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Comprehensive Determination of the Phenolic Compound Contents and Antioxidant Potentials of Leaves and Roots of *Peucedanum japonicum* Harvested from Different Accessions and Growth Periods

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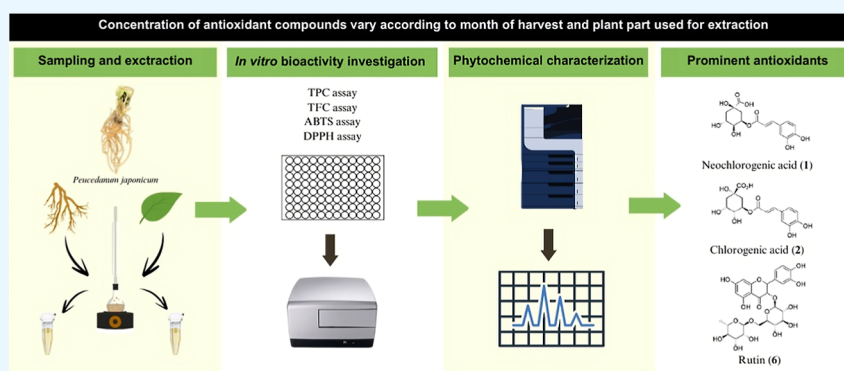
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ABSTRACT: *Peucedanum japonicum* Thunberg, a medicinal plant, remains understudied despite its potential therapeutic benefits. This study aimed to determine the phytochemical profiles and antioxidant capacities in the extracts of different accessions of *P. japonicum* by measuring the total polyphenol and flavonoid content of the *P. japonicum* extracts coupled with DPPH and ABTS$^+$ assays. In addition, phytochemical screening via LC−MS/MS and high-performance liquid chromatography analysis quantified nine compounds wherein chlorogenic acid (CA) was found to be the most abundant in all compounds while hyperoside and peucedanol were the least. Results showed variation in these compounds' content among accessions (2.01−21.31 mg/g CA) and plant parts (0.34−19.57 mg/g CA), with leaves generally showing higher antioxidant activity. The abundance of these compounds These integrated analyses provide insights into the phytochemical composition and antioxidant activity of this understudied plant, contributing to advances in natural product chemistry and potential therapeutic applications.

1. INTRODUCTION

Secondary metabolites are compounds synthesized by organisms that are not directly involved in growth or reproduction, but confer selective advantages, such as defense against predators or environmental stressors.[1] These bioactive compounds, whether used alone or in combination, have medicinal properties that often surpass those of synthetic drugs. In addition, they can enhance the therapeutic effects of other compounds in drug formulations.[2] In plants, secondary metabolites serve primarily as antioxidants, protecting them from oxidative damage caused by various stressors.[3]

Plants generally contain a wide variety of these metabolites which vary in concentration and type from plant to plant.[4] Even different individuals of the same plant species will almost always have different concentrations and types of metabolites in them. This is because these metabolites are strongly controlled by environmental factors.[5] Plants growing in stressful environments often produce higher concentrations of these metabolites because they need them to avoid oxidative

stress.[6] Therefore, phytochemical profiling is very important in the field of natural product research. However, there are still plants that have received little attention or interest.

One such plant is *Peucedanum japonicum* Thunberg. of the family Apiaceae also known as coastal hog fennel. It is a hardy perennial that grows well in a variety of environments, including sandy soils, rocky crevices, and coastal dunes.[7] It has a tall stature with an erect stem that branches out, reaching heights of 60 to 100 cm, and its strong roots provide stability and nutrition even in harsh conditions. The compound leaves are arranged alternately along the stem, with each leaf having three leaflets (ternate) that may be further divided into three

Table 1. Accession Names, Harvest Date, and Tissues of *P. japonicum* Used for Metabolite Analysis

no	accession name	harvest date of leaves						
		Jun 27 (A)	Jul 20 (B)	Aug 17 (C)	Sep 07 (D)	Oct 05 (E)	Nov 07 (F)	Nov 23 (R)
S1	SNU-59	S1-A	S1-B	S1-C	S1-D	S1-E	S1-F	S1-R
S2	SNU-5	S2-A	S2-B	S2-C	S2-D	S2-E	S2-F	S2-R
S3	SNU-18	-[a]					S3-F	S3-R
S4	SNU-6						S4-F	S4-R
S5	SNU-25						S2-F	S2-R
S6	SNU-31						S6-F	S6-R

[a]Not used for the metabolite analysis.

(A)

(B)

Figure 1. Images of the cultivation field (A) and root morphologies of the six selected accessions (B). The leaves and roots of 60 of the *P. japonicum* accessions pictured in (A) were selected based on their good yield during the last 5 years of these 60 accessions, six elite accessions were used for the metabolite analysis.

leaflets (biternate). These leaves have a unique sheen due to their hairless, shiny surface. Several parts of Korea, including Gyeonggi, Gangwon, Chungnam, Jeonnam, Gyeongbuk, Gyeongnam, and Jeju, are home to this plant,[8] which can also be found in the Philippines, China, Taiwan, and Japan.[9]

In Korea, *P. japonicum* young leaves and stems are consumed as vegetables,[10] but are also valued for their therapeutic effects in some Asian countries.[8,11] Specifically, in Korea, the roots are used to treat headaches and coughs, while in Japan, the leaves are used as an herbal remedy to treat coughs.[12] The plant has also been shown to have antiobesity,[13] antidiabetic,[14] and antiosteoporosis[15] properties. These medicinal properties can be attributed to the numerous phytochemicals found in this species. Such bioactive compounds from plants have received increasing attention in recent years.[16] Prior studies that detected and quantified the phytochemical constituents of this plant are scant. Phytochemical constituents like neo-chlorogenic acid (**1**), chlorogenic acid (CA) (**2**), crypto-
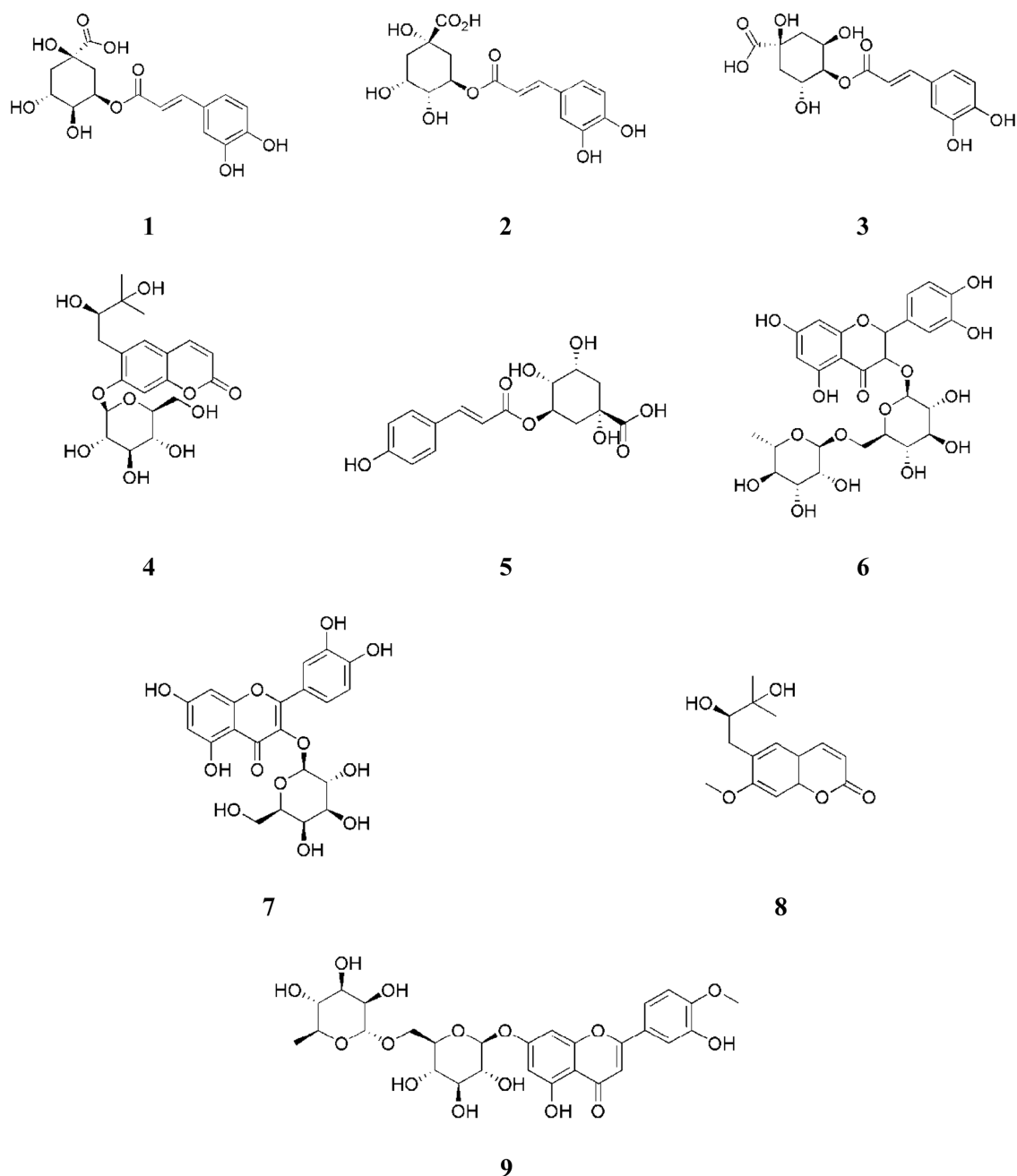
Figure 2. Chemical structures of neochlorogenic acid (**1**), CA (**2**), cryptochlorogenic acid (**3**), peucedanol 7-*O*-glucoside (**4**), 3-*O*-coumaroylquinic acid (**5**), rutin (**6**), hyperoside (**7**), peucedanol (**8**), and diosmin (**9**).

chlorogenic acid (**3**), peucedanol 7-*O*-glucoside (**4**), rutin (**6**), and peucedanol (**8**) previously isolated from the leaves of this plant.[10] The present work also wanted to check if the same compounds previously isolated from the leaves are also in the roots of the plant.

