Regular Article
pISSN: 2288-9744, eISSN: 2288-9752
Journal of Forest and Environmental Science
Vol. 37, No. 3, pp. 226-234, September, 2021
https://doi.org/10.7747/JFES.2021.37.3.226

JFES
Journal of Forest and Environmental Science

Analysis of Total Phenolic, Flavonoid Contents, and Antioxidant Capacity Extract from Leaves of Selected Accessions of Two Wild Pear Species, *Pyrus pyrifolia* and *P. ussuriensis*

Si Woong Yang[1], Hwa Lee[1], Jae Mo Song[2], Sun Eun Choi[3] and Eun Ju Cheong[1,*]
[1]*Department of Forest Environment System, College of Forest & Environmental Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea*
[2]*Gangwon Forest Science Institute, Chuncheon 24341, Republic of Korea*
[3]*Department of Forest Biomaterials Engineering, College of Forest & Environmental Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea*

Abstract

Two species, *P. pyrifolia* and *P. ussuriensis*, of the genus Pyrus native to Korea, are valuable genetic resources that can be used for food, dietary supplement, pharmaceutical, and cosmetics. Bioactive compounds of the plant leaves are the main components that are used for the products. Farmers had cultivated a few individuals from the wild to produce fruits or leaves for traditional remedy or tea; however, bioactive components of their leaves are not tested. We selected some trees from the natural stand that have distinct traits for the improvement program. We investigated the bioassay on the extracts' bioactive compounds and antioxidant capacity from the selected accessions and other accessions, including newly developed cultivars. The contents of the phenolic compounds and flavonoids from the leaf extracts of the selected accessions were higher than the commonly cultivated trees in both species but lower than 'Sanhyang' in *P. ussuriensis*. The antioxidant capacity was measured using two assay methods, DPPH and ABST. The selected cultivars also had higher inhibitory activity than common trees. The selected accession 'Cultivar 3' in *P. pyrifolia* had the highest radical scavenging activity than others. Although leaves of the accessions were used only in this study, all three selected individuals have the potential for cultivar in containing high bioactive compounds and antioxidant capacity.

Key Words: *Pyrus pyrifolia*, *Pyrus ussuriensis*, wild pear, bioactive compound, antioxidant capacity

Introduction

P. pyrifolia (Burm.f.) Nakai and *P. ussuriensis* are naturally growing in the Korean peninsula, known as 'Korean pear.' These are different from an improved cultivar bearing large fruits, which is introduced from Japan. These belong to the Rosaceae family and the genus of Pyrus (Oh et al. 2015). They grow deciduous tall or shrub and have resistance to the cold (Yu 1989). These have distinctive bright white colors, and some were developed for ornamentals in landscape and potting plants. Characteristics in flowers and inflorescence and fruits are distinct from each other. Fruits

Received: June 6, 2021. Revised: July 8, 2021. Accepted: July 20, 2021.

Corresponding author: Eun Ju Cheong
Department of Forest Environment System, College of Forest & Environmental Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea
Tel: +82-33-250-8312, Fax: +82-33-259-5621, E-mail: ejcheong@kangwon.ac.kr

of *P. pyrifolia* have been used to treat cough, asthma, fever, and hangover in oriental remedy for a long time. Therefore, it has been developed to improve fruit characteristics, sweeter or larger size (Yu 1989). While the ripe fruits of *P. pyrifolia* are brown with no calyx, *P. ussuriensis* is yellow and calyx attached (Lee 2003). Fruits have been used for medicinal purposes rather than table-eating raw because they are small and rugged (Lee 1996).

Plants are essential materials for the pharmaceutical and cosmetic industries (Faccio 2020). In addition, there is a rapidly growing demand for natural resources in their own country after joining the Nagoya Protocol. In this context, these two species have good potential for the use of bio-industries. Several researchers reported the effectiveness of the extracts of pear leaves on severe gastroenteritis because of the components arbutin and tannin (Kim 1998). Extracts from Ussurian pear (Sandolbae, *P. ussuriensis*) effectively prevent cancer and bacterial infection (Lee et al. 2010). Also, the extracts from the Korean pear (Dolbae, *P. pyrifolia*) had an effect on protecting skin cells from UV light (Koh et al. 2016).

Bioactive compounds are found in small quantities in various plant parts (Yahia et al. 2020). As they are providing health benefits, researchers are more interested in natural resources. Common examples of bioactive compounds from plants are plastids such as carotenoids and secondary metabolites like phenolic. Phenolic compounds, including flavonoids, have antioxidant capacity (Hamzaliglu and Gökmen 2016). Many plants have bioactive compounds; however, wild plants have higher nutritional compounds and antioxidants than the cultivated vegetables because cultivated plants were improved to have the sweetness or gentle texture (Luta et al. 2020).

It is similar in the case of pear. This species had been bred to produce the sweet and large size fruit, contents of bioactive compounds were not considered for the breeding purpose. Most researches were conducted using common plant materials. Selecting some accessions which have good characteristics such as high bioactive elements would beneficial to the producers. The content of bioactive components of some selected individuals was measured for the wild pear breeding program. We analyzed the bioactive compounds and their antioxidant capacity of the selected accessions compared with the commonly cultivated ones by farmers such as 'Sanhyang' and 'Moonbae' and developed cultivars before the future breeding program.

Materials and Methods

Plant materials

Three accessions of *P. pyrifolia* and four of *P. ussuriensis* were used for the study. Plants were grafted to maintain the maternal characteristics, and they have been grown in a nursery in the Institute of Forest Science of Gangwon-do located in Chuncheon-si, since 2019. A common accession of each species was included as a control. Three selected cultivars 'Sanhyang', '*P. ussuriensis* var. *seoulensis* named 'Moonbae,' and candidate 'cultivar 1' of *P. ussuriensis*, and two candidates named 'cultivar 2' and 'cultivar 3' of *P. pyrifloia* were examined in this study.

'Cultivar 1' has unique flower characteristics, which are double petals. Leaves of 'Cultivar 1' is wide serration angle than common *P. ussuriensis* and leaf width is narrow. The fruit of 'Cultivar 2' is different from a common *P. pyrifolia*, which is bottle shape. The fruit of 'Cultivar 3' has no core and stone cells, ripe in November, relatively later than usual. Leaves of 'Cultivar 2' and 'Cultivar 3' have narrow serration angle and wider width than common *P. pyrifolia*.

Pretreatment for extract

Leaves collected in June were washed and dried at room temperature for 24 hours in the shade. They were placed in a 5L Erlenmeyer flask, and 60% fermented alcohol for food and beverage (Ethanol Supplies World Co., Ltd) was added 20 times the dry weight of leaves. It was extracted for 72 hours at room temperature. The extractions were filtered using a Buchner funnel and filter paper (Hyundai Micro No. 20). After that, leached substances of the filtrate were removed through the concentration with a rotary vacuum evaporator (RE-501). The extract in the form of an aqueous solution was pre-frozen in a -18°C freezer for 1-2 days and then dried in a freeze dryer (Ilshin Biobase, Seoul, Korea) at -88°C for three days. Powdered samples were stored in a freezer before the experiments. The yield of each extract was calculated as a percentage of the leaves' dry weight used for preparation by the extract's dry weight of the lyophilized.

Contents of total polyphenols

Total polyphenol contents (TPC) were determined by the modified Folin-Denis method (Suzuki et al. 2002). Four substances, Methyl Gallete (MG), Ethyl Gallete (EG, Tokyo Chemical Industry Co., LTD), Gallic acid (GA), and Tannic acid (TA, Sigma-Aldrich, USA), were used for standard. Extracts were diluted in different concentrations, 1, 10, 25, 50, 100, and 250 mg/μL, in a stock solution. Standard substances were also prepared with the same concentrations as extracts. The reaction solution was mixed with 60 μL of each extract and standard and 0.2 M Folin-Denis reagent (Sigma-Aldrich, USA) in a 96-well plate. Reaction solutions were placed in darkness at room temperature for 3 hours. Sixty μL of 2% sodium carbonate solution (Sigma-Aldrich, USA) were added to the reaction then leave 47 minutes in the darkness. Absorbance at 750 nm was measured from each reaction using Microplate Reader (Tecan, Switzerland), all measurements were repeated three times. The total polyphenol content of the extract was calculated based on each substance's standard curve, i.e. MG ($y=0.0103x+0.0007$, $R^2=0.9964$), EG ($y=0.0095x+0.0015$, $R^2=0.9981$), GA ($y=0.0119x+0.0045$, $R^2=0.9966$), TA ($y=0.0075x+0.0161$, $R^2=0.9983$).

Content of total flavonoid

We followed the modified method by Moreno et al. (2000) to quantify the total flavonoid content. The reaction solution was prepared in the order of 100 μL of sample stock solutions of various concentrations (1, 10, 25, 50, 100, 250, 500, and 1,000 mg/μL), 20 μL of 10% aluminum nitrate (Sigma-Aldrich, USA), 20 μL of 1M potassium acetate (Sigma-Aldrich, Missouri, USA), and 860 μL of 95% ethanol (Duksan, Korea). It was placed at dark for 40 minutes, then 200 μL were distributed into a 96-well plate. Absorbance at 415 nm was measured from each reaction using Microplate Reader (Tecan Infinite 200 Pro, Switzerland), all measurements were repeated three times. A standard curve ($y=0.0023x-0.0322$, $R^2=0.9997$) was draw up based on quercetin (Tokyo Chemical Industry Co., LTD). The total flavonoid content of the extract was calculated based on the standard curve of quercetin.

DPPH radical scavenging activity

The DPPH free radical scavenging activity was determined by modifying the method described in Blois (1958). The 1,1-diphenyl-2-picryl-hydrazyl (DPPH, Sigma-Aldrich, Missouri, USA) solution (0.2 mM) was dissolved in 99% Ethyl alcohol (Samchun, Korea) and calibrated the value of molar extinction coefficient at 0.95-0.99 at 515 nm. Ascorbic acid (Samchun, Korea) was used as a positive control. Extracts and standard substances were diluted to 31.25, 62.5, 125, 250, 500, and 1,000 μg/mL concentrations for the measurement. A hundred eighty DPPH solution was added to a well containing 20 μL of each extracted sample and ascorbic acid. Absorbance was read using Microplate Reader at 515 nm, and measurements were repeated three times per sample. The formula for calculating % scavenging activity by DPPH is as follows, then the value of IC_{50} was calculated from the scavenging activity curve.

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{OD\ Control - OD\ Sample}{OD\ Control}\right) - OD\ Blank$$

'OD Sample'=Absorbance values of DPPH radicals after treatment with sample

'OD Blank'=Absorbance values of H_2O

'OD Control'=Absorbance values of DPPH radicals

ABTS radical scavenging activity

For ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity, we referred to the method of Van den Berg et al (1999). ABTS solution was mixed 1:1 (v/v) of 7.0 mM 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma-Aldrich, USA) and 2.45 mM potassium persulfate (Samchun, Korea) and placed for 12-16 hours for reaction. Absorbance at 730 nm was controlled to 0.7 ± 0.02 using PBS buffer (pH 7.4, Welgene, Korea). Ascorbic acid was used as a positive control, and it was diluted to 31.25, 62.5, 125, 250, 500, and 1,000 μg/mL, same as samples. Twenty mL of each extracted sample and positive control with 180 μL of ABTS solution dispensed in a 96-well plate then reacted for 15 minutes at room temperature. Absorbance was measured at 750 nm, and measurements were repeated three times for

each sample. ABTS radical scavenging activity was calculated based on the formula below, and IC_{50} was calculated.

$$\text{ABTS radical scavenging activity (\%)} = \left(\frac{OD\ Control - OD\ Sample}{OD\ Control}\right) - OD\ Blank$$

'OD Sample'=Absorbance values of ABTS radicals after treatment with sample

'OD Blank'=Absorbance values of H_2O

'OD Control'=Absorbance values of ABTS radicals

Statistical analysis

Statistical analyses were carried out on the collected data using one-way or two-way ANOVA with Duncan's post-hoc test and Pearson's correlation with SPSS ver. 24 (IBM).

Results and Discussion

Extraction yield

The yield of all the accessions was presented in Table 1. There were significant differences among the accessions in the extraction yield. Two cultivars, 'Sanhyang' and 'Moonbae,' had the highest yield, 20.79%, and 17.56%, respectively, among *P. ussuriensis*. The commonly grown by farmers and selected accession had as low as 7.58%. Among the *P. pyrifolia*, while the general accession had the highest, 22.2%, two selected accessions had low at 13.88% and 5.5%, respectively. When Lee et al. (2011) used 80% ethanol to extract from the fruits of two species, the average harvesting rate was less than 10 mg/mL, and Kim et al. (2020) used hot water (80°C) to extract from *P. ussuriensis* leaves and took about 23.7-32.7%, which is relatively high. However, the yield of extractions would be different from the method used (Kim et al. 2018). They had the highest yield of polyphenols when 70% ethanol was used than others, hot water or methanol. Generally, the yield of phenolic compounds was higher when methanol or acetone was used; however, these are not appropriate for food or supplements due to the hazardous compounds (Lee et al. 2020). The edible 60% fermented alcohol is a little lower percentage of alcohol than other researches, but we had relatively good extractions.

Table 1. Extraction yield of 60% Fermentation Ethanol for Food Grade Extract of leaf collected from various *P. pyrifolia* and *P. ussuriensis*

Species	Cultivar name	Extraction yield (%)
P. ussuriensis	Common cultivar	8.75
	cv. 'Sanhyang'	20.79
	Munbae	17.56
	Cultivar 1	7.58
P. pyrifolia	Common cultivar	22.20
	Cultivar 2	5.50
	Cultivar 3	13.88

Extraction yield (%)={Extract (g)/Dry leaf (g)}×100.

Total phenolic and flavonoid contents

Various functional antioxidant compounds, phenolic compounds (methyl gallete, ethyl gallete, gallic acid, and tannic acid), and total flavonoids were compared among the accessions. Total polyphenol contents of selected accessions of *P. ussuriensis* were significantly higher than the common cultivars. There was no difference between the common cultivar and selected accessions. Cultivar candidate 'Cultivar 2' of *P. pyrifolia* had more polyphenols than the common cultivar. The cultivar 'Sanhyang' had the highest polyphenols, followed by 'Moonbae,' 'Cultivar 1', and the common cultivar had the lowest contents of all phenolic compounds (Table 2). The selected accession of *P. pyrifolia* had a good amount of phenolic compounds, although it was slightly lower than 'Sanhyang'.

It was reported that the various cultivars of pear had different concentrations of polyphenols in different parts. The highest one, 'Chuhwangbae', had 145 mg/100 g extract (Choi et al. 2006) from fruit peel. Compare to this report, the accessions in this study had higher contents than any parts of the pears, such as fruit skin, core, and flash, which are mainly cultivated to produce fruits. Plant leaves are considered the best material to provide antioxidants beneficial to human health because they contain more precursors of phenolic compound biosynthetic pathways than other plants (Anderson and Jorheim 2006).

Flavonoid is a phenolic compound with high antioxidant activity and can help regulate cellular activity and free radicals that cause oxidative stress (Panche et al. 2016). Therefore, more attention was given to it, not only in food

Table 2. Total polyphenol content (μg/mg extract) of *P. pyrifolia* and *P. ussuriensis*

Species	Cultivar name	Methyl gallete	Ethyl gallete	Gallic acid	Tannic acid
P. ussuriensis	Common	19.31 ± 0.41^{g}	17.15 ± 5.10^{c}	13.30 ± 1.19^{e}	24.22 ± 1.09^{e}
	cv. 'Sanhyang'	34.14 ± 0.56^{a}	32.16 ± 5.56^{a}	28.65 ± 2.76^{a}	41.25 ± 1.24^{a}
	cv, 'Munbae'	29.33 ± 0.22^{b}	27.29 ± 5.71^{ab}	23.63 ± 1.59^{b}	35.73 ± 0.48^{b}
	Cultivar 1	26.15 ± 0.45^{d}	24.06 ± 5.39^{abc}	20.37 ± 1.92^{c}	32.07 ± 1.15^{c}
P. pyrifolia	Common	22.12 ± 0.01^{f}	17.15 ± 0.98^{c}	16.18 ± 1.04^{de}	27.45 ± 0.64^{d}
	Cultivar 2	28.59 ± 0.13^{c}	26.54 ± 5.58^{abc}	22.87 ± 1.74^{bc}	34.88 ± 0.71^{b}
	Cultivar 3	23.04 ± 0.19^{e}	20.92 ± 5.42^{bc}	17.14 ± 1.34^{d}	28.50 ± 0.86^{d}

Values are mean±SE and mean separation within columns by Duncan's multiple range test, $p < 0.05$.
Same letters are not different in each parameter at level of $p=0.05$ by Duncan's test.

Table 3. Total flavonoids contents comparison of *P. pyrifolia* and *P. ussuriensis*

Species	Name	Total flavonoid contents (μg QE/mg extract.)
P. ussuriensis	Common cultivar	8.75
	cv. 'Sanhyang'	20.79
	cv, 'Munbae'	17.56
	Cultivar 1	7.58
P. pyrifolia	Common cultivar	5.50
	Cultivar 2	22.20
	Cultivar 3	13.88

Concentration: 1,000 μg/mL.
Values are mean±SE and mean separation within columns by Duncan's multiple range test, $p < 0.05$.
Same letters are not different in each parameter at level of $p=0.05$ by Duncan's test.

or supplement but also in the cosmetic and pharmaceutical industries. The content of flavonoids is significantly related to bioactivity. Each accession's total flavonoid content was indicated as μg/mg using the standard substance, quercetin. The relative content of each accession was shown in Table 3. It was similar to total polyphenols, and 'Moonbae' had the highest (127.08 μg/mg extract), followed by 'Sanhyang' (124.03 μg/mg extract) and 'Cultivar 2' (118.96 μg/mg extract). These values were ten times higher than raspberry leaf extracted by various methods (Lee 2016). However, 'Cultivar 1' had a low flavonoid content (60.51 μg/mg extract), it had a high polyphenols content. It was often observed in other plants, e.g., valerian with a high polyphenol and a low flavonoid (Kim et al. 2012).

In contrast, some plants, such as barley, mistletoe grew on an oak tree, and plantain had a high flavonoid but low polyphenols. It is because polyphenols are a large group that includes flavonoids and non-flavonoid polyphenols. It is thought that the 'Cultivar 1' had more non-flavonoids than flavonoids, which suggests the contents of polyphenol and the flavonoid do not necessarily have a positive correlation.

Antioxidant activity analysis

A substance that helps protect cells from the damage caused by free radicals. The damage by the free radicals may increase the risk of cancer and other diseases. Most antioxidant substances derived from plants and the radical scavenging activity play an essential role in protecting health (Jo et al. 2014). We conducted two assays for the radical scavenging activity of the extracts from the accessions. First, the DPPH assay is screening the antioxidant activity of plant extracts. The hydrogen atom donating ability of the plant extracts was determined by the decolorization of methanol solution of DPPH. DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants (Wang et al. 2013). Although the antioxidant activity of polar and non-polar samples cannot be measured simultaneously by DPPH assay because the radical has low solubility in water to be applied only to the organic phase, it has excellent repeatability. Also, it allows for a quick comparison of the antioxidant activity (Re et al. 1999).

We compared the IC_{50} value of ascorbic acid and all other extracts from the accessions, and it showed in Table 4.

Table 4. DPPH radical scavenging activity and IC_{50} of *P. pyrifolia* and *P. ussuriensis*

Species	Name	Inhibitory activity (%)	IC_{50} (μg/mL)
P. ussuriensis	Common cultivar	77.44 ± 0.41^{d}	294.93 ± 35.62^{bc}
	cv. 'Sanhyang'	79.34 ± 0.73^{bc}	227.13 ± 7.48^{b}
	cv, 'Munbae'	80.98 ± 0.25^{a}	287.49 ± 12.37^{c}
	Cultivar 1	78.03 ± 1.01^{cd}	338.25 ± 9.61^{d}
P. pyrifolia	Common cultivar	81.10 ± 0.25^{a}	327.84 ± 28.58^{cd}
	Cultivar 2	77.04 ± 1.39^{d}	327.58 ± 7.27^{cd}
	Cultivar 3	79.99 ± 0.44^{ab}	225.47 ± 9.55^{a}
Positive control	Ascorbic acid	90.55 ± 0.12	46.25 ± 0.64

Concentration: 1,000 μg/mL.
IC_{50} : The values indicate the concentration required to scavenger 50% of ABTS radical under the standard conditions.
Same letters are not different in each parameter at level of $p=0.05$ by Duncan's test.

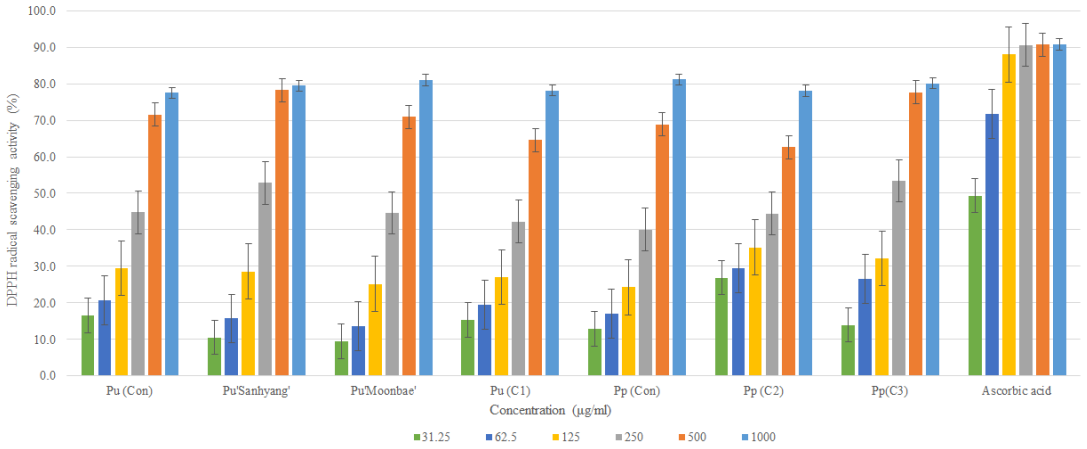

Fig. 1. DPPH radical scavenging activity of *P. pyrifolia* and *P. ussuriensis* extract at various concentration. Pu, *Pyrus ussuriensis*; Py, *Pyrus pyrifolia*, Con, common cultivar; C 1, 2, 3, Cultivar 1, 2, 3.

The inhibitory activity of all accessions is relatively high, which ranges from 77 to 81%. The DPPH radical scavenging activity of the common cultivar of *P. pyrifolia* and 'Moonbae' has the highest activity. The IC_{50} values of 'Sanhyang' and 'Cultivar 3' of *P. pyrifloia* were lower than others. Among the accessions, 'Cultivar 3' had the best DPPH radical scavenging activity. When the concentrations of the extracts are increased, DPPH radical scavenging activity increased (Fig. 1).

ABTS radical scavenging is a helpful method that utilizes the degree to which antioxidant substances remove cationic ABTS radicals generated by reaction with potassium persulfate. The radicals having a bluish-green color are discolored to transparent white due to the radical inhibitory action of antioxidants on both polar and non-polar samples (Awika et al. 2003). It is difficult to measure the antioxidant capacity of the hydrophilic compound using the DPPH because it is dissolved in organic solvents. However, ABTS had the maximum absorbance at 645, 735, and 815 nm, and it enables to measure the antioxidant capacity (Kwon and Youn 2017). ABTS radical scavenging activity of all accessions in this study had increased as a higher concentration, and it was similar to the standard substance, ascorbic acid, at 250-1,000 μg/mL (Table 5 and Fig. 2). The IC_{50} values of 'Moonbae' (55.33 μg/mL) and the 'Cultivar 2' (61.72 μg/mL) were as low as ascorbic acid (32.71 μg/mL). It is supposed to have a high antioxidant capacity. All samples showed high values of antioxidant ac-

Table 5. ABTS radical scavenging activity and IC_{50} of *P. pyrifolia* and *P. ussuriensis*

Species	Name	Inhibitory activity (%)	IC_{50} (μg/mL)
P. ussuriensis	Common cultivar	94.08 ± 0.16^{cd}	92.94 ± 2.08^{cd}
	cv. 'Sanhyang'	94.13 ± 0.22^{c}	72.30 ± 11.47^{b}
	cv, 'Munbae'	94.60 ± 0.00^{b}	55.33 ± 0.86^{a}
	Cultivar 1	93.90 ± 0.00^{d}	89.97 ± 1.77^{c}
P. pyrifolia	Common cultivar	94.75 ± 0.00^{b}	77.40 ± 1.15^{b}
	Cultivar 2	94.27 ± 0.08^{c}	61.72 ± 1.91^{a}
	Cultivar 3	95.03 ± 0.14^{a}	99.56 ± 1.08^{d}
Positive control	Ascorbic acid	95.08 ± 0.00	32.71 ± 0.52

Concentration : 1,000 μg/mL.
IC_{50}: The values indicate the concentration required to scavenger 50% of ABTS radical under the standard conditions.
Same letters are not different in each parameter at level of $p=0.05$ by Duncan's test.

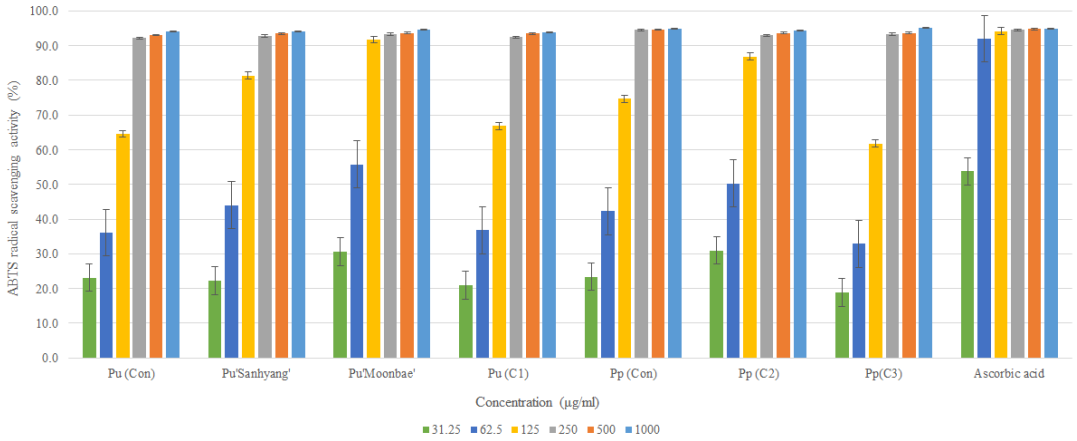

Fig. 2. ABST radical scavenging activity of *P. pyrifolia* and *P. ussuriensis* extract at various concentration. Pu, *Pyrus ussuriensis*; Py, *Pyrus pyrifolia*; Con, common cultivar; C 1, 2, 3, Cultivar 1, 2, 3.

tivity using ABTS rather than DPPH, and the IC_{50} values were also 4-5 times lower than the ones of DPPH. However, it tends to show similar but not the same results by two methods.

DPPH measures free radicals, but ABTS measures cationic radicals so that the inhibitory ability may differ depending on the phenolic substances that bind to each radical. According to the type of phenolic substances, the binding degree to the two substrates is different, and in the end, scavenging capacity may differ (Wang et al. 1998). We analyzed the total phenolic compounds without dividing the types in this study, and it showed different antioxidant capacities depending on the method. It needs to investigate the type of phenolic compounds of the samples as Chojnaki and Vogtman (1984) reported isoprenoid alcohol and polyphenol from *P. ussuriensis*.

Correlation of Bioactive compounds and antioxidant capacity

The correlation of bioactive compounds (total polyphenol and flavonoids), DPPH radical scavenging activity, and ABTS radical scavenging activity was shown in Table 6. The contents of polyphenols and flavonoids have a positive correlation of 0.644, and the radical scavenging activity of the two methods (DPPH and ABTS) also has a positive correlation of 0.622. However, the correlation between the contents of bioactive compounds and antioxidant capacity was not high. In the case of *P. pyrifolia*, while it has a

Table 6. Correlationship of total polyphenol contents, total flavonoids contents, DPPH radical scavenging activity and ABTS radical scavenging activity of *P. pyrifolia* and *P. ussuriensis*

		Total polyphenols	Total flavonoids	DPPH radical scavenging	ABTS radical scavenging
Total polyphenols	Pearson's correlation	1			
	p-value	-			
Total flavonoids	Pearson's correlation	0.644**	1		
	p-value	0.002	-		
DPPH radical scavenging	Pearson's correlation	0.006	-0.025	1	
	p-value	0.979	0.913	-	
ABTS radical scavenging	Pearson's correlation	-0.239	0.019	0.622**	1
	p-value	0.297	0.934	0.003	-

**, significant at $p < 0.01$.

low content of polyphenol, 20.73 μg/mg extract, the IC_{50} value by ABTS assay was relatively high. In contrast, 'Moonbae' contains high polyphenol (29 μg/mg extract) and flavonoids (128.07 μg/mg extract), the IC_{50} value by DPPH assay was lower than other accessions. The correlation between the contents of bioactive compounds and the antioxidant capacity differs from the accession.

Generally, the higher phenolic compounds in plants are interpreted as having higher antioxidant capacity. The results of our study agreed with the report by Shon et al. (2008), which stated specific phenolic compounds might contribute to the antioxidant capacity. Lee et al. (2011) also had similar results that the total polyphenols did not influence the antioxidant capacity. It suggests that each cultivar or accession might have specific components effective to the antioxidant capacity. Some common indigenous individuals were collected by the specific characters such as fruit texture or sweetness rather than the phenolic compounds or flavonoids. Although we selected others based on the morphology, we found that the selected accessions have relatively high bioactive compound contents in the leaves than the common accessions. It would have the potential to develop a new cultivar with a further investigation of the specific compounds.

References

Andersen OM, Jordheim M. 2006. The Anthocyanins. In: Flavonoids: Chemistry, Biochemistry and Applications (Andersen OM, Markham KR, eds). CRC Press, Boca Raton, pp 471-551.

Awika JM, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos L. 2003. Screening methods to measure antioxidant activity of sorghum (*sorghum bicolor*) and sorghum products. J Agric Food Chem 51: 6657-6662.

Blois M. 1958. Antioxidant Determinations by the Use of a Stable Free Radical. Nature 181: 1199-1200.

Choi JH, Lee EY, Kim JS, Choi GB, Jung SG, Ham YS, Seo DC, Heo JS. 2006. Physiological Activities According to Cultivars and Parts of Ulsan Pear. Appl Biol Chem 49: 43-48.

Chojnacki T, Vogtman T. 1984. The occurrence and seasonal distribution of C50-C60-polyprenols and of C100-and similar long-chain polyprenols in leaves of plants. Acta Biochim Pol 31: 115-126.

Faccio G. 2020. Plant Complexity and Cosmetic Innovation. iScience 23: 101358.

Hamzaliglu A, Gökmen V. 2016. Interaction between Bioactive Carbonyl Compounds and Asparagine and Impact on Acrylamide. In: Acrylamide in Food: Analysis, Content and Potential Health Effects (Gökmen V, ed). Academic Pres, London, pp 355-376.

Jo HJ, Kim JW, Yoon JA, Kim KI, Chung KH, Song BC, An JH. 2014. Antioxidant Activities of Amaranth (*Amaranthus spp. L.*) Flower Extracts. Korean J Food Nutr 27: 175-182.

Kim DS, Choi MH, Shin HJ. 2018. Polyphenol contents and antioxidant activities of domestic bamboo leaves with different extraction solvents. J Adv Eng Technol 11: 7-13.

Kim EJ, Choi JY, Yu MR, Kim MY, Lee SH, Lee BH. 2012. Total Polyphenols, Total Flavonoid Contents, and Antioxidant Activity of Korean Natural and Medicinal Plants. Korean J Food Sci Technol 44: 337-342.

Kim H, Kim TH, Jeon B, Bang MH, Kim WJ, Park JW, Chung DK. 2020. Evaluation of the Physiological Activity on the Skin and Identification of the Active Ingredient of Leaf Extract from Sanhyang Sandolbae (*Pyrus ussuriensis*) as a New Variety. J Korean Soc Food Sci Nutr 49: 35-45.

Kim JH. 1998. Agriculture Technology Series 34. The Latest Culture of Pear: New Cultivar, New Technology, New Agriculture. Osung Publisher, Seoul, 421 pp.

Koh A, Choi S, Kim Y, Park G. 2016. Protective Effects of *Pyrus pyrifolia* NAKAI Leaf Extracts on UVB-induced Toxicity in Human Dermal Fibroblasts. J Soc Cosmet Sci Korea 42: 87-94.

Kwon Y, Youn KS. 2017. Antioxidant and Physiological Activities of *Hijikia Fusiforme* by Extraction Methods. Korean J Food Preserv 24: 631-637.

Lee CE, Kim YH, Lee BG, Lee DH. 2010. Antioxidant Effect of the Fraction Isolated from Pyrus ussuriensis Leaves. J Korean For Soc 99: 885-890.

Lee CH, Shin SL, Kim NR, Hwang JK. 2011. Comparison of Antioxidant Effects of Different Korean Pear Species. Korean J Plant Res 24: 253-259.

Lee DY, Lee HS, Jo JH, Yi YW, Kim SJ, Kang K, Kwon TW, Yang SG, Lee IH. 2020. Comparison of Ingredients and Activities of Danggwisoo-san and Jakyakgamcho-tang by Extraction Method. J Korean Med Rehabi 30: 31-39.

Lee TB. 2003. Coloured Flora of Korea. Hyangmunsa, Seoul.

Lee WC. 1996. Coloured Standard Illustrations of Korean Plants. Academy Book, Seoul, 624 pp.

Lee YJ. 2016. Antioxidant and anti-inflammatory response of stem and leaf extracts from *Rubus coreanus* Miquel. MS thesis. Hanyang University, Seoul, Korea. (in Korean)

Luta G, Gherghina E, Balan D, Israel-Roming F. 2020. Bioactive Compounds and Antioxidant Properties of Some Wild Plants with Potential Culinary Uses. Rev Chim 71: 179-184.

Moreno MIN, Isla MI, Sampietro AR, Vattuone MA. 2000. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. J Ethnopharmacol 71: 109-114.

Oh Y, Shin H, Kim K, Han H, Kim YK, Kim D. 2015. Researches of pear tree (*Pyrus* spp.) genomics. J Plant Biotechnol 42: 290-297.

Panche AN, Diwan AD, Chandra SR. 2016. Flavonoids: an overview. J Nutr Sci 5: e47.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26: 1231-1237.

Shon HK, Lee YS, Park YH, Kim MJ, Lee KA. 2008. Physico-chemical Properties of Gugija (*Lycii fructus*) Extracts. Korean J Food Cookery Sci 24: 905-911.

Suzuki K, Suzuki M, Akamatsu H, Matsungaga K. 2002. Allergic contact dermatitis from minoxidil: study of the cross-reaction to minoxidil. Am J Contact Dermat 13: 45-46.

Van den Berg R, Haenen GRMM, van den Berg H, Bast A. 1999. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chem 66: 511-517.

Wang H, Liu YM, Qi ZM, Wang SY, Liu SX, Li X, Wang HJ, Xia XC. 2013. An overview on natural polysaccharides with antioxidant properties. Curr Med Chem 20: 2899-2913.

Wang M, Li J, Rangarajan M, Shao Y, LaVoie EJ, Huang TC, Ho CT. 1998. Antioxidative Phenolic Compounds from Sage (*Salvia officinalis*). J Agric Food Chem 46: 4869-4873.

Yahia Y, Benabderrahim MA, Tlili N, Bagues M, Nagaz K. 2020. Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two *Ziziphus* Mill. species. PLoS One 15: e0232599.

Yu TJ. 1989. The Food Guide. Munundang, Seoul, pp 166.