; Yuan, 2018).

3.1.2. Quantitative and comparable analysis on anthocyanins of blue honeysuckle, bilberry, and cranberry

Fourteen anthocyanins were identified in blue honeysuckle through HPLC mass spectrometry analysis (Table 1). Cyanidin-3-glucoside was the predominant anthocyanin, constituting 96% of the total anthocyanins with a concentration of 5686.28 mg/100 g DW, consistent with previous studies (Gorzelany et al., 2023; Guo et al., 2023; Zhang et al., 2023). The contents of other anthocyanins in blue honeysuckle berries were as follows: cyanidin-3,5-diglucoside (96.21 mg/100 g DW), peonidin-3,5-diglucoside (0.61 mg/100 g DW), peonidin-3-glucoside (7.76 mg/100 g DW), cyanidin-3-rutinoside (5.33 mg/100 g DW), pelargonidin-3-glucoside (5.93 mg/100 g DW), peonidin-3-rutinoside (0.52 mg/100 g DW), cyanidin-3-xyloside (70.33 mg/100 g DW), peonidin-3-glucoside pyruvic derivative (0.06 mg/100 g DW), cyanidin-3-(6″-acetyl)-glucoside (0.02 mg/100 g DW), delphinidin-3-sambubioside (0.02 mg/100 g DW), cyanidin-3-sambubioside (0.85 mg/100 g DW), delphinidin-3-rutinoside (39.82 mg/100 g DW), and delphinidin-3-glucoside (0.36 mg/100 g DW). The total anthocyanin content in blue honeysuckle was 5914.09 mg/100 g DW. In comparison, the predominant anthocyanin in bilberry and cranberry was cyanidin-3-galactoside, with concentrations of 864.98 mg/100 g DW and 211.20 mg/100 g DW, respectively. The total anthocyanin content in bilberry and cranberry was 1286.04 mg/100 g DW and 467.48 mg/100 g DW, respectively.

3.2. Detection and comparison of total phenolic content (TPC), and total flavone content (TFC) of blue honeysuckle, bilberry, and cranberry

The content of TPC and TFC were measured in blue honeysuckle, bilberry, and cranberry, as shown in Fig. 2A, Blue honeysuckle exhibited the highest TPC, reaching 196.84 mg GAE/g DW, which significantly exceeded that of bilberry (17.82 mg GAE/g DW) and cranberry (13.39 mg GAE/g DW). These findings are consistent with previous studies (Juríková et al., 2020; Li et al., 2019; Urbonaviciene et al., 2022). The TPC in bilberry was higher than in cranberry, with cranberry showing the lowest TPC among the three. Similarly, blue honeysuckle had the highest TFC content, at 495.72 mg CE/g DW, which was significantly higher than that of bilberry (59.71 mg CE/g DW) and cranberry (52.61 mg CE/g DW). The TPC content in cranberry is comparable to previous reports (Oszmiański et al., 2017), and the TFC content in bilberry was higher than in cranberry. These results align with those of previous studies on blue honeysuckle, bilberry, and cranberry (Abeywickrama et al., 2016; Chen et al., 2014; Çolak et al., 2017; Ștefănescu et al., 2020).

