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Original Research Article

Antioxidant Activity, Total Phenolic Content, Total Flavonoid Content, and Total Anthocyanin Content of *Vaccinium oldhamii* Miq. Collected from 11 Regions of South Korea

Da Hyun Lee[1], Jun Hyeok Kim[1], Chung Youl Park[2], Kyeong Min Kim[1], Hyeon Min Kim[1], Un Seup Shin[1], Do Hyun Kim[1], Chae Sun Na[1] and Wan Geun Park[3]*

[1]Researcher, Forest Bioresource Deparment, Baekdudaegan National Arboretum, BongHwa 36209, Korea
[2]Researcher, National Institute of Horticultural & Herbal Science, RDA, Wanju 55365, Korea
[3]Professor, Division of Forest Science, Kangwon National University, Chuncheon 24341, Korea

Abstract - We studied antioxidant activities of *Vaccinium oldhamii* Miq. from 11 regions in South Korea and blueberries, domestically produced and imported. Correlation analysis between *V. oldhamii* habitats, environmental factors, and antioxidant properties was conducted. DPPH RC_{50} values ranged from 220.44 to 902.38㎍/mL, ABTS from 524.29 to 1230.97 ㎍/mL, and FRAP from 1783.71 to 2235.78 ㎛ Fe (II)/g. *V. oldhamii* from Gumi showed highest DPPH activity, Taean and Haenam for ABTS, and Gimcheon for FRAP. *V. oldhamii* exhibited superior antioxidant activities compared to blueberries. Meteorological conditions correlated positively with ABTS and DPPH activities, negatively with wind speed and humidity affecting DPPH and phenolic, flavonoid, and anthocyanin contents. Based on these findings, it is suggested that *V. oldhamii* fruits collected from Gimcheon and Gumi regions can be effectively utilized as natural antioxidants derived from plant materials.

Key words – ABTS, Anthocyanin content, Antioxidant activity, DPPH, Flavonoid content, FRAP, Phenolic content, *Vaccinium oldhamii* Miq.

Introduction

As human life expectancy has increased, there have been significant increases in the prevalences of chronic diseases, such as diabetes, dementia, cancer, and cardiovascular disease, which are often referred to collectively as lifestyle-related diseases (Tanaka *et al*., 2000). These increases have been attributed to an abnormal increase in the production of reactive oxygen species (ROS), because of factors such as environmental pollution, westernized dietary habits, alcohol consumption, and smoking (Wiseman, 1996).

For the prevention of ROS-induced damage, there has been a growing interest in antioxidants. While synthetic antioxidants including butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been predominantly used therapeutically, concerns have arisen regarding their potential side effects. This has led to greater interest in the possibility of administering natural antioxidants (Amarowicz and Raab, 1997; Fang *et al*., 2002; Gowri and Vasantha, 2010; Lee *et al*., 2021).

Alongside the growing interest in antioxidants, the consumption of berries has also been increasing (An *et al*., 2015). Blueberries, a representative type of berry, belong to the Ericaceae family, *Vaccinium* genus, and are primarily found in North America. They have been reported to have various health benefits, including antioxidant effects (Sellappan *et al*., 2002), anti-cancer effects (Seeram *et al*., 2006), anti-diabetic properties (Martineau *et al*., 2006), and potential anti-dementia effects (Papandreou *et al*., 2009). They have gained recognition as one of the top ten foods that prolong longevity globally, and the commercial cultivation of blueberries began in South Korea after 2000 (Chae, 2021).

Crop wild relatives (CWRs) are wild plant species that are closely related to cultivated crops and have evolved to survive despite environmental challenges such as dry condi-

*Corresponding author. E-mail : wgpark@kangwon.ac.kr
Tel. +82-033-250-8315

tions, high humidity, high temperatures, and poor soil. These can serve as a source of new genetic diversity for the breeding of more resilient crops. There has been a global initiative to conserve CWRs since 2011 that has prioritized the collection, preservation, pre-breeding, and information systems for wild plant genetic resources. The genetic resources associated with indigenous crops, including the "native blueberry," *Vaccinium oldhamii* Miq, have been conserved in South Korean forests since 2021.

V. oldhamii, a species in the Ericaceae family and *Vaccinium* genus, is a deciduous shrub that grows to 1~4 m in height. It is primarily found in China, and Japan, and particularly in South Korea, south of Gyeryongsan, but also along the western coast, extending to Anmyeondo (Chung and Hyun, 1989). *V. oldhamii* produces flowers with 5~15 yellowish-red or reddish-brown racemes between May and June, and then yields black or purplish, round fruits (4~6 ㎜) between September and October (Plants of the World Online, 2022).