In this study, the leaf extracts of two *P. japonicum* accessions (S1 and S2) and the leaf and root extracts of six accessions (S1−S6) were used to compare the total polyphenol (TPC) and total flavonoid (TFC) contents, coupled with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonate) (ABTS⁺) assays to determine their antioxidant activities. In order to gain insight into the nature of the compounds present in the extracts, phytochemical screenings were performed using liquid chromatography-tandem mass spectrometry (LC−MS/MS), and the com-

pounds detected were quantified by high-performance liquid chromatography (HPLC). To the best of our knowledge, this is the first study that investigated the differences in the phytochemical content among the different parts of this plant, as well as the effects of harvest time. Previous studies only focused on either stems and leaves or the roots alone. The results of this study can be used in the development of functional foods, and novel medicinal ingredients, and as a reference for future phytochemical studies.

2. MATERIALS AND METHODS

2.1. Plant Materials. *P. japonicum* germplasms were obtained from various locations, including Goheung, Geumo Island, Wando, Jeju Island, and Ulleung Island, Korea. Pedigree selection for the development of inbred lines was

carried out at the Seoul National University farm in Suwon, Gyeonggi-do, Korea.[17] From a pool of genetically diverse conserved resources, 60 accessions were carefully selected based on their superior yield and cultivated according to a standard cultivation protocol. Each accession was systematically planted in blocks of 25 individual plants spaced 14 cm apart.

2.2. Harvest of the Leaves and Roots of *P. japonicum*. From the 60 groups, six accessions (S1–S6) known for their robust yields were selected for detailed leaf and root sampling. Leaves of S1 and S2 of accessions were collected from June 27 to October 5 at 3 to 4 week intervals and from all six accessions on November 7, and roots were collected from all six accessions on November 23, as documented in Table 1 and Figure 1. After harvest, the leaves and roots were processed by drying and powdering.

2.3. Instruments and Reagents. The HPLC analysis was performed using an Agilent 1260 Infinity II Quat Pump (Santa Clara, CA, USA) equipped with an Agilent variable wavelength (VW) detector and a YMC Pack-Pro C18 column (250 mm × 4.6 mm, 5 μm). The HPLC grade solvents trifluoroacetic acid (TFA) and acetonitrile (ACN) were purchased from J. T. Baker (Radnor, PA, USA). The standards, neochlorogenic acid (**1**), CA (**2**), cryptochlorogenic acid (**3**), peucedanol 7-*O*-glucoside (**4**), 3-*O*-coumaroylquinic acid (**5**), rutin (**6**), hyperoside (**7**), peucedanol (**8**), and diosmin (**9**) were generously provided by the Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Korea (Figure 2).

2.4. Extraction. Ten grams of each powdered sample was combined with 200 mL of 95% ethanol (EtOH) and subjected to three reflux extractions. After each extraction, the extracts were filtered through Whatman qualitative filter paper, grade 1 (Bernolsheim, France), and concentrated using an Eyela N-1210BVF rotary evaporator (Tokyo, Japan). Concentrated extracts were stored until further use.

2.5. TPC Analysis. The TPCs of the *P. japonicum* extracts were measured following the same procedures as described in our previous publication.[18] Briefly, 60 μL of each extract was dispensed into a 96-well microplate. Then, 40 μL of Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 100 μL of 7.5% sodium carbonate (Na$_2$CO$_3$) solution were added to the same wells. The plate was vortexed using a Micromixer MX4 microplate reader (FINEPCR, Gunpo, Republic of Korea). It was then incubated for 30 min at room temperature in the dark to allow the reaction to occur. The absorbance of the solutions was then measured at 760 nm using a microplate reader (Epoch; BioTek, Winooski, VT, USA). The TPC expressed as mg tannic acid equivalent (TAE)/g *P. japonicum* extract, was determined using a standard curve constructed from different concentrations of tannic acid.

2.6. TFC Analysis. As with the TPC assay, the TFC of the *P. japonicum* extracts was measured using a method described in the same study.[18] One hundred μL of each extract was dispensed into a 96-well microplate and added to 100 μL of 2% aluminum chloride hexahydrate (AlCl$_3$·6H$_2$O) solution. The plate was briefly vortexed using a Micromixer MX4 microplate reader (FINEPCR, Gunpo, Korea) and then incubated for 10 min at room temperature. After incubation, the absorbance of the samples was read at 430 nm using an Epoch microplate reader (BioTek). The TFC was determined using a standard curve derived from different concentrations of quercetin and

expressed as mg quercetin equivalent (QE)/g *P. japonicum* extract.

2.7. DPPH Radical Scavenging Assay. The methodology used in this study was adapted from our previously published work.[18] The experimental procedure began with the preparation of a 0.2 mM DPPH working solution, obtained by diluting the initial DPPH stock solution with 95% EtOH. Next, 10 μL of the plant extracts were combined with 200 μL of the DPPH working solution in the wells of a 96-well plate, with three replicates to ensure precision. After thorough mixing on a microplate shaker, the solutions were incubated in the dark for 30 min. Then, their absorbance was measured at a wavelength of 514 nm (OD$_{514}$) using ascorbic acid as a standard for comparison. The rate of DPPH radical scavenging was then calculated using the equation

$$\text{DPPH radical scavenging activity (\%)}$$
$$= (\text{blank OD}_{514} - \text{Sample OD}_{514}) / \text{blank OD}_{514}$$
$$\times 100$$

2.8. ABTS$^+$ Radical Scavenging Assay. The methodology used in this assay was also adapted from our previous work.[18] First, the ABTS stock solution was prepared and was subsequently diluted with water to make the ABTS working solution. Next, 10 μL of each plant extract was combined with 200 μL of ABTS working solution in the wells of a 96-well plate with three replicates. After thorough mixing and incubation for 30 min in the dark, absorbance was recorded at 734 nm (OD$_{734}$) using ascorbic acid as a comparative standard. The ABTS radical scavenging rate was calculated using the equation

$$\text{ABTS}^+ \text{ radical scavenging activity (\%)}$$
$$= \frac{(\text{blank OD}_{734} - \text{Sample OD}_{734})}{\text{blank OD}_{734}} \times 100$$

2.9. LC–MS/MS Conditions. The LC–MS/MS was performed on an LC system comprising a Thermo Vanquish UHPLC instrument equipped with a Waters Cortex T3 column (150 mm × 2.1 mm, particle size 1.6 μm). The temperature was maintained at 45 °C. The mobile phase consisted of 0.1% HCOOH in water (eluent A) and 0.1% HCOOH in ACN (eluent B). A gradient was applied and the flow rate was set at 0.25 mL/min. A Triple TOF 5600+ system (AB SCIEX, USA) with a heated electrospray ion source (H-ESI) was used for mass spectrometric analysis. Using a quadrupole system with a resolution setting of 70,000, the mass spectrometer produced survey full-scan MS spectra (*m/z* 100–1500) while operating in both positive and negative ion modes. For the positive ion mode, the spray voltage was set to 3.5 kV. MS2 fragmentation was performed on the 15 most energetic precursor ions, and spectra were acquired at 17,500 resolutions. Additional MS parameters included a capillary temperature of 320 °C, sheath gas at 50 AU, sweep gas at 1 AU, and auxiliary gas flow rate at 10 AU.

2.10. HPLC Sample and Standard Solutions Preparation. The *P. japonicum* extracts were dissolved in HPLC-grade methanol to obtain a concentration of 30 mg/mL. Similarly, the nine standard compounds (neochlorogenic acid, CA, cryptochlorogenic acid, peucedanol 7-*O*-glucoside, 3-*O*-cou-maroylquinic acid, rutin, hyperoside, peucedanol, and diosmin) were also dissolved in the same solvent to obtain a
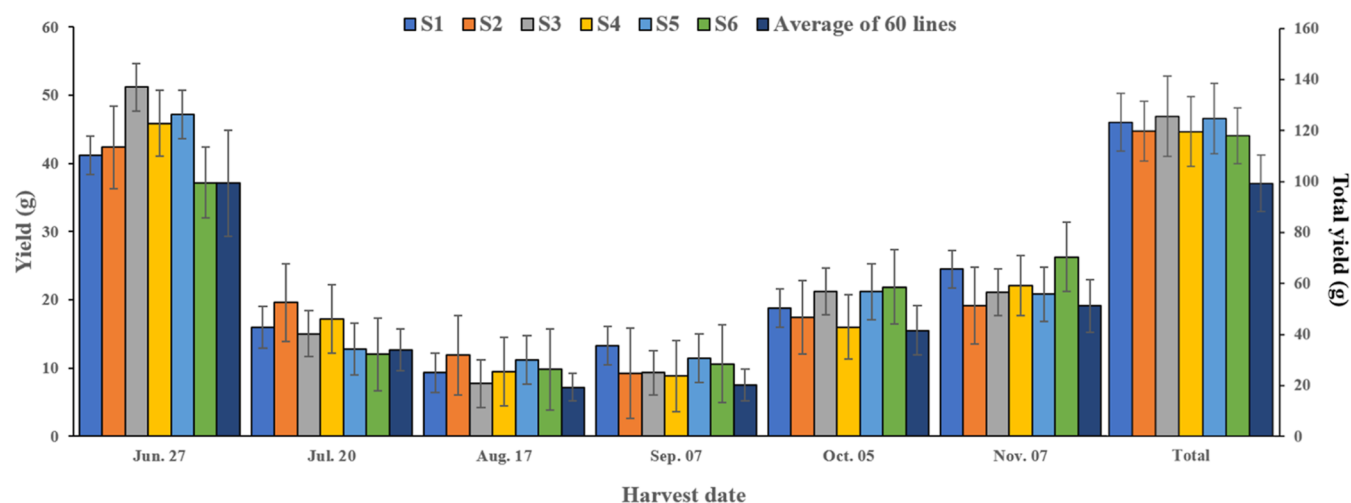
Figure 3. Leaf yields for six *P. japonicum* accessions harvested at different dates. Fully expanded leaves were harvested from six accessions which were collected from 25 individual plants in a 1 × 1 m block for each accession.
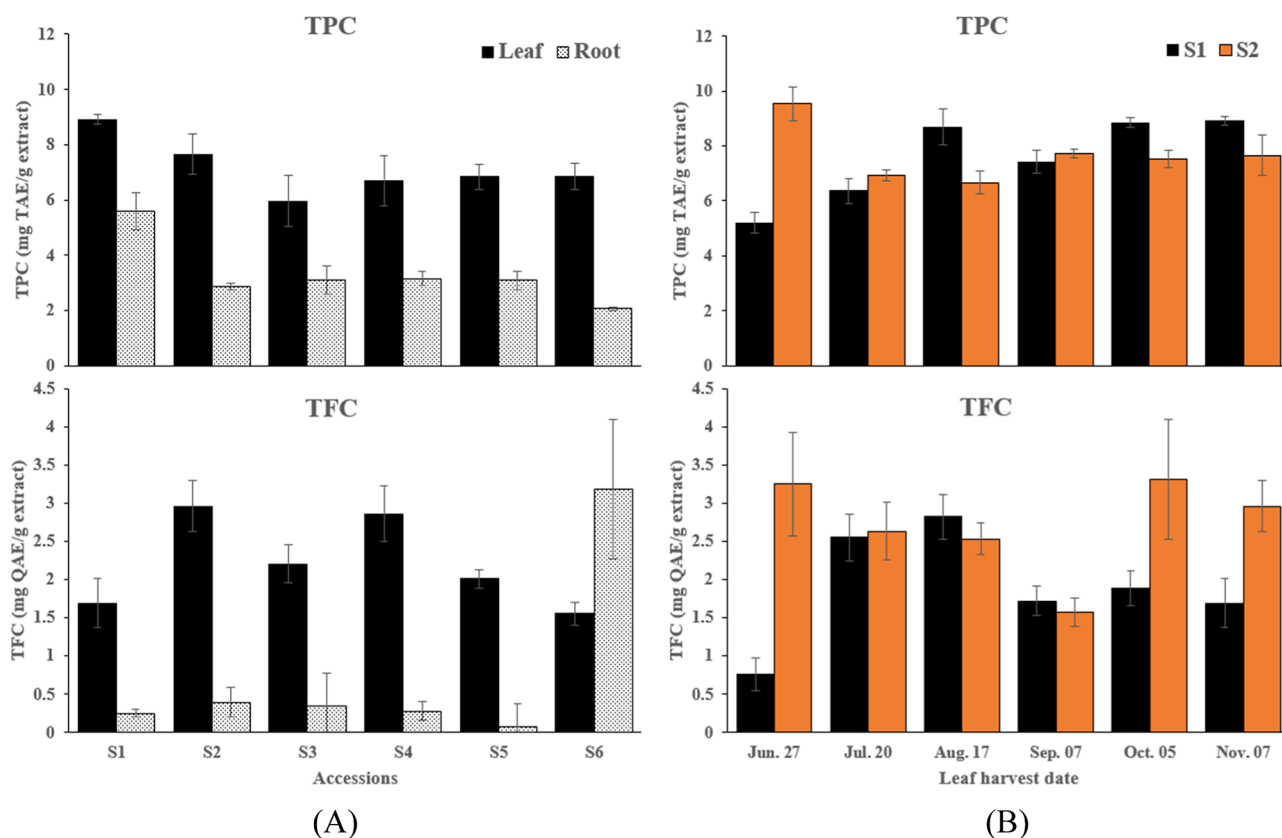

Figure 4. Total phenolic content (TPC; top) and TFC content (TFC; bottom) of the leaf and root extracts of six accessions (S1−S6); (A) and leaf extracts of accessions S1 and S2 at six harvest dates (B). The leaves and roots for the extracts in (A) were harvested on November 7 and 23. The results are expressed as the mean ± SD (n = 3).

concentration of 1 mg/mL. All solutions were then sonicated for 15−20 min and filtered through a polyvinylidene fluoride (PVDF) membrane filter with a pore size of 0.45 μm. The standard solutions were then diluted to obtain five concentrations (0.5−0.03125 mg/mL) suitable for calculations in the quantitative analysis.

2.11. HPLC Conditions. The *P. japonicum* extracts were subjected to quantitative HPLC analysis performed using an Agilent 1260 Infinity II Quat Pump (Santa Clara, CA, USA) equipped with an Agilent VW detector (Santa Clara, CA,

USA) and a YMC Pack-Pro C18 column (250 mm × 4.6 mm, 5 μm). The mobile phase used was a gradient elution consisting of 0.1% TFA in water (A) and ACN (B). The gradient elution conditions included these steps: 90% A from 0 to 10 min, decreasing to 40% A at 40 min and to 0% A at 45 min, and then returning to 90% A at 60 min and holding until 70 min. The injection volume was 10 μL and the flow rate was 1.0 mL/min. The detector wavelength was set at 330 nm.

2.12. Calibration Curve Construction. The calibration curves were constructed for each standard solution using the
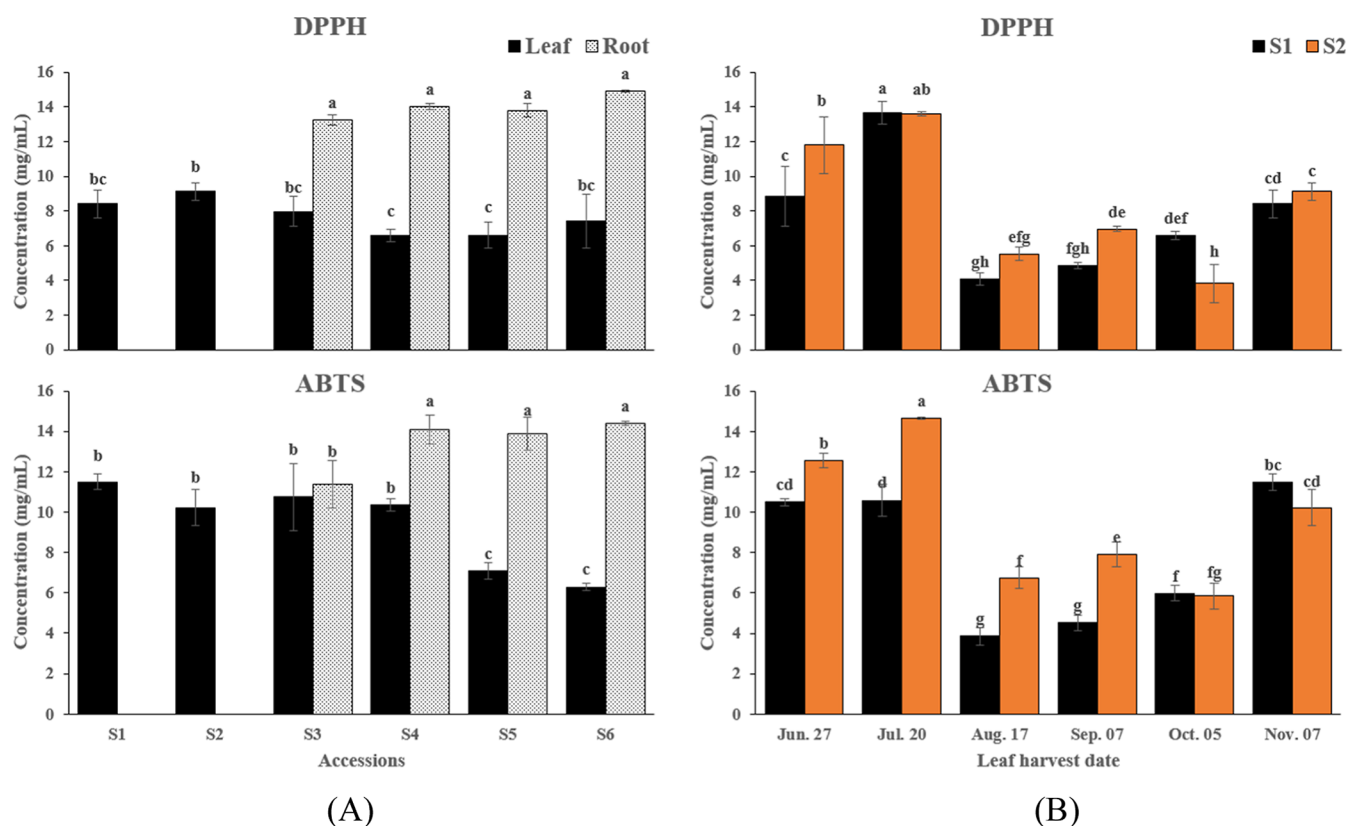
Figure 5. Results of DPPH (top) and ABTS (bottom) assays of the leaf and root extracts of six accessions (S1−S6); (A) and leaf extracts of accessions S1 and S2 at six harvest dates (B). The leaves and roots for the extracts in (A) were harvested on November 7 and 23. The results are expressed as the mean IC$_{50}$ concentrations ± SD ($n = 3$), and the lowercase letters indicate significance groupings: means associated with the same letter are not significantly different ($p < 0.005$).

five concentrations obtained through dilution. The linearity of the calibration curves was then evaluated using the coefficient of determination (R^2), and the content of the target compound was calculated using the equation derived from the calibration curve. For the calibration correction function of the standard compound, the solution concentration (in $\mu g/mL$) was plotted on the x-axis, while the peak area was plotted on the y-axis. The mean ($n = 3$) ± standard deviation (SD) was used as the value to be substituted. To calculate the lowest detectable amount or concentration of the analyte (LOD) and minimum quantifiable amount of analyte with acceptable repeatability and accuracy (LOQ), the following formulas were used: LOD = 3.3 (σ/S) and LOQ = 10 (σ/S). These formulas were based on the SD values of the intercept (σ) and the slope (S).

2.13. Statistical Analysis. Results were obtained from three independent experiments (triplicates) and are presented as mean ± SD for all analyses except LC−MS/MS. The data were tested for normality and lognormality before being treated. Statistical significance was assessed using a one-way analysis of variance followed by Tukey's post hoc test based on an acceptable type-1 error rate of $p < 0.05$. All statistical analyses and graphs except the calibration curves, were performed using GraphPad Prism 8.0.2 statistical software (GraphPad Software, Boston, MA 02110, USA). The calibration curves were generated were generated using the Agilent OpenLab software.

3. RESULTS AND DISCUSSION

3.1. Leaf Growth and Yield of Six Accessions. In this study, 25 individual plants per accession were grown in a 1 × 1 m block, spaced 14 cm apart. Leaves from 60 accessions were regularly harvested at three-week intervals for a total of six harvests. Of the 60 accessions, six were specifically selected for detailed metabolite analysis. During the growing season from June to October, leaf regrowth was observed within 3 to 4 weeks after harvest in all lines. The volume of leaf growth was consistent in five of the accessions, S1−S5, but was significantly lower in S6. The total yield of the six harvests was approximately 120 g for S1−S5, while that for S6 was less than 100 g as shown in Figure 3. This difference in growth and yield highlights the variability in metabolic performance and growth dynamics among the selected accessions.

3.2. TPC and TFC Assays. Polyphenols and flavonoids, which are abundant in plant foods and herbal medicines, are known for their potent bioactive properties.[19] These compounds exhibit antioxidant, anti-inflammatory, anticancer, antimicrobial, and cardioprotective effects, scavenge free radicals; modulate inflammatory pathways, inhibit tumor growth; and combat microbial infections.[20,21] Their multiple health benefits make them promising candidates for disease prevention and treatment. Therefore, the determination of TPC and TFC is critical for the characterization of natural products and extracts, providing insight into their phytochemical composition and potential health benefits. By quantifying the concentrations of polyphenolic and flavonoid compounds, TPC and TFC assays provide an estimate of the sample's
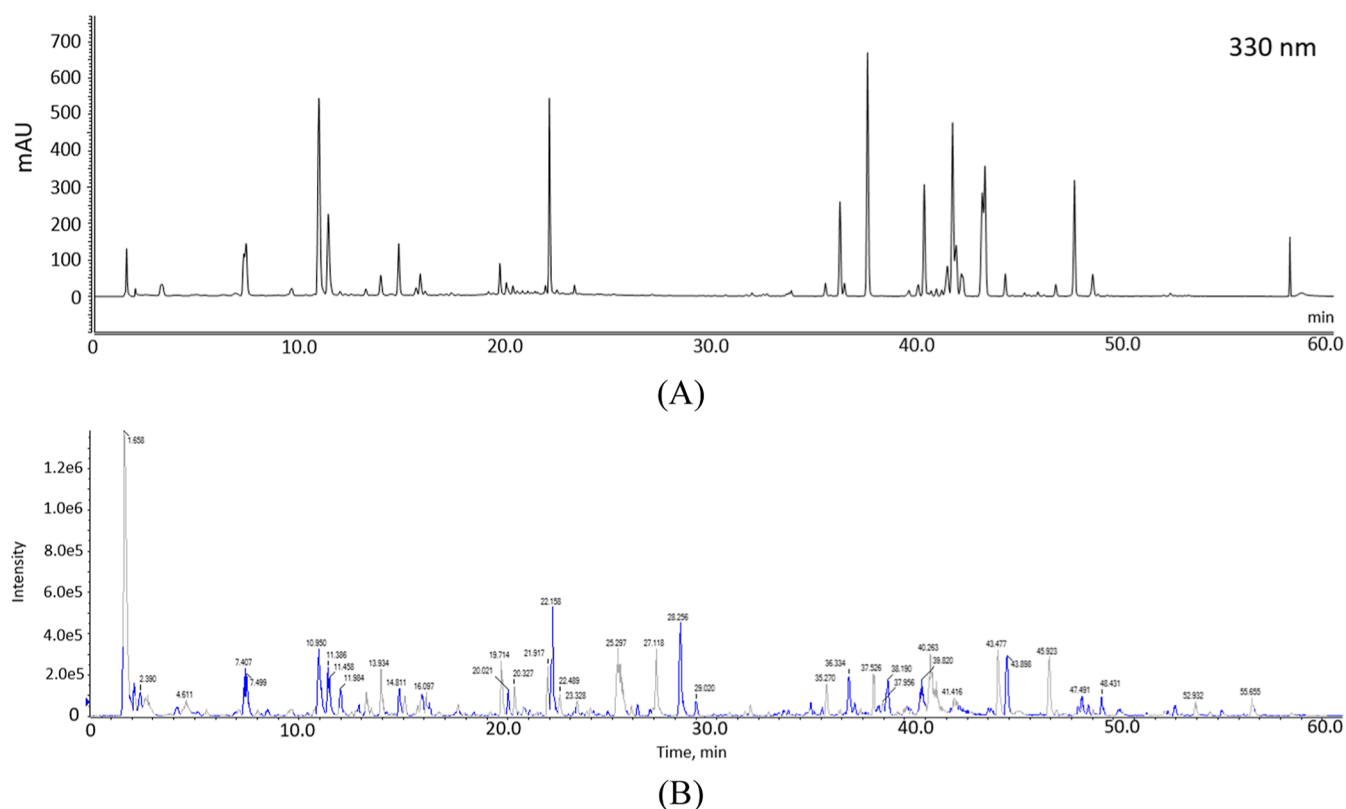
Figure 6. UV chromatogram of S2-A at 330 nm (A) and the base peak chromatogram of S2-A that was analyzed in negative ion mode (B).

antioxidant capacity, which is critical in combating oxidative stress and preventing cellular damage.[22]

In this study, the TPC and TFC of leaves and roots of six different *P. japonicum* accessions harvested at the same time of year and the leaves of two harvested over six months were measured using colorimetric methods (Figure 4). The results showed that the TPC and TFC of accessions S1 and S2 fluctuated over the six-month period, and no clear trend could be deduced from the data itself. However, the average TPC of S1 (7.67 mg TAE/g extract) was higher than that of S2 (7.57 mg TAE/g extract) over the six months of the growth period with S1-F having the highest TPC of 8.91 mg TAE/g extract while S2-A having 9.54 mg TAE/g extract. A similar observation was made with respect to TFC, where S2 had an average TFC of 2.70 mg QE/g extract, while that of S1 was only 1.90 mg QE/g extract. Among S1 samples, S1-C had the highest TFC with 2.82 mg QE/g extract while S2-E had the highest at 3.31 mg QE/g extract in S2 samples. Thus, accession S2 generally had more polyphenols and flavonoids compared to its counterpart. There was no clear relationship or trend observed among the samples.

On the other hand, the roots of the six different accessions generally had lower TPC and TFC compared to their leaf counterparts. The highest TPC was recorded from S2 leaves (9.54 mg TAE/g extract), while the lowest was from S6 roots (2.06 mg TAE/g extract). Regarding TFC, S2 leaves still showed the highest content with 2.96 mg QE/g extract, while the lowest was recorded from S4-R with 0.28 mg QE/g extract. The results are consistent with those of our previous study.[18] This is because the leaves or aerial parts of the plant are more involved in photosynthesis which is associated with reactive oxygen species formation and thus generate more secondary metabolites that act as antioxidants compared to the roots.

In a previous study, the TPC and TFC of *P. japonicum* root extracts were investigated using both distilled water and EtOH as solvents.[23] The results showed that EtOH extract had the highest TPC of 5.64 mg GAE/g and TFC of 664.17 μg QE/g compared to water extract with values of 4.75 mg GAE/g and 541.61 μg QE/g, respectively. Despite the use of EtOH for extraction in the present study, lower TPC and TFC results were still observed. This discrepancy may be due to differences in extraction methods, which include differences in extraction time and temperature, all of which can affect the efficiency of compound extraction.[24,25] In addition, differences in analytical techniques and quantification standards may also contribute to inconsistent results.[26] These discrepancies highlight the need to consider various factors in experimental design and interpretation to ensure accurate and reliable comparisons between studies.

3.3. DPPH and ABTS$^+$ Radical Scavenging Assays. As mentioned above, polyphenols and flavonoids, the primary constituents quantified by TPC and TFC assays, are well-known for their potent antioxidant properties. Therefore, the evaluation of radical scavenging activity using DPPH and ABTS$^+$ assays serves as a functional assessment of the antioxidant capacity of the sample.[27] Figure 5 shows the differences in IC$_{50}$ values of all samples used in this study.

The DPPH radical scavenging assay showed significant differences in the IC$_{50}$ values of each sample. Accession S1, harvested at the third month (S1-C), showed superior antioxidant activity (4.09 mg/mL) than S1 samples harvested at different months. For accession S2, the samples harvested on the fifth month (S2-E in Table 1) outperformed the other S2 samples with an IC$_{50}$ value of 3.82 mg/mL. However, in general, S1 samples have lower IC$_{50}$ values compared to their S2 counterparts. For the leaves and roots of the six *P.*

japonicum accessions harvested at the same time of the year, the leaves showed superior antioxidant activity compared to their root counterparts. The IC_{50} values of all the root samples are above 10 mg/mL while those of all the leaf samples were below. There was not much difference in the IC_{50} values of all the root samples, while in the leaf samples, S4 outperformed its counterparts. Two of the root samples showed no DPPH activity.

In the $ABTS^+$ assay, the same trend was observed when comparing the S1 and S2 accessions: S1 produced better average IC_{50} values than S2. For the S1 accession samples, those harvested in the fourth month (S1-D) showed a superior $ABTS^+$ radical scavenging ability (4.52 mg/mL), whereas, for the S2 accession samples, the best IC_{50} value was observed in S2-E (5.84 mg/mL). For the six *P. japonicum* accessions harvested at the same time of the year, the leaf samples again showed better antioxidant activity than the root samples. Specifically, the lowest IC_{50} value recorded from the leaf samples was 6.29 mg/mL from the leaves of accession S6, while the lowest IC_{50} value from the root samples was observed from accession S3 (11.36 mg/mL). As seen in the DPPH assay, S1 and S2 roots did not show any antioxidant activity.

A previous study using *P. japonicum* showed that the root extracts of this plant had strong antioxidant activity when evaluated by DPPH and $ABTS^+$ assays.[23] Specifically, the DPPH radical scavenging assay of the extracts was 95.5% at 1000 μg/mL, while the $ABTS^+$ radical scavenging assay was 87.2% at 2000 μg/mL. These results are not consistent with the results of the present study as the root samples here showed relatively low antioxidant activities. The contrasting results warrant careful consideration.[23] Several factors may contribute to these differences, including variations in extraction methods, sample preparation, plant maturity, environmental factors, and genetic variability among plant populations.[28] It is also important to consider potential differences in the chemical composition of the extracts, which could affect their antioxidant properties.

High levels of TPC and TFC are often associated with strong radical scavenging activity as they indicate the presence of bioactive compounds capable of counteracting oxidative damage and promoting cellular health.[29] However, although S2 had higher TPCs and TFCs, S1 still generally produced lower IC_{50} values in both antioxidant assays. This could be due to compounds found in the samples that are not polyphenolic and therefore were not detected by the TPC and TFC assays. This underscores the importance of characterizing the relationship between phytochemical composition and antioxidant activity by measuring both TPC and TFC and DPPH and $ABTS^+$ radical scavenging activity, as it provides a comprehensive understanding of the potential health-promoting effects of the sample and its suitability for various applications in preventive and therapeutic interventions.[30]

3.4. LC−MS/MS and HPLC Results. To characterize the compounds responsible for the antioxidant activities observed, phytochemical profiling was performed by LC−MS/MS (Figure 6). A representative sample was analyzed in both positive and negative ionization modes, and the negative mode was able to detect 12 compounds compared to only five in the positive ionization mode (Table 2). This is because compounds that are inherently more polar or acidic ionize better in the negative mode.[31]

Table 2. Proposed Compounds Based on LC−MS/MS Analysis in Negative (MW [M − H]⁻) Mode

retention time (min)	MW [M − H]⁻	tentative identification
5.57	154.0	protocatechuic acid
7.43	354.1	neochlorogenic acid
9.67	338.1	5-coumaroylquinic acid
10.96	354.1	chlorogenic acid
11.38	354.1	cryptochlorogenic acid
13.23	354.1	1-caffeoylquinic acid
13.93	338.1	3-*O*-coumaroylquinic acid
19.70	610.2	rutin
20.03	464.1	hyperoside
21.93	610.2	hesperidin
22.10	608.2	diosmin

Nine compounds of interest were then quantified using an HPLC analysis, namely neochlorogenic acid (**1**), CA (**2**), cryptochlorogenic acid (**3**), peucedanol 7-*O*-glucoside (**4**), 3-*O*-coumaroylquinic acid (**5**), rutin (**6**), hyperoside (**7**), peucedanol (**8**), and diosmin (**9**) with good linearity and retention times (Table 3). The methods used in the analysis

Table 3. Calibration Curve Equations and Statistics for Compounds 1−9

compound[a]	t_R (min)	regression equation	R^2
1	7.6	$y = 22578.951x + 43.529$	0.9998
2	13.8	$y = 23601.509x + 123.089$	0.9991
3	15.4	$y = 21705.563x + 29.058$	0.9999
4	17.8	$y = 9364.747x + 43.074$	0.9993
5	18.7	$y = 20345.459x + 65.135$	0.9998
6	22.0	$y = 9220.838x + 33.914$	0.9995
7	22.5	$y = 12876.292x + 47.843$	0.9996
8	23.5	$y = 23315.967x + 219.979$	0.9948
9	24.4	$y = 14228.972x + 19.781$	0.9999

[a]Neochlorogenic acid (**1**), CA (**2**), cryptochlorogenic acid (**3**), peucedanol 7-*O*-glucoside (**4**), 3-*O*-coumaroylquinic acid (**5**), rutin (**6**), hyperoside (**7**), peucedanol (**8**), and diosmin (**9**).

yielded good compound separation (Figure 7) and retention times. These compounds are known to have bioactive properties that contribute to improved heart health, blood circulation, and protection against chronic diseases.[32] They also have significant potential for pharmaceutical and nutritional applications due to their diverse therapeutic effects. The detection of these compounds can explain the antioxidant activities observed from the previous assays.

3.5. Concentration of the Nine Compounds in the Roots and Leaves of the Six Accessions. Analysis of the HPLC data revealed several notable trends and relationships among the samples (Figure 8). The content of several compounds showed significant differences between leaves and roots. Six compounds (neochlorogenic acid, CA, cryptochlorogenic acid, 3-*O*-coumaroylquinic acid, rutin, and diosmin) were particularly abundant in the leaves, while peucedanol 7-*O*-glucoside, although measured in both leaves and roots, was more abundant in the roots.9.

On the other hand, hyperoside (**7**) and peucedanol (**8**) were detected at varying concentrations in the roots but were absent from the leaves of six accessions. Trace amounts of hyperoside (**7**) were detected in the roots of accessions S1, S2, and S6, but S3 was rich in the compound. Regarding peucedanol (**8**),
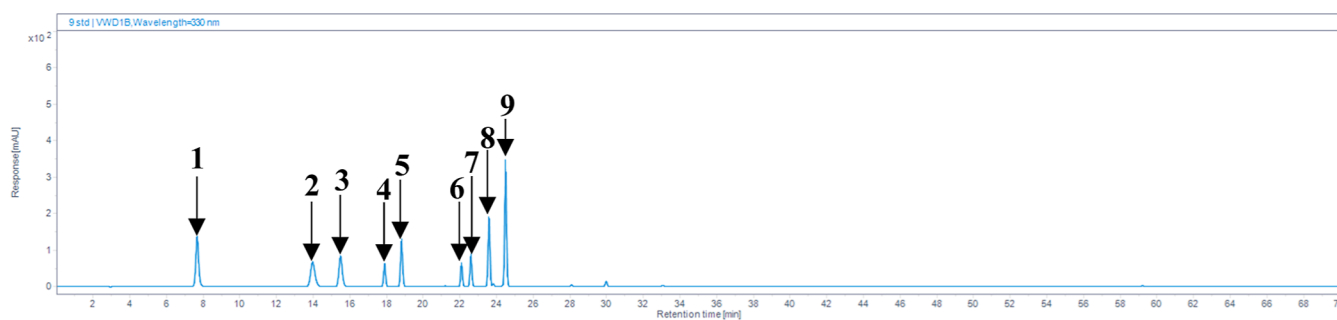
Figure 7. HPLC chromatogram of the nine standard solutions showing the peaks of neochlorogenic acid (**1**), CA (**2**), cryptochlorogenic acid (**3**), peucedanol 7-*O*-glucoside (**4**), 3-*O*-coumaroylquinic acid (**5**), rutin (**6**), hyperoside (**7**), peucedanol (**8**), and diosmin (**9**).

significantly large amounts were detected in the roots of S1, but only low amounts were detected in S2 and S3, and trace amounts were detected in S4, S5, and S6.

A large variation in the contents of compounds was detected among the six accessions, which could be due to environmental influences[33] or differences in gene regulation for the biosynthetic pathway of each compound. This variation highlights the importance of considering tissue- and genotype-specific variation in phytochemical composition for gaining a comprehensive understanding of plant metabolism.

In relation to the antioxidant assays conducted, the HPLC quantification of compounds revealed that CA (**2**) and rutin (**6**) were present in significant amounts in samples with notable antioxidant activities.

Among the leaf extracts, S4-F showed the highest antioxidant activity with the lowest DPPH IC_{50} value of 6.58 mg/mL. This sample had a notably high content of CA (**2**) (17.30 mg/g) and a significant amount of rutin (**6**) (2.60 mg/g). Another highly active leaf extract, S6-F, exhibited the lowest ABTS IC_{50} value of 6.29 mg/mL and also contained high levels of CA (**2**) (18.48 mg/g) and rutin (**6**) (2.31 mg/g). These results suggest that CA (**2**) and rutin (**6**) are major contributors to the antioxidant activity of leaf extracts. In contrast, S2-F exhibited the lowest antioxidant activity among leaf samples with a DPPH IC_{50} value of 9.12 mg/mL, correlating with its relatively lower CA (**2**) content (11.60 mg/g).

Conversely, root extracts generally showed lower antioxidant activities compared to the leaf extracts. The highest antioxidant activity among the root extracts was observed in S3-R, with a DPPH IC_{50} value of 13.25 mg/mL and an ABTS IC_{50} value of 11.36 mg/mL. This sample had a moderate CA (**2**) content (1.43 mg/g) and significant amounts of other compounds such as peucedanol 7-*O*-glucoside (**4**) (6.38 mg/g) and hyperoside (**7**) (0.55 mg/g). On the other hand, S1-R displayed no measurable antioxidant activity, indicating a lack of significant amounts of potent phenolic compounds, with a total compound content of only 3.32 mg/g. This disparity suggests that the higher concentrations of CA (**2**) and rutin (**6**) in the leaf extracts contribute to their stronger antioxidant properties. These results are also consistent with the TPC and TFC assays where the leaves generally had more phytochemicals. As mentioned above, the roots contain a lesser amount of these bioactive compounds because leaves have more substantial photosynthetic activity compared to the roots.[34] Comparison between the leaves and roots of this plant has never been documented as of this writing.

3.6. Concentration of the Nine Compounds in the Leaves of Two Accessions Harvested Monthly for Six

Months. Variations in the contents were observed in leaf samples collected every month during the growth period. Accessions S1 and S2 showed similar patterns over time, with S1 generally containing higher amounts of compounds. When comparing accessions S1 and S2, S1 had consistently higher CA levels than S2 in all the leaf samples harvested from June to November. For example, S1-A's CA (**2**) content was 7.67 mg/g, while S2-A had only 5.70 mg/g (Figure 9). Similarly, S1 generally contained higher levels of cryptochlorogenic acid (**3**) than S2. In sample S1−C, the cryptochlorogenic acid (**3**) content was 4.35 mg/g, while in S2-C, it was 4.06 mg/g. Notably, hyperoside (**7**) and peucedanol (**8**) were not detected in any of the leaf samples at any time. Peucedanol 7-*O*-glucoside (**4**) showed no substantial changes in all six leaf samples over the sampling period. The other six compounds such as neochlorogenic acid (**1**), CA (**2**), cryptochlorogenic acid (**3**), 3-*O*-coumaroylquinic acid (**5**), rutin (**6**) and diosmin (**9**) were abundant in the leaves. Among them, diosmin (**9**) shows relatively little change over the season in accession S2, but it decreases dramatically in August in accession S1. However, the other five compounds such as neochlorogenic acid (**1**), CA (**2**), cryptochlorogenic acid (**3**), 3-*O*-coumaroylquinic acid (**5**), and rutin (**6**) dramatically increased between July and August. These variations could be attributed to seasonal changes, variations in nutrient availability, or other environmental factors. Specifically, the combined content of the compounds in S1 leaves harvested in August is 46.29 mg/g, much higher than that in other S1 samples. This abrupt increase may be indicative of specific environmental conditions or a change in the developmental stage of the plants during this particular period.

A similar study analyzed the antiobesity effects of *P. japonicum*.[35] To investigate which compounds were responsible for the bioactivity shown by the extracts, they used an HPLC analysis. The authors detected 18 compounds, three of which were also detected in the present study (neochlorogenic acid, CA, and rutin). Another study analyzed the differences in the amount of CA (**2**), rutin (**6**), hesperidin, and diosmin (**9**) in different parts of *P. japonicum* plants grown in different types of soil, and all compounds were detected under all experimental conditions.[36] However, they noted that diosmin (**9**) was found in the leaves,[36] as reported in previous literature.[37] This coincided with the results of the present study, where only the leaf samples contained diosmin (**9**), while in the root samples, it was either in trace amounts or not detected at all.

Similar to the previous section, the HPLC quantification of compounds also revealed that neochlorogenic acid (**1**), CA (**2**), and rutin (**6**) were present in significant amounts in
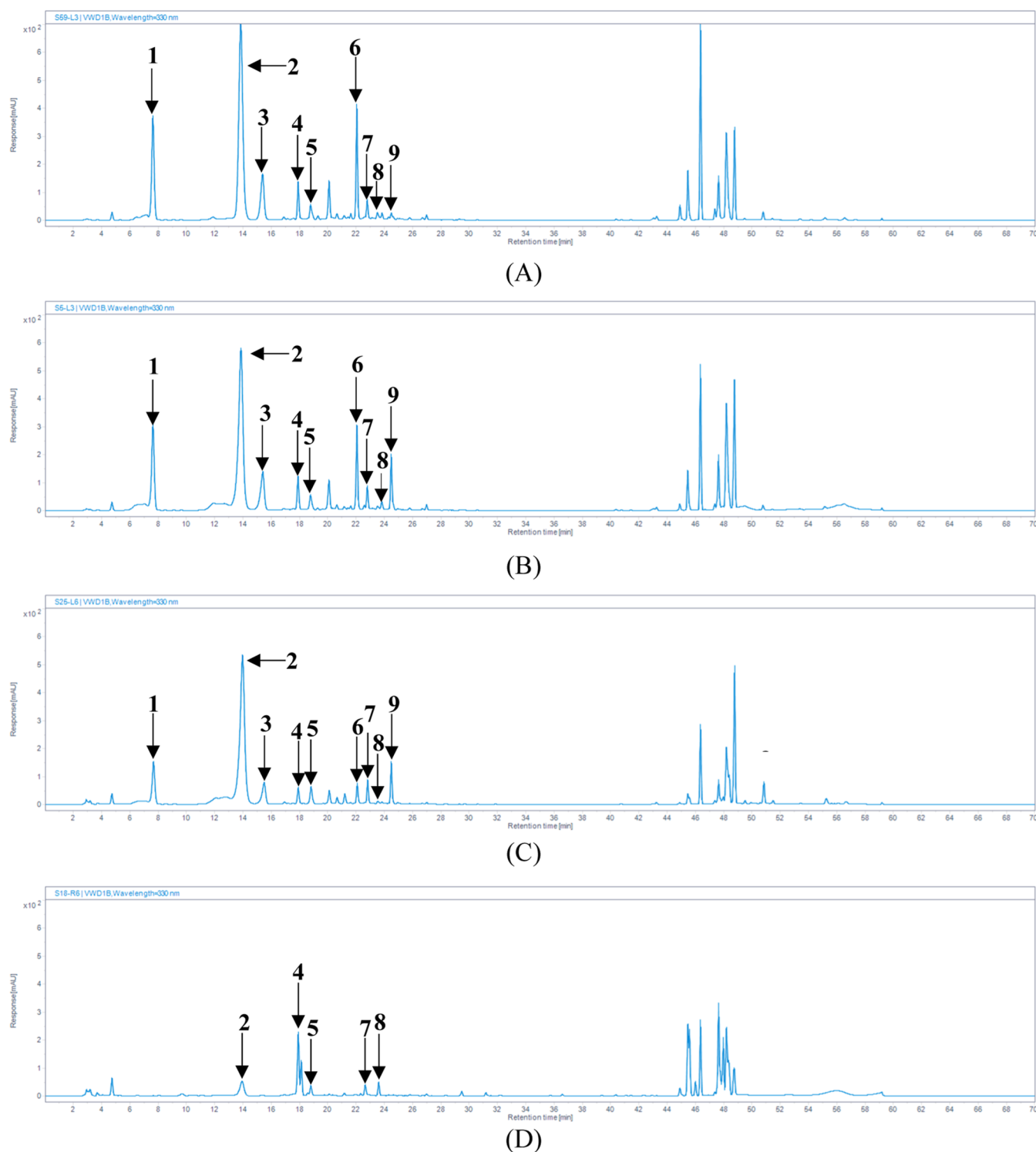
Figure 8. HPLC chromatograms of S1-C (A), S2-C (B), S5-F (C), and S3-R (D).

samples with strong antioxidant activities. Specifically, sample S1-C contained 6.84 mg/g of neochlorogenic acid (**1**), 21.27 mg/g of CA (**2**), and 9.56 mg/g of rutin (**6**). Similarly, sample S1-D had 6.95 mg/g of neochlorogenic acid (**1**), 21.31 mg/g of CA (**2**), and 4.37 mg/g of rutin (**6**). Sample S2-E contained 5.42 mg/g of neochlorogenic acid (**1**), 20.14 mg/g of CA (**2**), and 3.34 mg/g of rutin (**6**). Conversely, the lowest antioxidant activities were recorded from S1-B and S2-B. Interestingly,

these samples both have the lowest concentration of neochlorogenic acid (**1**), CA (**2**), and rutin (**6**).

The results indicate a strong correlation between the presence of specific phenolic compounds and the antioxidant activity of S1 and S2 extracts. Neochlorogenic acid (**1**), CA (**2**), and rutin (**6**) were consistently found in higher concentrations in extracts that exhibited stronger antioxidant activities, as evidenced by their lower IC_{50} values in both DPPH and ABTS assays.
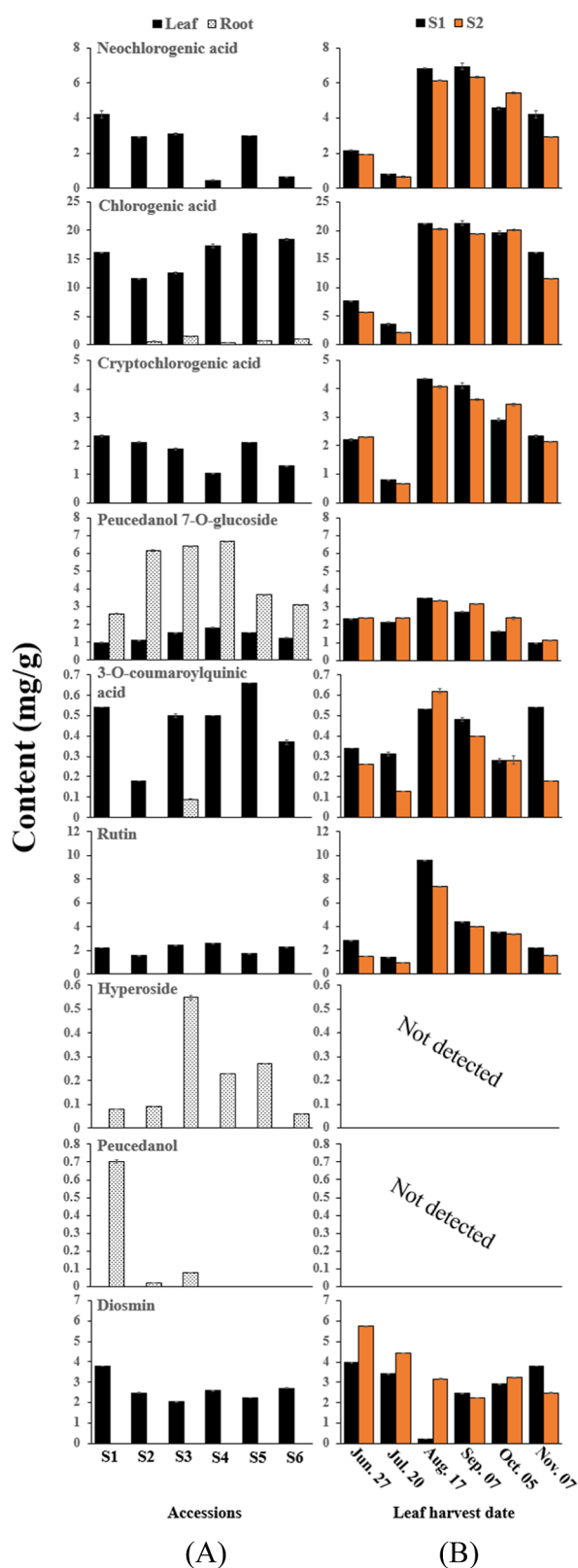
Figure 9. Quantifications of the nine metabolites in the leaf and root extracts of six accessions (S1−S6); (A) and leaf extracts of accessions S1 and S2 at six harvest dates (B). The leaves and roots for the extracts in (A) were harvested on November 7 and 23. The tested metabolites are represented as means ± SD (n = 3).

Neochlorogenic acid (**1**) and CA (**2**) are known for their potent antioxidant properties, which are attributed to their

ability to donate hydrogen atoms and stabilize free radicals.[38] Rutin (**6**), a flavonoid, enhances antioxidant activity through its free radical scavenging ability and chelation of metal ions.[39] If we observe closely, the only difference that set S1-C and S1-D is the higher rutin (**6**) concentration found in S1-C which might suggest that rutin (**6**) is the compound responsible for the antioxidant activity. This could also suggest that rutin (**6**) works synergistically with the other two compounds in demonstrating its antioxidant activity.

These differences in the concentration of the compounds might have something to do with the month of harvest. A previous study found that the phytochemicals were all significantly affected by the harvesting period.[40,41] When compared to shoots taken during the rainy season, those harvested during the dry season often had a much higher amount of bioactive chemicals and, as a result, better antioxidant activity.[41] These findings coincide with the results of the present study. The samples S1-C, S1-D, and S2-E were all harvested during the dry months of South Korea except S1-C which was harvested in the middle of August, which marks the transition of the summer to autumn. Another study also found that harvest time affects the bioactive compound concentration in the plant and cited the importance of determining the proper harvest time to maximize the bioactive compounds present in the plants.[40]

The integration of LC−MS/MS and HPLC analyses, TPC and TFC measurements, and radical scavenging assays enhances the depth of phytochemical characterization and antioxidant evaluation.[42] The LC−MS/MS and HPLC analyses provide critical insight into the identities and concentration of the bioactive compounds responsible for the observed antioxidant activity.[43,44] These techniques allow researchers to elucidate the complex phytochemical profiles of natural products and identify not only known antioxidants but also novel bioactive compounds with potential health benefits. In addition, LC−MS/MS and HPLC facilitate the comparison of phytochemical compositions among different samples, accessions, or extraction methods, thus aiding in the selection of sources with superior bioactivity for further development.[45]

4. CONCLUSION

By linking the results obtained from TPC and TFC assays, radical scavenging assays, LC−MS/MS, and HPLC analyses, this study provides a comprehensive understanding of the relationship between phytochemical composition and antioxidant activity in *P. japonicum*. This integrated approach allows researchers to elucidate the mechanisms underlying the observed bioactivities and optimize extraction and formulation strategies to enhance the bioavailability and efficacy of natural products. These findings contribute to the advancement of research in natural product chemistry, nutraceutical development, and evidence-based medicine, with potential implications for disease prevention and health promotion. Overall, the findings have broad applications in food science, medicine, and pharmaceuticals, advancing the development of natural product-based treatments and disease management strategies.

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Notes

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■ REFERENCES

(1) Xu, Z.; Ullah, N.; Duan, Y.; Hou, Z.; Liu, A.; Xu, L. Editorial: Plant secondary metabolites and their effects on environmental adaptation based on functional genomics. *Front. Genet.* **2023**, *14*, 1211639.

(2) Petruk, G.; Del Giudice, R.; Rigano, M. M.; Monti, D. M. Antioxidants from plants protect against skin photoaging. *Oxid. Med. Cell. Longevity.* **2018**, *2018*, 1454936.

(3) Chen, D.; Mubeen, B.; Hasnain, A.; Rizwan, M.; Adrees, M.; Naqvi, S. A. H.; Iqbal, S.; Kamran, M.; El-Sabrout, A. M.; Elansary, H. O.; Mahmoud, E. A.; Alaklabi, A.; Sathish, M.; Din, G. M. U. Role of promising secondary metabolites to confer resistance against environmental stresses in crop plants: Current scenario and future perspectives. *Front. Plant Sci.* **2022**, *13*, 881032.

(4) Labarrere, B.; Prinzing, A.; Dorey, T.; Chesneau, E.; Hennion, F. Variations of secondary metabolites among natural populations of sub-antarctic Ranunculus species suggest functional redundancy and versatility. *Plants* **2019**, *8*, 234.

(5) Liu, W.; Yin, D.; Li, N.; Hou, X.; Wang, D.; Li, D.; Liu, J. Influence of environmental factors on the active substance production and antioxidant activity in Potentilla fruticosa L. and its quality assessment. *Sci. Rep.* **2016**, *6*, 28591.

(6) Sharifi-Rad, M.; Anil Kumar, N. V.; Zucca, P.; Varoni, E. M.; Dini, L.; Panzarini, E. P.; Rajkovic, J.; Tsouh Fokou, P. V.; Azzini, E.; Peluso, I.; Prakash Mishra, A.; Nigam, M.; El Rayess, Y.; Beyrouthy, M. E.; Polito, L.; Iriti, M.; Martins, N.; Martorell, M.; Docea, A. O.; Setzer, W. N.; Calina, D.; Cho, W. C.; Sharifi-Rad, J. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Front. Physiol.* **2020**, *11*, 694.

(7) Song, H.-S.; Cho, W. Growth pattern and species composition by landform and seaside distribution of Peucedanum japonicum Thunb. Community group in Korea. *Kor. J. Env. Eco.* **2007**, *21*, 74−81.

(8) Lee, C.-D.; Cho, H.; Shim, J.; Tran, G. H.; Lee, H.-D.; Ahn, K. H.; Yoo, E.; Chung, M. J.; Lee, S. Characteristics of phenolic compounds in Peucedanum japonicum according to various stem and seed colors. *Molecules* **2023**, *28*, 6266.

(9) Chen, C.; Agrawal, D. C.; Lee, M.; Lee, R.; Kuo, C.; Wu, C.; Tsay, H.; Chang, H. Influence of LED light spectra on in vitro somatic embryogenesis and LC-MS analysis of chlorogenic acid and rutin in Peucedanum japonicum Thunb.: A medicinal herb. *Bot. Stud.* **2016**, *57*, 9.

(10) Hisamoto, M.; Kikuzaki, H.; Nakatani, N. Constituents of the leaves of Peucedanum japonicum Thunb. and their biological activity. *J. Agric. Food Chem.* **2004**, *52*, 445−450.

(11) Deng, J.; Cho, H. S.; Paul, N. C.; Lee, H. B.; Yu, S. H. A novel Alternaria species isolated from Peucedanum japonicum in Korea. *Mycobiology* **2014**, *42*, 12−16.

(12) Hwang, D.; Ryu, H. W.; Park, J. W.; Kim, J.; Kim, D.-Y.; Oh, J.; Kwon, O.; Han, S.; Ahn, K. S. Effects of 3′-isovaleryl-4′-senecioylkhellactone from Peucedanum japonicum Thunberg on PMA-stimulated inflammatory response in A549 human lung epithelial cells. *J. Microbiol. Biotechnol.* **2022**, *32*, 81−90.

(13) Okabe, T.; Toda, T.; Nukitrangsan, N.; Inafuku, M.; Iwasaki, H.; Oku, H. Peucedanum japonicum Thunb inhibits high-fat diet-induced obesity in mice. *Phytother. Res.* **2011**, *25*, 870−877.

(14) Choi, R. Y.; Nam, S. J.; Ham, J. R.; Lee, H.-I.; Yee, S. T.; Kang, K. Y.; Seo, K. I.; Lee, J. H.; Kim, M.; Lee, M. K. Anti-adipogenic and anti-diabetic effects of cis −3′,4′-diisovalerylkhellactone isolated from Peucedanum japonicum Thunb leaves in vitro. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4655−4660.

(15) Kim, J.; Erkhembaatar, M.; Lee, G.; Lee, J.; Noh, E.; Lee, M.; Song, H. K.; Lee, C. H.; Kwon, K.; Kim, M. S.; Lee, Y. Peucedanum japonicum Thunb. ethanol extract suppresses RANKL-mediated osteoclastogenesis. *Exp. Ther. Med.* **2017**, *14* (1), 410−416.

(16) Mitra, S.; Naskar, N.; Chaudhuri, P. A review on potential bioactive phytochemicals for novel therapeutic applications with special emphasis on mangrove species. *Phytomed. Plus.* **2021**, *1*, 100107.

(17) Kim, J. T.; Joh, H. J.; Yu, H. S.; Park, Y. S.; Kim, J.-S.; Jeong, Y. H.; Yang, T.-J. Flowering, fertilization characteristics and speedy breeding in a protandry plant Peucedanum japonicum Thunb. *Korean J. Plant Res.* **2023**, *36*, 402−412.

(18) Uy, N. P.; Kim, H.; Ku, J.-J.; Lee, S. Regional variations in Peucedanum japonicum antioxidants and phytochemicals. *Plants* **2024**, *13*, 377.

(19) Zhang, Z.; Li, X.; Sang, S.; McClements, D. J.; Chen, L.; Long, J.; Jiao, A.; Jin, Z.; Qiu, C. Polyphenols as plant-based nutraceuticals: Health effects, encapsulation, nano-delivery, and application. *Foods* **2022**, *11*, 2189.

(20) Ullah, A.; Munir, S.; Badshah, S. L.; Khan, N.; Ghani, L.; Poulson, B. G.; Emwas, A.; Jaremko, M. Important flavonoids and their role as a therapeutic agent. *Molecules* **2020**, *25*, 5243.

(21) Afnan; Saleem, A.; Akhtar, M. F.; Sharif, A.; Akhtar, B.; Siddique, R.; Ashraf, G. M.; Alghamdi, B. S.; Alharthy, S. A. Anticancer, cardio-protective and anti-inflammatory potential of natural-sources-derived phenolic acids. *Molecules* **2022**, *27*, 7286.

(22) Abreu-Naranjo, R. A.; Paredes-Moreta, J. G.; Granda-Albuja, G.; Iturralde, G.; González-Paramás, A. M.; Alvarez-Suarez, J. M. Bioactive compounds, phenolic profile, antioxidant capacity and effectiveness against lipid peroxidation of cell membranes of Mauritia flexuosa L. fruit extracts from three biomes in the Ecuadorian Amazon. *Heliyon* **2020**, *6*, No. e05211.

(23) Lim, H.; Kim, I.; Jeong, Y. Antioxidant Activities of Peucedanum japonicum Thunberg root extracts. *J. Korean Soc. Food Sci. Nutr.* **2019**, *48*, 32−39.

(24) Ponphaiboon, J.; Krongrawa, W.; Aung, W. W.; Chinatangkul, N.; Limmatvapirat, S.; Limmatvapirat, C. Advances in natural product extraction techniques, electrospun fiber fabrication, and the integration of experimental design: A comprehensive review. *Molecules* **2023**, *28*, 5163.

(25) Spigno, G.; Tramelli, L.; De Faveri, D. M. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng.* **2007**, *81*, 200−208.

(26) Koraqi, H.; Petkoska, A. T.; Khalid, W.; Sehrish, A.; Ambreen, S.; Lorenzo, J. M. Optimization of the extraction conditions of antioxidant phenolic compounds from strawberry fruits (Fragaria x ananassa Duch.) using response surface methodology. *Food Anal. Methods* **2023**, *16*, 1030−1042.

(27) Baliyan, S.; Mukherjee, R.; Priyadarshini, A.; Vibhuti, A.; Gupta, A.; Pandey, R. P.; Chang, C.-M. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. *Molecules* **2022**, *27*, 1326.

(28) Li, H.; Tsao, R.; Deng, Z. Factors affecting the antioxidant potential and health benefits of plant foods. *Can. J. Plant Sci.* **2012**, *92*, 1101−1111.

(29) Zhao, H.; Fan, W.; Dong, J.; Lu, J.; Chen, J.; Shan, L.; Lin, Y.; Kong, W. Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chem.* **2008**, *107*, 296−304.

(30) Muflihah, Y. M.; Gollavelli, G.; Ling, Y.-C. Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 Indonesian indigenous herbs. *Antioxidants* **2021**, *10*, 1530.

(31) Liigand, P.; Kaupmees, K.; Haav, K.; Liigand, J.; Leito, I.; Girod, M.; Antoine, R.; Kruve, A. Think negative: Finding the best electrospray ionization/MS mode for your analyte. *Anal. Chem.* **2017**, *89*, 5665−5668.

(32) Gutiérrez-del-Río, I.; López-Ibáñez, S.; Magadán-Corpas, P.; Fernández-Calleja, L.; Pérez-Valero, Á.; Tuñón-Granda, M.; Miguélez, E. M.; Villar, C. J.; Lombó, F. Terpenoids and polyphenols as natural antioxidant agents in food preservation. *Antioxidants* **2021**, *10*, 1264.

(33) Li, Y.; Kong, D.; Fu, Y.; Sussman, M. R.; Wu, H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant. Physiol. Biochem.* **2020**, *148*, 80−89.

(34) Roy, A. C.; Prasad, A.; Singh, S.; Priya, K.; Ghosh, I. Comparative bioactivity analysis of hydro-methanolic extracts of root, leaf, and inflorescence of Tupistra nutans Wall. ex Lindl: An in vitro study on antioxidant and anti-hepatocellular carcinoma activity. *Biocatal. Agric. Biotechnol.* **2023**, *52*, 102819.

(35) Nukitrangsan, N.; Okabe, T.; Toda, T.; Inafuku, M.; Iwasaki, H.; Oku, H. Effect of Peucedanum japonicum Thunb extract on high-fat diet-induced obesity and gene expression in mice. *J. Oleo Sci.* **2012**, *61*, 89−101.

(36) Suwa, R.; Tajima, H.; Gima, S.; Uehara, N.; Watanabe, K.; Yabuta, S.; Tominaga, J.; Kawamitsu, Y. Polyphenol production in Peucedanum japonicum Thunb. varies with soil type and growth stage. *Hort. J.* **2018**, *87*, 382−388.

(37) Tamura-Norimatsu, A.; Mori, H. Anti-edema Effect of a Drink Containing Peucedanum Japonicum Thunb. in Healthy Japanese Women. *Nippon Shokuhin Kagaku Kogaku Kaishi* **2012**, *59*, 509−514.

(38) Woźniak, D.; Nawrot-Hadzik, I.; Kozłowska, W.; Ślusarczyk, S.; Matkowski, A. *Caffeoylquinic Acids. Handbook of Dietary Phytochemicals.* 1st ed.; Springer: Singapore, 2021; pp 1065−1104.

(39) Rodríguez-Arce, E.; Saldías, M. Antioxidant properties of flavonoid metal complexes and their potential inclusion in the development of novel strategies for the treatment against neuro-degenerative diseases. *Biomed. Pharmacother.* **2021**, *143*, 112236.

(40) Ghimire, B. K.; Seo, J.; Kim, S.; Ghimire, B.; Lee, J.; Yu, C.; Chung, I. Influence of harvesting time on phenolic and mineral profiles and their association with the antioxidant and cytotoxic effects of Atractylodes japonica Koidz. *Agronomy* **2021**, *11*, 1327.

(41) Mahmoudi, M.; Boughalleb, F.; Maaloul, S.; Zaidi, S.; Bakhshandeh, E.; Abdellaoui, R. The effect of seasonality on the phytochemical composition of two Limonium species naturally growing in a Mediterranean arid-salt marsh: Harvesting time optimization by modeling approach. *Sci. Hortic.* **2023**, *309*, 111616.

(42) Yilmaz, M. A.; Ertas, A.; Yener, I.·.; Akdeniz, M.; Cakir, O.; Altun, M.; Demirtas, I.; Boga, M.; Temel, H. A comprehensive LC-MS/MS method validation for the quantitative investigation of 37 fingerprint phytochemicals in Achillea species: A detailed examination of A. coarctata and A. monocephala. *J. Pharm. Biomed. Anal.* **2018**, *154*, 413−424.

(43) Kumar, B. Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (GLVs). *J. Pharm. Anal.* **2017**, *7*, 349−364.

(44) Wen, C.; Wang, D.; Xing, L.; Huang, T.; Huang, C.; Hu, K. