Comparative analysis of the polyphenols profiles and the antioxidant and cytotoxicity properties of various blue honeysuckle varieties

Abstract: The polyphenol profile, antioxidant activity (particularly peroxyl radical-scavenging capacity (PSC) and cellular antioxidant activity (CAA)) and cytotoxicity of extracts from seven varieties of blue honeysuckle were compared in this study. Moreover, an analysis of correlations between individual polyphenol profiles and antioxidant activities was also conducted. Seventeen components were found in the investigated blue honeysuckle extracts, with anthocyanins being the prominent bioactive components among polyphenols. Fruit peel contained higher concentrations of individual anthocyanins compared to the fruit pulp. Beilei blue honeysuckle had the highest antioxidant activity. Correlation analysis showed that antioxidant activity values were highly associated with cyanidin-3-glucoside levels. Blue honeysuckle extracts were not cytotoxic over the range of doses tested. Among the varieties analyzed, Beilei possessed the highest antioxidant properties and was found to be the most appropriate source of natural antioxidants.

Keywords: blue honeysuckle; polyphenol profiles; antioxidant activity; cytotoxicity.

1 Introduction

Berry fruits have been regarded as rich sources of nutrients and phytochemicals, such as phenolic acids, flavonoids, and anthocyanins. These phytochemicals have been reported to exhibit powerful antioxidant, anti-inflammatory, anticancer, antimicrobial, and anti-aging activities [1-3]. Additionally, some polyphenols have been found to act as neurohormetics by stimulating several cellular signaling pathways [4-7]. Hence, long-term intake of berries rich in nutrients and phytochemicals reduces the incidence of cardiovascular and degenerative disorders and has a positive impact on human health [8].

Blue honeysuckle (Lonicera caerulea L.), belonging to the family Caprifoliaceae, is mainly found in Russia, Japan, Korea, Inner Mongolia, northeast China, and parts of North America [9]. Blue honeysuckle is rich in polyphenols, contributing to its potential bioactive properties; its polyphenol content is higher than that of some other berries, such as blueberry and raspberry [10,11]. The main identified components of blue honeysuckle are shikimic acid, 5-O-cafeoylquinic acid, 3-O-cafeoylquinic acid, cyanidin-3-glucoside, cyanidin-3,5-diglucoside,peonidin-3,5-diglucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside [12]. Additionally, iridoid compounds including loganic acid, 7-epi-loganic acid, 8-epi-loganic acid, 7-O-pentoside, loganan, sweroside, secologanin and pentoxyloganic acid, which have anti-inflammatory properties and reduce the risk of metabolic diseases and cardiovascular disease, were identified in blue honeysuckle [13,14]. Blue honeysuckle extracts have been found to exhibit extensive positive biological effects, such as anti-inflammatory [15,16,17], anti-microbial [18], anti-radiation activities [19]. However, even in different cultivars within a species or different tissues within a fruit, the composition of phytochemicals can vary widely due to genetic variation, growth conditions, degree of maturity and harvesting season, which results in differences in their bioactive properties [20,21]. The in vitro or in vivo activities of different varieties of blueberry and sea buckthorn vary dramatically, as do their phytochemical profiles [22,23].

Although the composition and some biological activities of blue honeysuckle have been reported [9,12,24,25], previous studies mainly focused on a limited number of varieties of wild blue honeysuckle. Moreover,
the antioxidant activities of these molecules have only been assessed chemically, usually in terms of oxygen radical absorbance capacity, peroxyl radical-scavenging capacity (PSC), DPPH radical-scavenging capacity, total oxygen radical-scavenging capacity, and ferric-reducing antioxidant power [26,27]. Hence, there is a need to identify polyphenols in more varieties of blue honeysuckle and to assay antioxidant activities using the cellular antioxidant activity (CAA) assay, which is a more accurate measure of CAA and accounts for the absorption, metabolism, and localization of antioxidants in cells [28].

The objectives of this study were (1) to identify and compare the polyphenols profiles, specifically focusing on anthocyanins, of seven varieties of blue honeysuckle; (2) to determine the antioxidant activities, including the PSC, CAA, and DPPH and ABTS radical-scavenging capacity in such varieties; and (3) to analyze the antiproliferative activity against HepG2 human liver cancer cells. In addition, a correlation analysis of these findings with extracellular and cellular antioxidant activities and polyphenols profile composition was performed. The data highlight blue honeysuckle as a potential functional and health food mainly owing to its polyphenol profiles.

2 Materials and methods

2.1 Chemicals and reagents

Analytical grade methanol, ethanol, hexane, ethyl acetate, hydrochloric acid, acetic acid, potassium chloride, sodium acetate, sodium carbonate, sodium hydroxide, potassium phosphate monobasic, and potassium phosphate dibasic were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, USA). 2′,7′-Dichlorofluorescein diacetate, fluorescein disodium salt, reagent grade sodium borohydride, analytical-grade chloranil and vanillin, catechin hydrate, Folin–Ciocalteu reagent, ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, chromatography-grade trifluoroacetic acid and acetonitrile, cholera toxin, hydrocortisone, penicillin, streptomycin, and gentamicin were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Analytical grade tetrahydrofuran was obtained from Fisher Scientific (Fair Lawn, NJ, USA), while gallic acid was purchased from ICN Biomedicals, Inc. (Aurora, OH, USA). 2,2′-Azobis(2-amidinopropane) dihydrochloride was purchased from Wako Chemicals (Richmond, USA), while HepG2 human hepatoma cells were obtained from the American Type Culture Collection (HB-8065). Williams' medium E, Hank's balanced salt solution, epidermal growth factor, heparin, insulin, and other cell culture reagents were purchased from Gibco U.S. Biotechnology Co. Inc. (Richmond, VA, USA).

2.2 Blue honeysuckle samples

Wild, L-5, Haskap, HSY-24, BKQE, BLS and Beilei blue honeysuckle were harvested in June 2017 from the planting station at the College of Horticulture, Northeast Agricultural University (45°44′25.18″N, 126°43′22.50″E), Haerbin, Heilongjiang province, China. The plants were transported to the College of Food Science, Shenyang Agricultural University, China and stored at −80°C before extraction.

2.3 Extraction of phenolic compounds

For HPLC-MS/MS, ABTS, DPPH, PCS, CAA and cytotoxicity analyses, the extracts were prepared using our previously described method with some modifications [11]. Berries were homogenized after thawing. Homogenate (300 g) was extracted in 600 mL 80% aqueous acetone for 90 min in an ultrasonic bath, and the mixture was clarified by vacuum filtration. The residue was re-extracted using the above procedure until the solvent remained colorless. Extract supernatants were then combined and subjected to rotary evaporation (RE-5203A, Shanghai Bilon Instruments Co., Ltd., China) at 45°C until no acetone remained. The concentrated solution was filtered and then separated through a 400 mL glass column loaded with nonionic polystyrene–divinylbenzene resin (D101, Shanghai, China) at 4°C. Deionized water was passed through the column to remove water-soluble substances, and acetone was used as the eluting solvent. After concentration in the rotary evaporator at 45°C, the collected liquid was freeze-dried into powder using a vacuum freeze dryer (LGO.2, Shenyang Aerospace Xinyang Quick Freezing Equip. Manuf. Co., Ltd., China). The obtained powder was placed in sealed 2-mL centrifuge tubes and stored at -20°C until analysis. The extraction yields of the seven varieties of blue honeysuckle ranged from 1.5% to 2.7% (Table 1).

2.4 Identification of phenolic compounds

Peel and pulp were separated using tweezers after the berries were thawed. Phenolic compounds in the peel and pulp of seven varieties of blue honeysuckle were extracted
Table 1: Extraction yields of the seven varieties of blue honeysuckle. Values are expressed as means ± SD (n = 5).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Extraction yield (% FW)</th>
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<tbody>
<tr>
<td>L-5</td>
<td>1.5 ± 0.27</td>
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<tr>
<td>Haskap</td>
<td>2.3 ± 0.25</td>
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<tr>
<td>Wild</td>
<td>2.7 ± 0.14</td>
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<tr>
<td>HSY-24</td>
<td>1.7 ± 0.31</td>
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<tr>
<td>BKQE</td>
<td>2.5 ± 0.47</td>
</tr>
<tr>
<td>BLS</td>
<td>1.8 ± 0.22</td>
</tr>
<tr>
<td>Beilei</td>
<td>2.4 ± 0.18</td>
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</tbody>
</table>

*Extraction yields (% FW) were calculated using the following formula: extraction yield (%) = W_{extract}/W_{fruits}×100% (n=5).

2.5 ABTS assay

Free radical-scavenging activity of the extracts on ABTS free radicals was analyzed according to the procedures previously described by Caprioli et al. [12]. ABTS was oxidized using MnO₂ in distilled water for 30 min in the dark to obtain ABTS•⁻ solution (5 mM). Next, 50 μL aliquots of extracts in different concentrations and 200 μL of ABTS•⁻ solution were added to wells in 96-well microtiter plates. After equilibration for 10 min at room temperature, the absorbance was recorded at 734 nm using a microplate reader (FLUOstar Optima, BMG Labtech microplate reader). The results were expressed as Trolox equivalents (TE) per gram of extract.

2.6 DPPH assay

The levels of DPPH radical-scavenging activity in the extracts were determined using appropriate commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions. The absorbance was determined at 517 nm. Results were expressed as TE per gram of extract.

2.7 PCS assay

Total antioxidant activity was chemically determined by the PSC assay [26]. In this assay, the thermal degradation of 2,2’-azobis(2-aminopropane) produces peroxyl radicals (ROO⁻) that oxidize nonfluorescent dichlorofluorescein to fluorescent dichlorofluorescein. Hence, the inhibition of dichlorofluorescein oxidation radicals is used as the basis for calculating antioxidant activity. Fluorescence was measured on a fluorescent spectrophotometer (Fluoskan Ascent FL, Thermo Electron Corp., Asheville, NC, USA) at an excitation wavelength of 485 nm and emission wavelength of 538 nm. Antioxidant activity was calculated as milligrams of vitamin C equivalents per 100 g fresh weight, and data are reported as mean ± SD of at least three replicates.

2.8 Cell culture

HepG2 cells were grown at 37°C and 5% CO₂ in Williams’ medium E supplemented with 5% fetal bovine serum, 10 mM HEPES, 2 mM L-glutamine, 5 μg/mL insulin, 0.05 μg/mL hydrocortisone, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamycin [29]. Cells between passages 18 and 28 were used for the experiments.

2.9 CAA assay

CAA was measured according to published methods [28]. Briefly, HepG2 cells were seeded at 6 × 10⁴ cells per well on a 96-well microplate containing 100 μL growth medium per well. The growth medium was removed by pipette 24 h post-seeding, and wells were washed with PBS. Triplicate wells were then treated for 1 h with 100 μL medium containing blue honeysuckle extracts and 25 μM dichlorofluorescein diacetate. The wells were then exposed to 600 μM 2,2’-azobis(2-aminopropane) in 100 μL Hank’s balanced salt solution. For comparison, some wells were washed with 100 μL PBS to remove
honeysuckle extracts prior to exposure to 2,2’-azobis(2-amidinopropane). Plates were then read every 5 min for 1 h at an excitation wavelength of 485 nm and an emission wavelength of 538 nm in a Fluoroskan Ascent FL plate reader (Thermo Electron Corp.) operating at 37°C.

2.10 Quantification of CAA

After subtraction of the blank and initial fluorescence, the area under the curve of fluorescence versus time graph was calculated to determine CAA. CAA was expressed as $1 - \frac{\int_{S}A}{\int_{C}A}$, where $\int_{S}A$ is the integrated area under the curve for the test sample, and $\int_{C}A$ is the integrated area under the curve for control samples. The median effective dose ($EC_{50}$) was calculated from a plot of log ($\frac{\int_{a}}{\int_{u}}$) versus log (dose), where $\int_{a}$ is the fraction affected by honeysuckle extracts (CAA units) and $\int_{u}$ is the unaffected fraction (1 – CAA units). Results are reported as micromoles of quercetin equivalents per 100 μg sample.

2.11 Cytotoxicity

Cytotoxicity was measured using the methylene blue assay. Briefly, HepG2 cells were seeded at $4 \times 10^4$ cells/well in a 96-well microplate containing 100 μL growth medium/well and allowed to adhere for 24 h at 37°C. The growth medium was then removed, and cells were washed with PBS. Subsequently, cells were incubated for 24 h at 37°C in 100 μL medium supplemented with or without (vehicle controls) different concentrations of blue honeysuckle extracts. Cells were then washed with PBS, stained for 1 h at 37°C with 50 μL methylene blue (98% Hank’s balanced salt solution, 0.67% glutaraldehyde, and 0.6% methylene blue), and washed in deionized water until the wash was clear. Subsequently, cells were extracted for 20 min on a tabletop oscillator using 100 μL 49% PBS, 50% ethanol, and 1% acetic acid, and the absorbance was measured at 570 nm in a microplate reader. Doses were considered cytotoxic if cell viability decreased by >10% in comparison to the control.

2.12 Inhibition of cell proliferation

Antiproliferative effects were assessed in HepG2 cells by the methylene blue colorimetric method. Briefly, HepG2 cells were seeded at $2.5 \times 10^4$ cells/well in central wells of a 96-well microplate, while peripheral wells were filled only with 100 μL medium. Plates were then incubated at 37°C for 4 h, at which point media were removed and replaced with 100 μL fresh media supplemented with or without 10, 20, 40, 60, 80, and 100 mg/mL blue honeysuckle extracts. Plates were then incubated at 37°C for 96 h and assayed in triplicate with methylene blue as described. Antiproliferative effects are reported as $EC_{50}$ values in milligrams of blue honeysuckle extract per milliliter.

2.13 Statistical analyses

Data were reported as the mean ± SD from three replicates per sample. Significant differences in PCS, CAA, ABTS radical-scavenging, DPPH radical-scavenging, cytotoxicity, and antiproliferative effects among the seven varieties were analyzed by ANOVA and Tukey’s test in SPSS (Statistics for Social Science) version 17.0, with statistical significance set at $p < 0.05$. The correlations between bioactive properties and the content of polyphenols profiles were also analyzed.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and discussion

3.1 Compositions of phenolic compounds in the peel and pulp

The compositions and contents of phenolic compounds detected in both the peel and pulp of blue honeysuckle berries are shown in Table 2. The chromatogram of Beili blue honeysuckle extracts is shown in Figure 1, and peaks 1-5 were identified as cyanidin-3-glucoside, quercetin rutinoside, gentistic acid, chlorogenic acid, and 5-caffeoylquinic acid, respectively. Anthocyanins [belonging to flavonoids] were the main phenolic compounds in all investigated blue honeysuckle samples. Nine anthocyanins, including cyanidin-3,5-dihexoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-acetylhexoside, peonidin-3,5-dihexoside, peonidin-3-glucoside, peonidin-3-rutinoside, pelargonidin-3-glucoside, and delphinidin-3-glucoside, were identified in all varieties of blue honeysuckle. In addition, the identities of three unknown substances, which had MS = 737 [M+H], 897 [M+H], and 765 [M+H] and were suspected to be cyanidin anthocyanins, need to be further confirmed by methods such as NMR. Cyanidin-3-glucoside was the major anthocyanin in the studied cultivars, with markedly higher amounts in the peel than...
Table 2: The identification of major phenolic compounds in extracts from the seven varieties of blue honeysuckle. The results are presented as means ± SD. Cyanidin-3-O-glucoside was used as the standard.

<table>
<thead>
<tr>
<th>Tentative identification</th>
<th>Molecular (m/z)</th>
<th>Fragment (m/z)</th>
<th>Contents (mg/g DW)</th>
<th>L-5 Peel</th>
<th>Pulp</th>
<th>Haskap Peel</th>
<th>Pulp</th>
<th>Wild Peel</th>
<th>Pulp</th>
<th>HSY-24 Peel</th>
<th>Pulp</th>
<th>BKQE Peel</th>
<th>Pulp</th>
<th>BLS Peel</th>
<th>Pulp</th>
<th>Beilei Peel</th>
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<tr>
<td>Anthocyanins</td>
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<tr>
<td>Cyanidin-3,5-dihexoside</td>
<td>611 [M+H]</td>
<td>449, 287</td>
<td>19.7 ± 2.3</td>
<td>41</td>
<td>5.5</td>
<td>0.8</td>
<td>3.9</td>
<td>1.2 ± 0.15</td>
<td>25.9</td>
<td>3.73</td>
<td>0.5</td>
<td>20.6</td>
<td>1.2</td>
<td>20.9</td>
<td>4.1</td>
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<tr>
<td>Cyanidin-3-O-glucoside</td>
<td>449 [M+H]</td>
<td>287</td>
<td>376.8 ± 15.91</td>
<td>78.7</td>
<td>179.3</td>
<td>71.7</td>
<td>437.2</td>
<td>122.4</td>
<td>416.4</td>
<td>68.1</td>
<td>337.5</td>
<td>101.1</td>
<td>408.9</td>
<td>129.1</td>
<td>408.7</td>
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<td>Cyanidin-3-rutinoside</td>
<td>595 [M+H]</td>
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<td>376.8 ± 15.91</td>
<td>78.7</td>
<td>179.3</td>
<td>71.7</td>
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<td>122.4</td>
<td>416.4</td>
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<td>337.5</td>
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<td>129.1</td>
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<td>95.1</td>
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<tr>
<td>Cyanidin-3-acetylhexoside</td>
<td>491 [M+H]</td>
<td>287</td>
<td>376.8 ± 15.91</td>
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<td>179.3</td>
<td>71.7</td>
<td>437.2</td>
<td>122.4</td>
<td>416.4</td>
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<td>129.1</td>
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<td>95.1</td>
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<tr>
<td>Peonidin-3,5-dihexoside</td>
<td>625 [M+H]</td>
<td>463, 301</td>
<td>0.8 ± 0.19</td>
<td>0.2</td>
<td>1.9</td>
<td>0.7</td>
<td>0.3</td>
<td>0.1 ± 0.08</td>
<td>0.2</td>
<td>0.14</td>
<td>0.10</td>
<td>0.21</td>
<td>0.15</td>
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<td>Peonidin-3-glucoside</td>
<td>463 [M+H]</td>
<td>301</td>
<td>0.8 ± 0.19</td>
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<td>Peonidin-3-rutinoside</td>
<td>609 [M+H]</td>
<td>463, 301</td>
<td>0.8 ± 0.19</td>
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<td>1.9</td>
<td>0.7</td>
<td>0.3</td>
<td>0.1 ± 0.08</td>
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<td>Pelargonidin-3-glucoside</td>
<td>433 [M+H]</td>
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<td>2.0 ± 0.36</td>
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<td>0.1</td>
<td>0.7</td>
<td>0.3</td>
<td>0.1 ± 0.08</td>
<td>0.2</td>
<td>0.14</td>
<td>0.10</td>
<td>0.21</td>
<td>0.15</td>
<td>0.13</td>
<td>0.21</td>
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<tr>
<td>Delphinidin-3-glucoside</td>
<td>465 [M+H]</td>
<td>303</td>
<td>2.0 ± 0.36</td>
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<td>0.7</td>
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<td>0.1 ± 0.08</td>
<td>0.2</td>
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<td>0.10</td>
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<td>Phenolic acid</td>
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<td>Chlorogenic acid</td>
<td>353 [M+H]</td>
<td>191, 179</td>
<td>1.6 ± 0.74</td>
<td>43.8</td>
<td>2.1</td>
<td>41.5</td>
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<td>53.1</td>
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<td>58.9</td>
<td>0.74</td>
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<td>Gentisic acid</td>
<td>153 [M+H]</td>
<td>109</td>
<td>1.6 ± 0.74</td>
<td>43.8</td>
<td>2.1</td>
<td>41.5</td>
<td>1.3</td>
<td>53.1</td>
<td>1.6</td>
<td>58.9</td>
<td>0.74</td>
<td>51.3</td>
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<tr>
<td>5-cafeoylquinic acid</td>
<td>377 [M+H]</td>
<td>355, 163</td>
<td>1.6 ± 0.74</td>
<td>43.8</td>
<td>2.1</td>
<td>41.5</td>
<td>1.3</td>
<td>53.1</td>
<td>1.6</td>
<td>58.9</td>
<td>0.74</td>
<td>51.3</td>
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<tr>
<td>Catechin</td>
<td>289 [M+H]</td>
<td>245, 203</td>
<td>6.3 ± 1.15</td>
<td>32.6</td>
<td>8.8</td>
<td>0.5</td>
<td>3.9</td>
<td>12.4</td>
<td>3.9</td>
<td>22.3</td>
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<td>20.9</td>
<td>4.1</td>
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<tr>
<td>Quercetin rutinoside</td>
<td>609 [M+H]</td>
<td>463, 301,</td>
<td>6.3 ± 1.15</td>
<td>32.6</td>
<td>8.8</td>
<td>0.5</td>
<td>3.9</td>
<td>12.4</td>
<td>3.9</td>
<td>22.3</td>
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<td>20.9</td>
<td>1.2</td>
<td>20.9</td>
<td>4.1</td>
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</tbody>
</table>

\[ ^a \text{[M+H] and [M-H] indicate identification in positive and negative ionization modes, respectively.} \]

\[ ^b \text{A trace amount was detected by MS.} \]

\[ ^c \text{‘n.d.’ means not detected.} \]
in the pulp. Cyanidin-3-acetylhexoside was exclusively located in the peel of Beilei blue honeysuckle. In general, the peel contained significantly higher concentrations of individual anthocyanins than did the pulp tissue, consistent with results reported by Stojanović et al. [30]. Wild blue honeysuckle (559.6 mg/g DW) contained the highest concentration of cyanidin-3-glucoside, followed by BLS (538 mg/g DW) and Beilei (503.8 mg/g DW), which were higher than those reported in our previous study [31]. Cyanidin was detected as the major anthocyanin both in the peel and pulp of blue honeysuckle. In addition, chlorogenic acid, gentistic acid, 5-caffeoylquinic acid, catechin and quercetin rutinoside were also identified, and they mostly exist in fruit pulp. The composition of phenolic compounds in this study is different from the previous studies reported by Kucharska et al. [13] and Auzanneau et al. [32]. The differences may be attributed to the use of different extraction methods and growth factors.

3.2 Extracellular and intracellular antioxidant activities

PSC was measured to estimate the extracellular antioxidant activity. As can be seen in Figure 2, PSC varied from 595.57 to 2708.67 μmol vitamin C equivalents/100 g fresh weight, with a mean of 1,078 μmol vitamin C equivalents/100 g fresh weight. The values were significantly higher for Beilei than for the other six varieties (p < 0.05). The PSC values were found to be 1057.57, 894.52, 855.36, 748.88, 685.16, and 595.57 μmol vitamin C equivalents/100 g fresh weight in BLS, wild blue honeysuckle, BKQE, L-5, Haskap, and HSY-24, respectively. The data suggested that Beilei and BLS blue honeysuckle have stronger antioxidant activities, presumably as a result of higher levels of polyphenols. We also found that cyanidin-3-glucoside was significantly related to the PSC results of blue honeysuckle extracts (p < 0.05). Notably, the PSC of Beilei blue honeysuckle was higher than that of cranberry (1019.9 μmol vitamin C equivalents/100 g fresh weight) and grape (2018.9 μmol vitamin C equivalents/100 g fresh weight). In addition, values for all honeysuckle varieties were universally higher than that of apple (309.2 μmol vitamin C equivalents/100 g fresh weight) [26]. Moreover, PSC exhibited a significant correlation with DPPH radical-scavenging capacity (R² = 0.867; p = 0.011).

Troxol equivalent antioxidant concentration (TEAC) values were measured in the blue honeysuckle extracts by the ABTS and DPPH radical-scavenging activity assays. As shown in Figure 2, Beilei blue honeysuckle extracts exhibited the highest ABTS radical-scavenging activity (497 μmol TE/g), and HSY-24 blue honeysuckle extracts had 25.8% lower activity. The trend of DPPH radical-scavenging power was similar to that of ABTS radical-scavenging power. In the DPPH assay, HSY-24 blue honeysuckle extracts exhibited a lower scavenging effect, with the IC₅₀ value 0.8 times higher than that of Beilei blue honeysuckle extracts (IC₅₀ = 3.0 μg/mL). Kusznierewicz et al. reported that the ABTS radical-scavenging activity (expressed as TEAC values) of several honeysuckle varieties cultivated in Poland varied from 12.65 to 49.73 mmol TE/100 g [33]. In particular, blue honeysuckle ethanol extracts showed comparable antioxidant activity in ABTS and DPPH tests, with TEAC values of 103 and
These differences in ABTS and DPPH radical-scavenging activities may be due to genetic and geographic factors, as well as on the extraction method. As the CAA assay accounts for the absorption, metabolism, and localization of antioxidants in cells, it is more biologically relevant than chemical methods, and is predictive of the behavior of substances in vivo [34]. Thus, we measured the CAA values of blue honeysuckle (Figure 2) and found that the values were the highest for Beilei (43.02 μmol quercetin equivalents/100 μg sample) and lowest for HSY-24 (7.88 μmol quercetin/100 μg sample), in line with the results obtained with the PSC assay. However, for Haskap, BLS, L-5, BKQE, and wild honeysuckle, the values were 37.76, 27.94, 25.58, 15.45, and 12.78 μmol quercetin/100 μg sample, respectively, which differed from the PSC trends. The discrepancy is likely due to the degradation of bioactive substances during absorption and metabolism. Of note, Liu et al. reported that the result of the CAA of the anthocyanin fraction from wild blue honeysuckle was 14.75 μmol quercetin equivalents/100 mg dry matter [34]. Wang et al. [22] reported that the results from the CAA of blueberry varieties ranged from 60.6 to 201.6 μmol quercetin equivalents/100 g fresh weight, of which 13.5–63.5 μmol quercetin equivalents/100 g fresh weight was taken up by cells.

### 3.3 Cell proliferation and cytotoxicity

As shown in Figure 3, proliferation in HepG2 cells, as measured by the methylene blue assay, decreased with exposure to increasing concentrations of honeysuckle extracts, with EC₅₀ values between 27.86 and 59.18 mg/mL (data not shown). In particular, the antiproliferative activity was the highest in wild blue honeysuckle, and the lowest in L-5. EC₅₀ values were 34.43, 38.41, 50.18, 50.27, and 55.17 mg/mL for Beilei, Haskap, BKQE, HSY-24, and BLS, respectively. Collectively, the data indicate that blue honeysuckle inhibits proliferation in HepG2 cells in a dose-independent manner, as previously noted [35]. Guo et al. reported that extracts from sea buckthorn (Hippophaë...
Yuehua Wang et al. *rhamnoides* L.) berries were strongly antiproliferative, with an EC₅₀ of 3.31 mg/mL [3].

In comparison to the control, all doses of blue honeysuckle extracts reduced cell viability by no more than 10% (Figure 3), indicating that the extracts were not cytotoxic. Furthermore, this result implies that the observed anticancer activity of blue honeysuckle is due to antiproliferative effects rather than cytotoxicity. Strikingly, the inhibition of cancer cell proliferation might be correlated with levels of polyphenols, flavonoids, and antioxidant activities.

### 4 Conclusions

In this study, we compared the polyphenol profiles, antioxidant activities, cytotoxicities, and antiproliferative activities of extracts from seven varieties of blue honeysuckle. Collectively, the results suggest that blue honeysuckle is a rich source of bioactive molecules, although the profiles of such molecules differed among varieties. Cyanidin, mainly located in the peel, was the prominent bioactive molecule in blue honeysuckle. The isolated molecules were antioxidative, as measured by PSC, CAA, ABTS, and DPPH assays, with the highest antioxidant activity in Beilei blue honeysuckle extracts. Moreover, blue honeysuckle extracts were found to inhibit the proliferation of HepG2 cells without causing cytotoxicity over the doses tested. Our results also indicated that the peel is a rich source of polyphenols profiles, particularly cyanidin-3-O-glucoside. Nevertheless, future research is necessary to investigate the mechanisms underlying these bioactive properties, as well as to develop health-related uses of blue honeysuckle.

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**Figure 3:** The cell proliferation and cytotoxicity of the seven varieties of blue honeysuckle. The results are presented as means ± SD (n=3).

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Conflict of interest: The authors declare that there are no conflicts of interest.

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References