A recent study (Chae, 2021) showed that *V. oldhamii* has a higher content of polyphenols, greater electron-donating ability, and greater ABTS+ radical scavenging ability than blueberries, which suggests that it could be used as a native blueberry alternative in functional food products. In the study by Kim *et al*. (2019), the pharmacological efficacy of *V. oldhamii*, indicated its potential for treating inflammation and bone disorders by regulating inflammation, osteoclastogenesis, and NF-κB and MAPK signaling in branches. According to Sekizawa *et al*. (2013), the fruit extract of *V. oldhamii* demonstrated inhibitory effects on the initial stages of Influenza virus (IFV) infection. Tsuda *et al*. (2013) reported that the fruit extract of *V. oldhamii* showed growth inhibitory effects on HL-60 human leukemia cells. In the research by Park and Kim (2005), four compounds were isolated from the MeOH extract of *V. oldhamii* branches in the ethyl acetate fraction, including (+)catechin, (-)-epicatechin, proanthocyanidin A-2: epicatechin-(2β→7, 4β→8)-epicatechin, and cinnamtannin B1: epicatechin-(2β→7, 4β→8)-epicatechin-(4α→8)-epicatechin. These compounds, mainly belonging to the catechin and proanthocyanidin groups, are reported to possess antioxidant and anti-inflammatory properties. These research findings suggest the diverse potential medicinal applications of *V. oldhamii*.

In the present study, we compared the antioxidant activities of *V. oldhamii* fruit collected from 11 different regions of South Korea and blueberries imported from three countries. This allowed us to compare the antioxidant activity and the contents of bioactive compounds of this plant, according to its growth environment. In this way, we aimed to provide fundamental data to facilitate the future development of superior varieties and the potential use of *V. oldhamii* as a functional ingredient.

Materials and Methods

Plant materials

The fruit of *V. oldhamii* used in the study were collected from 11 different regions of South Korea between September and October 2022 (Fig. 1, Table 1); Chungcheong-do (Nonsan, Buyeo, and Taean), Jeolla-do (Gwangju, Haenam, and Gwangyang), Gyeongsang-do (Hamyang, Gumi, and Gimcheon), and Jeju-do (Jeju and Seogwipo). In addition, the fruit of domestically-grown, U.S.-grown, Canadian-grown, and Chilean-grown blueberries that were available commercially

Fig. 1. The 11 regions of *Vaccinium oldhamii* Miq. samples collected for the study.

Table 1. The 11 regions of *Vaccinium oldhamii* Miq. samples collected for the study.

No.	Locality	Collect date
1	Jungsan-ri, Yangchon-myeon, Nonsan-si, Chungcheongnam-do, Republic of Korea	2022.9.7
2	Bokgeum-ri, Chunghwa-myeon, Buyeo-gun, Chungcheongnam-do, Republic of Korea	2022.9.7
3	Gonam-myeon, Taean-gun, Chungcheongnam-do, Republic of Korea	2022.9.7
4	Samsan-myeon, Haenam-gun, Jeollanam-do, Republic of Korea	2022.10.6
5	Doggok-ri, Ongnyong-myeon, Gwangyang-si, Jeollanam-do, Korea	2022.9.12
6	Ungok-ri, Seoha-myeon, Hamyang-gun, Gyeongsangnam-do, Republic of Korea	2022.9.13
7	Geumosan-ro, Gumi-si, Gyeongsangbuk-do, Republic of Korea	2022.9.23
8	1100-ro, Jeju-i, Jeju-do, Republic of Korea	2022.9.28
9	Jungmun-dong, Seogwipo-si, Jeju-do, Republic of Korea	2022.9.29
10	Daehang-myeon, Gimcheon-si, Gyeongsangbuk-do, Republic of Korea	2022.10.6
11	Ullim-dong, Dong-gu, Gwangju, Republic of Korea	2022.10.7

were purchased and used for comparison. The fruits were freeze dried, ground into powder using a liquid nitrogen-cooled mill, and stored at −20°C until use.

Climate data

The mean temperature, maximum temperature, minimum temperature, daily temperature range, mean wind speed, and mean humidity of the 11 regions between March and September 2022 were obtained from the Korea Meteorological Administration's Weather Data Open Portal (https://data.kma.go.kr/) and compared.

Extraction of plant components

The fruit samples underwent freeze-drying for 72 hr, then the dried samples were finely ground, and 70% ethanol solution (Macron Fine Chemicals, Randor, PA, USA) was added at a ratio of 20 times the mass of each dried sample (w/v). This mixture was then extracted at room temperature for 48 h in a maceration extractor.

The yield of each extract was calculated on the basis on the mass of each dried sample. The generated seed extracts were filtered using a 0.45 ㎛ syringe filter (PVDF syringe filter, 13 ㎜, Futecs, Seoul, Korea) and then concentrated using a vacuum centrifugal concentrator (CVE-3110, Eyela, NY, USA) to completely remove the solvent. The concentrated extract was then dissolved in dimethyl sulfoxide (DMSO) (Sigma, Saint Louis, MO, USA) to achieve a concentration of 30,000 ppm and stored at −20°C. It was further diluted according to the requirements of the experimental protocol.

To measure the total anthocyanin content, a mixture of methanol 99.8% (Avantor, J. T. Baker, USA), hydrochloric acid (Titripur, Germany), and distilled water at a 60:1:30 ratio was added to the powdered samples. The mixtures were then extracted at room temperature for 24 hr, the extracts were filtered through a PVDF syringe filter (0.45 ㎛), and the resulting solutions were used in the experiments.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was evaluated using a modification of Blois's method (Blois, 1958). In this assay, 50 μL of fruit extract was mixed with 150 μL of 0.1 mM DPPH (Alfa Aesar, Haverhill, MA, USA) radical solution and allowed to react for 30 min at room temperature. After this, the absorbance of the reaction mixture was measured at 517 ㎚ using a microplate reader (EPOCH2, Biotek, USA). To determine the DPPH radical scavenging activity, a blank was prepared by adding DMSO (the solvent used for the dilution of the extracts) instead of the fruit extract, and its absorbance was also measured. The DPPH radical scavenging activity was calculated as a percentage of the DPPH radical reduced by the extract using the following formula:

DPPH radical scavenging activity (%) = (1 − Absorbance of the test sample / Absorbance of the blank) × 100

The RC_{50} value, representing the concentration of seed extract required to scavenge 50% of the DPPH radicals, was determined

using this formula. Ascorbic acid (Fujifilm Wako Pure Chemical Co., Osaka, Japan) was used as a positive control.

ABTS radical scavenging activity

The ABTS radical scavenging activity was evaluated using a modification of the method developed by Miller *et al*. (Miller *et al*., 1993). To prepare the ABTS solution, 20 ㎎ of ABTS diammonium salt (Sigma Chemical Co.) was added to 5 mL of distilled water containing 88 μL of 140 mM potassium persulfate (Acros Organics, Geel, Belgium). This mixture was left in the dark at room temperature for 14−16 hr until an absorbance of 0.70 ± 0.02 was achieved at 734 ㎚ after dilution with ethanol. For the assay, 10 μL aliquots of fruit extract were mixed with 190 μL aliquots of the prepared ABTS solution in a 96-well plate. The reaction was allowed to proceed for 2 min 30 sec, after which the absorbances of the reaction mixtures were measured at 734 ㎚ using a microplate reader. A blank was prepared as for the DPPH assay: DMSO (the solvent used for the dilution of the extracts) was added instead of the fruit extract, and its absorbance was measured. The ABTS radical scavenging activity was calculated as a percentage of the reduction in ABTS radicals by the extract using the following formula:

ABTS radical scavenging activity (%) = (1 − Absorbance of the test sample / Absorbance of the blank) × 100

The RC_{50} value, representing the concentration of the fruit extract required to scavenge 50% of the ABTS radicals, was determined using this formula. Ascorbic acid (Fujifilm Wako Pure Chemical Co.) was used as a positive control.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was conducted using a modification of the method developed by Molina-Díaz *et al*. (1998). In order to prepare FRAP solution, a mixture of 40 mM HCl (Fujifilm Wako Pure Chemical Co.), 10 mM 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ), and 20 mM iron chloride (Sigma Chemical Co.) at a ratio of 10:1:1 was mixed in a 300 mM acetate buffer ㏗ 3.6 (Samchun, Korea, Pyeongtaek) solution. For the assay, 10 μL of the seed extract was mixed with 200 μL of the prepared FRAP solution, the reaction was allowed to proceed for 4 min at 37°C, and the absorbance of the reaction mixture was measured at 593 ㎚ using a microplate reader. The FRAP value is expressed in terms of the capacity of Fe (II) and was determined using a calibration curve created by preparing dilutions of a ferrous sulfate heptahydrate (Sigma Chemical Co.) standard.

Total phenolic content

The total phenolic contents of the samples were measured using a modification of the method developed by Singleton *et al.* (1999). In this assay, 20 μL of the fruit extract was mixed with 40 μL of 0.4 N Folin & Ciocalteu's phenol reagent (Sigma Chemical Co.) dissolved in distilled water and allowed to stand for 5 min. Next, 140 μL of 700 mM Na_2CO_3 (Daejung, Siheung-si, Korea) was added to the mixture, and the reaction was allowed to proceed at room temperature for 2 hr. The absorbance of the reaction mixture was then measured at 765 ㎚ using a microplate reader.

To determine the total phenolic content, a calibration curve was created using gallic acid (Sigma Chemical Co.) as a standard substance. The total phenolic content in the fruit extract was expressed in terms of gallic acid equivalents (GAEs).

Total flavonoid content

The total flavonoid contents of the samples were measured using a modification of the method developed by Christ and Müller (1960). In this assay, 100 μL of fruit extract was mixed with 300 μL of ethanol (Daejung, Siheung-si, Korea), 20 μL of 1 M potassium acetate (Junsei Chemical Co., Tokyo, Japan), 20 μL of 10% aluminum chloride (Junsei Chemical Co., Tokyo, Japan), and 560 μL of distilled water. After mixing, the reaction was allowed to proceed for 30 min at room temperature, then 200 μL aliquots of the mixtures were transferred to wells of a 96-well plate, and the absorbances of the reaction mixtures were measured at 415 ㎚ using a microplate reader. A calibration curve was created using quercetin (Sigma Chemical Co.) as a standard. The total flavonoid content of each fruit extract is expressed in terms of quercetin equivalents (QEs).

Total anthocyanin content

The total anthocyanin contents of the samples were measured

using an adapted pH differential method based on the AOAC method (Lee, 2005). For this, 1 mL of each extract was mixed with 1 mL of potassium chloride buffer 0.025 M (pH 1.0) and 1 mL of sodium acetate buffer 0.4 M (pH 4.5). The absorbances of the mixtures were measured at 510 nm and 700 nm using a microplate reader. Dilutions of the extracts were prepared to ensure that the absorbances fell within the range of 0.2 to 1.4, and the absorbances were measured between 20 and 50 min after the start of the reaction. The standard used was cyanidin 3-glycoside, and the concentration of anthocyanin in the extracts of *V. oldhamii* fruit were calculated using its molecular weight (MW = 449.2) and molar absorptivity (ε = 26,900). The concentrations of anthocyanin in the extracts were calculated using the following equation:

$$\text{Concentration of monomeric anthocyanin pigment (mg CGE/L)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times 1}$$

* Calculation : cyanidin-3-glucoside equivalents [MW = 449.2 g/mol, e = 26,900 L/cm·mol]
* $A = (A_{510\,nm} - A_{700nm})_{pH\,1.0} - (A_{510nm} - A_{700nm})_{pH\,4.5}$
* DF = Dilution factor

Statistical analysis

The data are expressed as mean ± standard deviation. The statistical analysis and correlation analysis were performed using R (http://www.r-project.org). Significance was accepted at the 5% level when using ANOVA followed by Duncan's multiple range test ($p < 0.05$). Pearson correlation analysis was used to analyze the relationships between antioxidant activities and environmental factors, according to region. The data was standardized using Z-scores and used in hierarchical cluster analysis using Ward's method, based on the squared Euclidean distance.

Results and Discussion

Comparisons of the antioxidant activity, total phenolic content, and total flavonoid content of *Vaccinium oldhamii*

We first assessed the antioxidant activity of *V. oldhamii* fruit samples collected from 11 regions of South Korea (Table 2). The RC_{50} values, which represent the concentrations of the extracts required to achieve 50% scavenging activity, were evaluated using DPPH and ABTS radical scavenging assays. The DPPH radical scavenging activity RC_{50} values ranged from 210.44 to 902.38 μg/mL, with a mean of 362.98 μg/mL. The ABTS radical scavenging activity RC_{50} values ranged from 524.29 to 1,230.97 μg/mL, with a mean of 930.89 μg /mL. The FRAP values ranged from 1,783.71 to 2,235.78 μg /mL, with a mean of 1,947.00 μg/mL. The total phenolic contents of the samples ranged from 10.66 to 36.43 mg GAEs per gram of fruit, with a mean of 18.34 mg GAE s/g fruit. The total flavonoid contents ranged from 2.23 to 5.21 mg QEs per gram of fruit, with a mean of 6.65 mg QE s/g fruit. The total

Table 2. Analysis of variance and descriptive statistics for the antioxidant activities, total phenolic contents, total anthocyanin contents, and total flavonoid contents of samples of *V. oldhamii* Miq. collected from 11 regions of South Korea.

	Min	Max	Median	Mean	SD	skew	kurtosis	SE
DPPH radical scavenging RC_{50} (μg/mL)	210.44	902.38	285.90	362.98	30.87	1.78	2.3	30.84
ABTS radical scavenging RC_{50} (μg/mL)	524.29	1,230.97	981.11	930.89	37.59	−0.46	−0.99	37.59
FRAP value (μm Fe (II)/g seed)	1,783.71	2,235.78	1,923.24	1,947.00	23.24	0.66	−0.65	23.25
Total phenolic content (mg GAE s/g fruit)	10.66	36.43	16.03	18.34	1.09	1.21	0.63	1.09
Total flavonoid content (mg QE s/g fruit)	2.23	5.21	3.46	6.65	0.14	0.33	−0.85	0.14
Total anthocyanin content (mg CE/100 g fruit)	189.70	781.30	392.31	434.62	27.93	0.73	−0.29	27.93

anthocyanin contents varied from 189.70 to 781.30 ㎎ cyanidin-3-glycoside equivalents (CEs) per 100 g fruit, with a mean of 434.62 ㎎ CE/100 g fruit. These results illustrate the diversity in the antioxidant properties and phytochemical contents of *V. oldhamii* collected from various regions of South Korea.

Antioxidant activity of *Vaccinium oldhamii* according to the location of collection, and a comparison with that of blueberries

Table 3 presents the RC_{50} values for DPPH radical scavenging activity of *V. oldhamii* fruits collected from 11 regions and blueberries. The analysis revealed significant variations in DPPH radical scavenging activity among the regions. The highest activity was observed in fruits from the Gumi region, with an RC_{50} value of 215.49±4.43 ㎍/mL, while the lowest activity was found in fruits from the Nonsan region, with an RC_{50} value of 792.84±166.94 ㎍/mL. Notably, there was considerable variability in the RC_{50} values of blueberries obtained from the four countries. The highest activity was found in domestically-grown blueberries, with RC_{50}s of 290.99±6.70 ㎍/mL, similar to those from Jeju, Seogwipo, Gimcheon, Taean, and Haenam, but lower than that of fruits from the Gumi region. As a reference, the RC_{50} value for ascorbic acid was 5.01±0.76 ㎍/mL, higher than all other samples.

The ABTS radical scavenging activity varied significantly among the regions. Fruits from Taean and Haenam regions exhibited the highest RC_{50} values (577.95±10.17 and 565.13±58.92 ㎍/mL, respectively), while fruits from Nonsan and Buyeo regions displayed lower activity, with RC_{50} values of 1,175.66±14.90 and 1,221.18±10.77 ㎍/mL, respectively. Among the blueberries from the four countries, American blueberries had the highest RC_{50} values, but these were lower than those of the best-performing regions for Korean blueberries, such as fruits from Gumi and Taean. The RC_{50} value for ascorbic acid was 6.00±0.10 ㎍/mL, higher than all other samples.