3.3. Comparison of antioxidant capacity in blue honeysuckle, bilberry, and cranberry

The data on antioxidant capacity in blue honeysuckle, bilberry, and

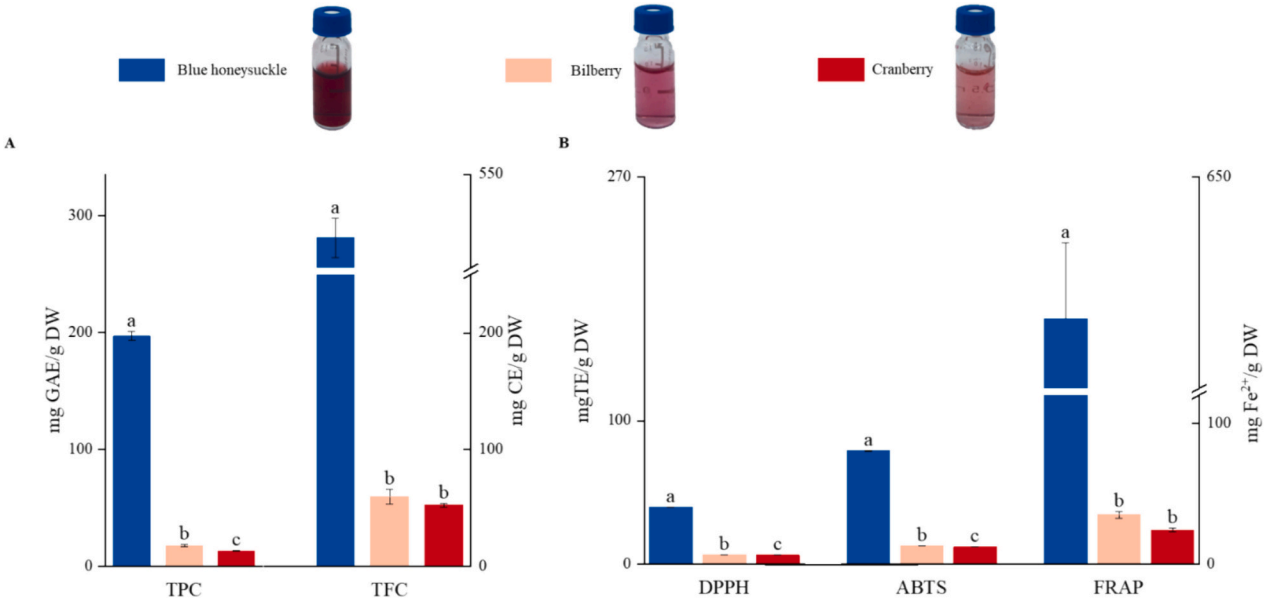


Fig. 2. (A) TPC and TFC of blue honeysuckle, bilberry, and cranberry, (B) DPPH, ABTS, and FRAP of blue honeysuckle, bilberry, and cranberry (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cranberry was shown in Fig. 2B and Fig. 5. Blue honeysuckle exhibited the highest antioxidant capacity compared to bilberry and cranberry, with values of 40.03 mg TE/g DW for DPPH, 79.12 mg TE/g DW for ABTS, and 549.52 mg $FeSO_4{\cdot}7H_2O$/g DW for FRAP. There were also significant differences in DPPH values among different varieties, indicating that different berries have varying antioxidant capacities (Česonienė et al., 2021). Bilberry had a DPPH antioxidant capacity of 6.61 mg TE/g DW, an ABTS antioxidant capacity of 12.94 mg TE/g DW, and a FRAP value of 35.2 mg $FeSO_4{\cdot}7H_2O$/g DW. Cranberry had a DPPH antioxidant capacity of 6.44 mg TE/g DW, an ABTS antioxidant capacity of 12.30 mg TE/g DW, and a FRAP value of 24.39 mg $FeSO_4{\cdot}7H_2O$/g DW. Both bilberry and cranberry had comparatively lower antioxidant capacities than blue honeysuckle. The differences in antioxidant capacities could be attributed to the varying levels of anthocyanins, TPC, and TFC, which align with previous reports. Additionally, the FRAP value of blue honeysuckle is higher than that of blueberry (De Silva & Rupasinghe, 2021).

3.4. Molecular docking analysis of SOD, POD, CAT and C3G

As shown in Fig. 3A. the activities of SOD, POD, and CAT in blue honeysuckle were higher than those in bilberry and cranberry. Similarly, the activities of SOD, POD, and CAT in bilberry were higher than those in cranberry. Among the three berries, CAT activity was the lowest, with values of 1043 U/g, 563.32 U/g, and 74.19 U/g for blue honeysuckle, bilberry, and cranberry, respectively. Conversely, POD activity was the highest, with values of 45,525.65 U/g, 35,544.64 U/g, and 10,717.82 U/g, respectively. In summary, the antioxidant enzyme activity varies significantly among different berries and even within different varieties of the same berry (Chen et al., 2022; Sun et al., 2018). The ligand-receptor complex posture of C3G was obtained through molecular docking with three antioxidant enzymes: SOD, POD, and CAT. The binding energies were − 0.26 Kcal/mol, −1.58 Kcal/mol, and − 0.24 Kcal/mol, respectively, as shown in Fig. 3B and C. Van der Waals forces form between C3G and SOD residues Lys27, Asp19, Glu22, Gly95, Ala94, and Pro93, affecting the selectivity and affinity of the molecular docking and binding process. A non-covalent interaction occurs between the π electron system of the aromatic ring of C3G and the alkyl chain of Ala20, enhancing the binding stability of C3G and SOD. The amino group of C3G forms a hydrogen bond with the POD residue Thr194, enhancing the stability between C3G and POD. A C—H bond forms with Gln201, and its binding stability is improved by van der Waals forces and hydrophobic interactions. Another non-covalent interaction occurs between the π electron system of the aromatic ring of C3G and the alkyl chain of Ala190 in POD. A stable covalent bond is preliminarily confirmed at Pro188. Additionally, Asp189 residues form unfavorable donor-donor interactions, which may affect the stability of intermolecular binding to some extent. C3G interacts with CAT receptor proteins mainly through amino acid residues in the active pocket, including Ala52, Glu53, Lys54, Asp223, Gly224, Lys226, Ala227, and Glu229. C3G binds to CAT receptor proteins via van der Waals forces, hydrogen bonding, and π-π stacking interactions. Specifically, the hydroxyl group of C3G forms a stable hydrogen bond with the D atom of Lys226. In conclusion, C3G exhibits lower binding energy and higher affinity with POD among the three antioxidant enzymes, which may explain the higher activity of the POD enzyme (Rahman et al., 2021; Sui et al., 2016).

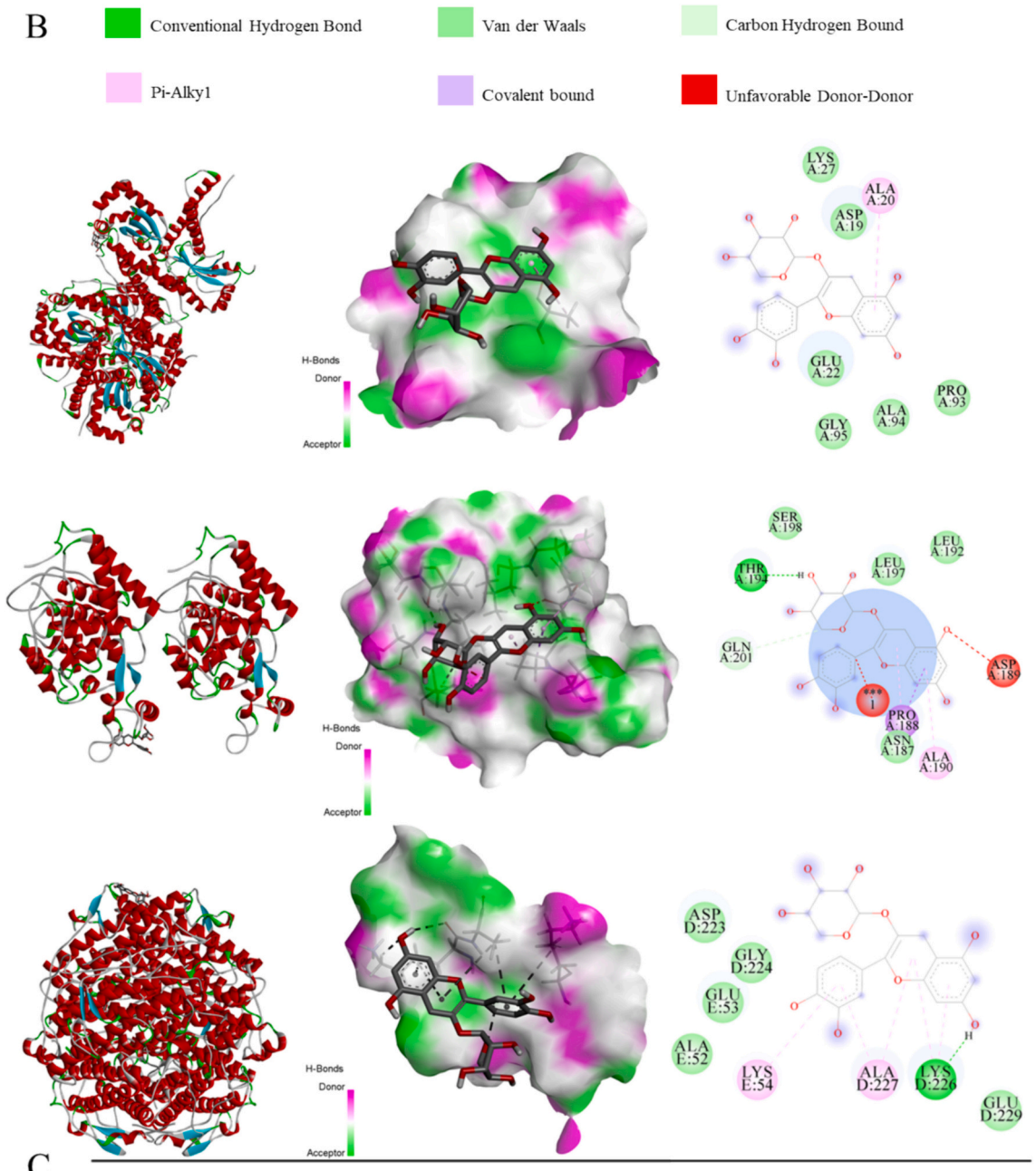
3.5. PCA and Correlation analysis of TPC, TFC, TAC, DPPH, ABTS, and FRAP

The initial principal component (PC1), which accounted for 88.27% of the variance, was positively correlated with SOD, POD, CAT, TAC, TPC, TFC, ABTS, FRAP, and DPPH. The second principal component (PC2), explaining 11.58% of the variance, was negatively correlated with the same variables, the results shown in Fig. 4A. The results of Pearson correlation analysis of TAC, TPC, TFC, DPPH, ABTS, FRAP, SOD, POD, and CAT were presented in Fig. 4B. TPC showed a strong correlation with TAC of blue honeysuckle berries, the result agrees with a previous report (Zhang et al., 2023). The correlation coefficient between FRAP and TPC was strong across all three types of berries, similar to previous findings (Chen et al., 2014; Kolarov et al., 2021). In cranberry berries, TPC showed the strongest correlation with ABTS, with an r value of 0.96, aligning with earlier reports (Kraujalytė et al., 2013; Oszmiański et al., 2017). The differences in antioxidant capacities among blue honeysuckle, bilberry, and cranberry may be attributed to variations in the composition of TPC, TFC, and anthocyanins in these berries (Gramza-Michałowska et al., 2019).

A

	Blue honeysuckle	Bilberry	Cranberry
SOD (U/g)	1761.17±107.33[a]	1462.31±77.59[b]	219.68±2.62[c]
POD (U/g)	45525.65±630.96[a]	35544.64±1852.87[b]	10717.82±61.07[c]
CAT (U/g)	1043.24±23.05[a]	563.32±10.95[b]	74.19±5.94[c]

B



C

	SOD	POD	CAT
Biding energy Kcal/mol	-0.26	-1.58	-0.24

Fig. 3. SOD, POD, CAT activity of blue honeysuckle, bilberry, cranberry (A). Molecular docking model for the C3G and SOD, POD, and CAT (B). Different letters represent significant differences ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

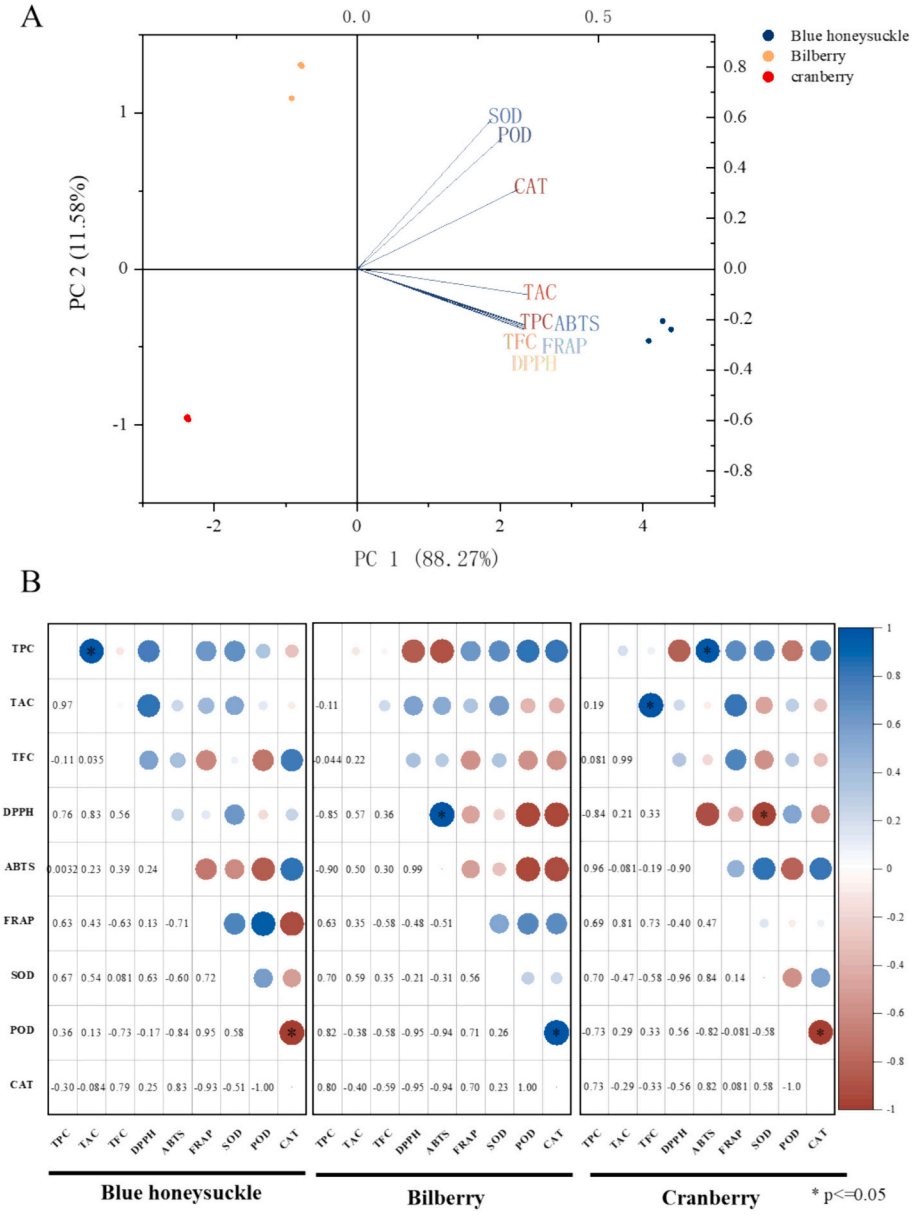


Fig. 4. PCA (A). Correlation coefficients of TPC, TFC, DPPH, ABTS, and FRAP (B).

4. Conclusions

Blue honeysuckle, bilberry, and cranberry contain different compositions of anthocyanins as tested by HPLC-ESI-QTOF-MS^2. Fourteen anthocyanins were detected in blue honeysuckle, fifteen in bilberry, and eight in cranberry. Cyanidin-3-glucoside was the predominant anthocyanin in blue honeysuckle. In cranberry, cyanidin-3-galactoside and peonidin-3-galactoside were the main anthocyanins, while bilberry exhibited the highest diversity of anthocyanins. Among the plant materials used in this experiment, blue honeysuckle had the highest TPC and TFC content compared to bilberry and cranberry. Consequently, the antioxidant capacity of blue honeysuckle was significantly stronger than that of bilberry and cranberry. The DPPH, ABTS, and FRAP values of blue honeysuckle were 4003.28 mg Trolox/100 g DW, 549.52 mg $FeSO_4{\cdot}7H_2O$/g DW, and 79.12 mg Trolox/g DW, respectively. Blue honeysuckle also showed higher antioxidant enzyme activity, with SOD, POD, and CAT values of 1761.17 U/g, 45,525.65 U/g, and 1043.24 U/g, respectively. C3G has a high affinity with POD, possibly explaining why blue honeysuckle has the highest antioxidant capacity. While there was a significant difference in total phenolic content between bilberry and cranberry, there was no significant difference in their antioxidant capacities.

CRediT authorship contribution statement

Liangchuan Guo: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Jinli Qiao:** Visualization, Investigation. **Muzyka Sergey Mikhailovich:** Investigation. **Limei Wang:** Visualization. **Yuxi Chen:** Investigation. **Xuefei Ji:** Investigation. **Haihui She:** Investigation. **Lijun Zhang:** Visualization. **Yan Zhang:** Methodology. **Junwei Huo:** Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

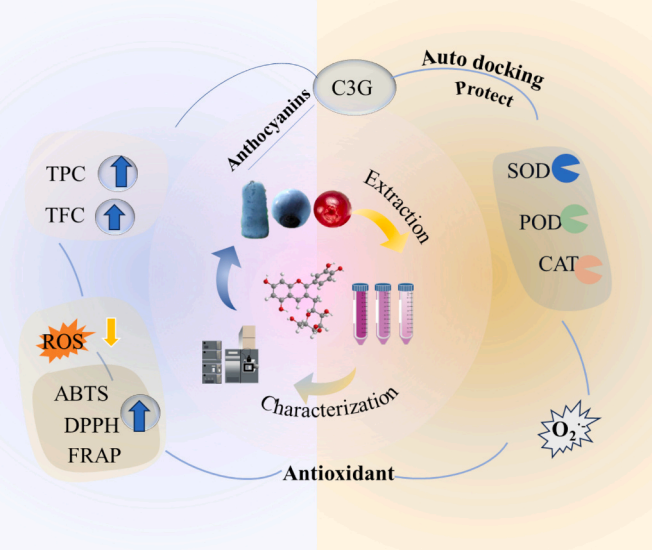


Fig. 5. The anthocyanins in three kinds of berries were qualitatively and quantitatively studied, C3G was molecule-docked with SOD, POD and CAT antioxidant enzymes, ABTS, DPPH, FRAP, TPC and TFC were determined for three types of berries.

Data availability

Data will be made available on request.

Acknowledgments

We gratefully acknowledge the financial support received from the National Key R&D Program of China (2022YFD1600500), and the Reform and Development Fund for Heilongjiang Provincial Undergraduate Universities Supported by the Central Government (High-level Talents Project), and China Agriculture Research System of MOF and MARA (CARS-29-10), and Heilongjiang Province "2023 College Students Entrepreneurship Assistance Plan".

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