The FRAP of the fruit extracts was measured to assess the antioxidant activity of Korean blueberries from various regions. The highest activity was observed in fruits from the Gimcheon region, with a value of 2,224.62±14.77 ㎛ Fe (II)/g fruit, while the lowest activity was found in fruits from the Hamyang region, with a value of 1,798.60±14.05 ㎛ Fe (II)/g

Table 3. RC_{50} values for DPPH and ABTS radical scavenging activities and FRAP value of *V. oldhamii* extracts and a comparison with blueberries.

		DPPH radical scavenging RC_{50} (㎍/mL)	ABTS radical scavenging RC_{50} (㎍/mL)	FRAP value (㎛ Fe (II)/g extract)
Vaccinium oldhamii Miq.	Nonsan	792.84±166.94b	1,175.66±14.90b	1,806.04±14.77gh
	Buyeo	595.85±70.56c	1,221.18±10.77b	1,882.31±48.76ef
	Taean	282.60±3.60fg	577.95±10.17g	1,928.82±64.36de
	Haenam	286.65±2.42fg	565.13±58.92g	1,949.29±19.60d
	Gwangyang	299.53±41.98efg	884.05±65.61ef	1,833.94±31.07fg
	Hamyang	398.00±39.37de	1,040.91±52.47cd	1,798.60±14.05gh
	Gumi	215.49±4.43g	792.47±30.28f	2,094.39±39.60b
	Jeju	274.33±21.24fg	956.53±168.54de	2,024.63±70.94c
	Seogwipo	264.78±11.89fg	1,002.45±63.39cde	2,019.98±28.64c
	Gimcheon	250.18±8.41fg	991.16±128.05cde	2,224.62±14.77a
	Gwangju	332.48±32.32def	1,032.35±44.64cd	1,854.41±50.64fg
Blueberries	Korea	290.99±6.70fg	1,002.99±22.08cde	1,729.76±37.16i
	America	298.06±9.15efg	984.07±78.75de	1,759.53±28.09hi
	Canada	1301.99±99.05a	1,729.78±85.31a	1,623.72±14.05j
	Chile	403.40±31.16d	1,121.44±40.95bc	1,761.39±20.12hi
Ascorbic acid		5.01±0.76	5.01±0.76	6.00±0.10

[z]The same letter in each column indicates no significant difference, according to Duncan's multiple range test, $p < 0.05$.

fruit. All four blueberry extracts from other countries showed lower activities than those of *V. oldhamii*, including those from the region with the lowest activity, Gimcheon.

A comparison of *V. oldhamii* with blueberries showed that the DPPH radical scavenging (RC_{50}), ABTS radical scavenging (RC_{50}), and FRAP (㎛ Fe (II)/g extract) antioxidant activities of *V. oldhamii* were higher than those of blueberries from domestically-grown, U.S.-grown, Canadian-grown, and Chilean-grown blueberries. The total phenolic and anthocyanin contents were also higher.

According to Kim *et al*. (2016), the antioxidant activity of fruits from four indigenous populations of *V. oldhamii* (Taean, Nonsan, Gumi, Gwangju) in Korea was investigated. The total phenolic content ranged from 4.40 to 10.58 ㎎ GAE/g, with the Gumi population showing the highest value. In terms of DPPH free radical scavenging activity, high antioxidant activity was observed at 800 ppm with an average of 94.6%, and at 400 ppm with an average of 76.4%. These results indicate morphological differences in fruit among the *V. oldhamii* populations, reflecting morphological diversity according to region.

The utility of *V. oldhamii* fruit was examined in a previous study (Kim *et al*., 2013) through antioxidant activity analysis such as DPPH free radical scavenging activity and reducing power using selected five specimens (Gwangju, Namhae, Nonsan, Muju, Taean). The DPPH free radical scavenging activity showed high activity with an overall average of 84.2% at a concentration of 400 ppm, with specimens collected from the Taean region exhibiting the highest scavenging activity at 91.4%. The reducing power was recorded as an average of 0.79 at 700 ppm, with specimens collected from the Taean region showing the highest reducing power at 0.96. These results confirm that the antioxidant activity of *V. oldhamii* fruit varies depending on the region and extraction conditions. According to Lee *et al*. (2023), variations in antioxidant activity, total phenolic content, and total flavonoid content of *Boehmeria nivea* var. *tenacissima* (Gaudich.) Miq. were attributed to differences in environmental conditions among collection sites.

Comparisons of the total phenolic content, total flavonoid content, and total anthocyanin content of *Vaccinium oldhamii*, according to the location of collection, and a comparison with blueberries

Table 4 presents the total phenolic, flavonoid, and antho-

Table 4. Total phenolic, flavonoid, and anthocyanin contents in *V. oldhamii*, expressed as equivalents to gallic acid, quercetin, and cyanidin-3-glucoside and a comparison with blueberries.

		Total phenolic content (㎎ GAE s/g fruit)	Total flavonoid content (㎎ QE s/g fruit)	Total anthocyanin content (㎎ CE/100 g fruit)
Vaccinium oldhamii Miq.	Nonsan	13.56±1.43fg[z]	2.78±0.19hi	327.15±46.54def
	Buyeo	15.35±0.75ef	3.89±0.06e	232.75±37.66f
	Taean	12.51±2.08g	2.41±0.27i	354.76±157.4cde
	Haenam	19.68±1.73c	3.50±0.10efg	461.33±33.24c
	Gwangyang	13.17±0.17fg	3.24±0.11fg	385.04±8.12cde
	Hamyang	14.53±0.35efg	3.37±0.09fg	366.34±26.04cde
	Gumi	26.34±2.01b	5.07±0.15bc	667.96±126.11ab
	Jeju	16.75±0.96de	3.57±0.28ef	468.46±59.75c
	Seogwipo	21.60±1.00c	4.47±0.11d	471.87±13.96c
	Gimcheon	32.62±3.56a	4.74±0.37cd	744.25±38.43a
	Gwangju	15.66±1.33ef	3.12±0.19gh	300.88±68.42ef
Blueberries	Korea	15.45±0.84ef	4.88±0.17cd	587.36±39.54b
	America	19.19±0.23cd	5.37±0.34b	434.62±44cd
	Canada	9.12±1.48h	3.51±0.11efg	88.62±2.23g
	Chile	24.73±1.39b	6.01±0.49a	458.37±35.6c

[z]The same letter in each row indicates no significant difference, according to Duncan's multiple range test, $p < 0.05$.

cyanin contents for *V. oldhamii* fruit collected from the 11 Korean regions and for blueberries. The fruit from the Gimcheon region exhibited the highest total phenolic content (32.62±3.56 ㎎ GAE s/g fruit) and the fruit from the Taean region displayed the lowest content (12.51±2.08 ㎎ GAE s/g fruit). The blueberries showed significant variation according to their country of origin. Chilean blueberries had the highest content, but this was lower than that of fruit from the Gimcheon region (32.62±3.56 ㎎ GAE s/g fruit. When comparing the regions with the highest content in both species, *V. oldhamii* showed approximately 1.7 times higher content compared to blueberries. Blueberries exhibited relatively lower content compared to *V. oldhamii* fruits.

The Gumi region yielded the fruit with the highest total flavonoid content (5.07±0.15 ㎎ QE s/g fruit), while those from the Taean region had the lowest content (2.41±0.27 ㎎ QE s/g fruit). Blueberries, and particularly the Chilean and American examples, showed higher total flavonoid contents, exceeding those of *V. oldhamii* fruit (6.01±0.49 and 5.37± 0.34 ㎎ QE s/g fruit, respectively).

The fruits from the Gimcheon region exhibited the highest total anthocyanin content (744.25±38.43 ㎎ CE/100g fruit), while fruits from the Buyeo region had the lowest (232.75±37.66 ㎎ CE/100g fruit). Blueberries showed considerable variation based on their country of origin, with Korean blueberries having the highest content (587.36±39.54 ㎎ CE/100g fruit) and Canadian blueberries having the lowest (88.62±2.23 ㎎ CE/100g fruit). Notably, *V. oldhamii* fruits from the Gimcheon and Gumi regions had higher anthocyanin contents than the blueberries. These findings offer insights into the variations in the contents of these bioactive compounds in *V. oldhamii* fruit across various regions of Korea and in comparison to blueberries.

The total flavonoid content of fruit from the Gumi region was particularly high, and the total anthocyanin content was particularly high in the fruit from the Gimcheon region.

According to Kim *et al*. (2016), the levels of physiological active substances in the fruits of four indigenous populations of *V. oldhamii* (Taean, Nonsan, Gumi, Gwangju) in Korea were investigated. The total phenolic content ranged from 4.40 to 10.58 ㎎ GAE/g, flavonoid content ranged from 2.22 to 8.09 ㎎ NE/g, and anthocyanin content ranged from 232.51 to 684.32 ㎎ CGE/100 g, with the Gumi population showing the highest values.

The utility of *V. oldhamii* fruit was examined in a previous study (Kim *et al*., 2013) through antioxidant activity analysis such as total phenolic content, DPPH free radical scavenging activity, and reducing power using selected five specimens (Gwangju, Namhae, Nonsan, Muju, Taean). The total phenolic content of *V. oldhamii* fruit averaged 17.9 ㎎/g, with specimens collected from the Taean region exhibiting the highest phenolic content at 19.8 ㎎/g. The results of this study showed similarity to the findings of previous research.

Relationships of the antioxidant activity, total phenolic content, total flavonoid content and total anthocyanin content with environmental conditions

The antioxidant activity of *V.oldhamii* fruit, along with the analysis of total phenolic, flavonoids, anthocyanins, and their correlation with meteorological conditions was conducted. Correlation coefficients were only indicated for cases with significant p-values in the correlation analysis (Fig. 2, 3). A significant correlation was observed between antioxidant activity, total phenolic content, and total flavonoid content, as indicated by the FRAP values.

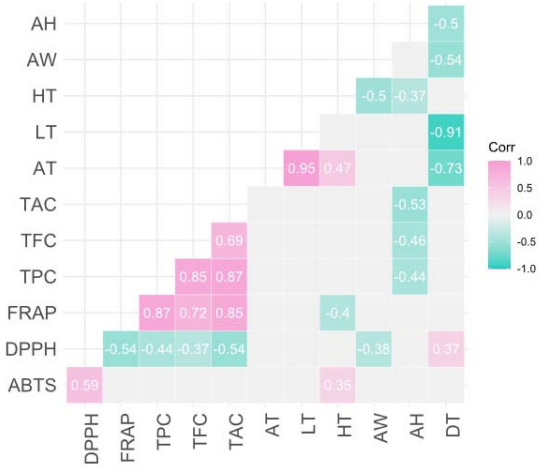

Fig. 2. Correlation coefficients for the relationships of antioxidant activity, total phenolic content, total flavonoid content, and total anthocyanin content with environmental parameters for each of the regions where *V. oldhamii* samples were collected. AT, mean temperature; HT, highest temperature; LT, lowest temperature; AW, mean wind speed; AH, mean humidity; DT, daily temperature change.

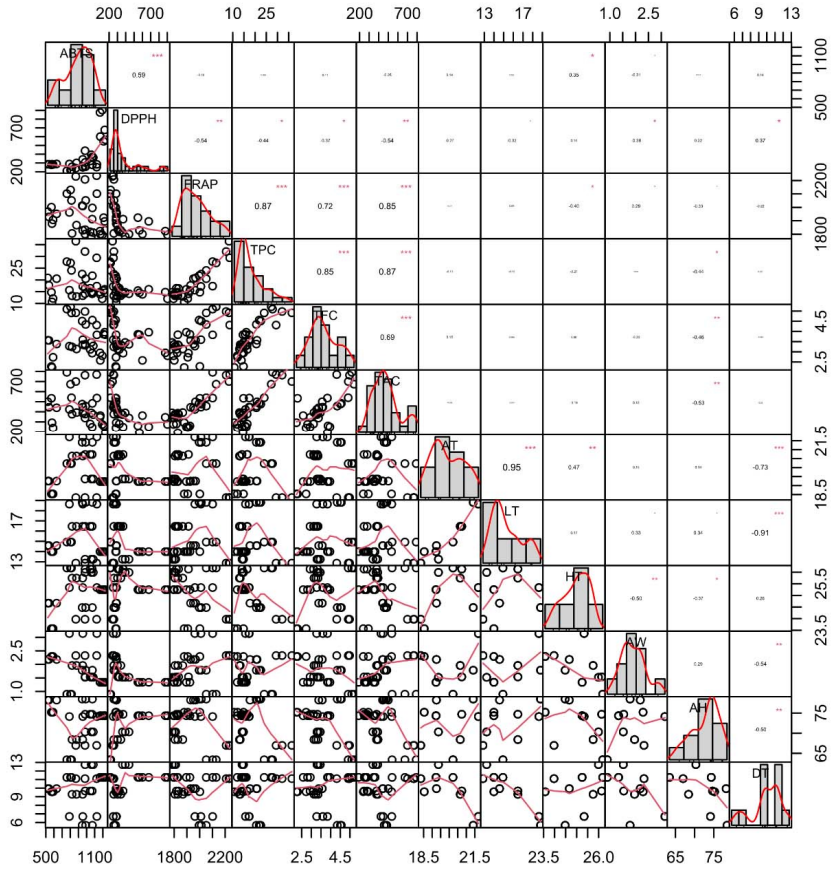

Fig. 3. Scatterplot of correlation coefficients between Antioxidant activity, Total phenolic, flavonoid, and anthocyanin content, and environmental factors in 11 regions of *V. oldhamii* samples. Environmental factors include Average Temperature (AT), Highest Temperature (HT), Lowest Temperature (LT), Daily Temperature Change (DT), Average Wind Speed (AW), and Average Humidity (AH).

A positive linear relationship was observed between the DPPH and ABTS radical scavenging abilities ($r=0.59$, $p < 0.001$). Strong positive linear relationships were also evident between FRAP and total phenolic content ($r=0.87$, $p < 0.0005$), total flavonoids ($r=0.72$, $p < 0.001$), and total anthocyanin content ($r=0.85$, $p < 0.001$). The total flavonoid content exhibited a strong positive linear relationship with total phenolic content ($r=0.85$, $p < 0.001$) and total anthocyanin content ($r=0.69$, $p < 0.001$). Similarly, a similar trend was observed between total phenolic content and total anthocyanin content ($r=0.87$, $p < 0.001$).

The correlation analysis between meteorological conditions and the antioxidant and physiological activities of *V. oldhamii* fruit revealed associations with various weather parameters. A positive linear relationship ($r=0.35$, $p < 0.5$) was observed between the maximum temperature (HT) and ABTS radical scavenging ability, while average wind speed (AW) and diurnal temperature range (DT) showed negative ($r=-0.38$, $p < 0.5$) and positive ($r=0.37$, $p < 0.5$) linear relationships, respectively, with DPPH radical scavenging ability.

Additionally, average humidity (AH) exhibited negative linear relationships with total phenolic content ($r=-0.44$, $p < 0.5$), total flavonoid content ($r=-0.46$, $p < 0.01$), and total anthocyanin content ($r=-0.53$, $p < 0.01$).

The analysis of the correlation between the antioxidant activity, total phenolic, flavonoid, anthocyanin content of *V. oldhamii* fruit, and meteorological conditions revealed positive correlations between maximum temperature (HT) and ABTS radical scavenging ability, as well as negative correlations between average wind speed (AW) and DPPH radical scavenging ability. A positive correlation was

observed between diurnal temperature range (DT) and DPPH radical scavenging ability. Additionally, average humidity (AH) showed negative correlations with total phenolic content, total flavonoid content, and total anthocyanin content.

Research on the specific correlation between these characteristics and the vegetation or environmental conditions of each region is still lacking, and the content of these phytochemicals can vary depending on the environmental and growth conditions of the plants. These differences in phytochemical content may manifest as differences in efficacy when consumed (Gololo, 2018). However, when antioxidant and physiological activity analyses of *V. oldhamii* fruits from 11 regions were correlated with meteorological conditions, no clear relationship was observed. These findings are similar to those of Lee *et al*. (2023) in their study on *Boehmeria nivea* var. *tenacissima* (Gaudich.) Miq. Regarding the correlation with environmental conditions, no significant correlation was observed between antioxidant and physiological activities and environmental factors such as average temperature, maximum temperature, minimum temperature, and diurnal temperature range.

Results of the hierarchical clustering analysis for *V. oldhamii* fruit, according to region

A clustering analysis was next performed using the antioxidant activities of *V. oldhamii* fruit from the 11 different regions of Korea. According to the DPPH radical scavenging (RC_{50}), ABTS radical scavenging (RC_{50}), FRAP (㎛ Fe (II)/g extract), total phenolic content (㎎ GAE s/g fruit), total flavonoid content (㎎ QE s/g fruit), and total anthocyanin content (㎎ CE/100g fruit) of the fruit, the regions were placed into three categories, as shown in Table 5 and Fig. 4.

Group 1 comprised the regions of Gumi and Gimcheon; Group 2 comprised Nonsan and Buyeo; and Group 3 comprised Taean, Seogwipo, Buan, Gwangju, Jeju, Gwangyang, and Hamyang. Thus, the antioxidant activities of *V. oldhamii* fruit extracts from the Gimcheon and Gumi regions, in Group 1, were higher than others.

As a result of hierarchical cluster analysis, the groups were divided into three. It was found that *V. oldhamii* fruit from Gimcheon and Gumi were found to be an important natural source of antioxidants. These data form a basis for the development of new varieties using locally grown native *V. oldhamii* species alongside blueberries in the future, which may help the adaptation to future food crises resulting from climate change.

This study aimed to compare the antioxidant and physiological activities of *V. oldhamii* with blueberries from 11 regions. Results showed that *V. oldhamii* exhibited higher antioxidant activity in terms of DPPH radical scavenging,

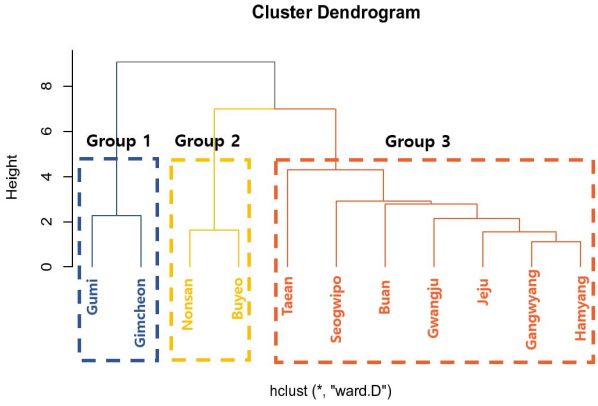

Fig. 4. Dendrogram showing the results of hierarchical clustering analysis using Ward's method, based on the antioxidant activity, total phenolic content, total flavonoid content, and total anthocyanin content of the fruit from the various regions of Korea.

Table 5. Comparison of antioxidant activities of the three groups identified using hierarchical clustering analysis according to antioxidant activity, total phenolic content, total flavonoid content, and total anthocyanin content.

Group	DPPH radical scavenging RC_{50} (㎍/mL)	ABTS radical scavenging RC_{50} (㎍/mL)	FRAP value (㎛ Fe (II)/g extract)	Total phenolic content (㎎ GAE s/g fruit)	Total flavonoid content (㎎ QE s/g fruit)	Total anthocyanin content (㎎ CE/100 g fruit)
1	232.82±8.14[z]	891.81±55.93	2,159.51±31.10	29.48±1.76	4.90±0.13	706.10±38.08
2	694.34±64.26	1,198.42±11.23	1,844.17±21.54	14.45±0.58	3.33±0.25	279.95±26.16
3	305.48±10.74	865.62±45.43	1,915.67±20.28	16.27±0.73	3.38±0.13	401.24±18.94

[z]The data are presented as mean ± SE.

ABTS radical scavenging, and FRAP compared to blueberries from the United States, Canada, and Chile. When comparing the highest content regions of *V. oldhamii* and blueberries, *V. oldhamii* showed approximately 1.3 times higher activity in FRAP, total phenolic compounds, and total anthocyanin content.

Hierarchical cluster analysis divided the regions into three groups, indicating that the fruits of *V. oldhamii* from Gimcheon and Gumi regions have a high potential for use as natural antioxidants.

Overall, fruits from Gimcheon and Gumi regions exhibited relatively superior characteristics in terms of antioxidant and physiological activities. Based on these results, it is believed that providing basic information through future selection breeding research can lead to the identification of high-quality *V. oldhamii* individuals. This study aimed to provide information necessary for cultivation and the development of new varieties for utilizing *V. oldhamii* as a functional food material in South Korea. This is an important step in exploring the commercial potential of *V. oldhamii* fruits and demonstrating their potential as effective plant materials contributing to the domestic food and health supplement industries. These research findings are expected to play a crucial role in improving the quality of *V. oldhamii* fruits and developing various utilization methods in the future.

Acknowledgements

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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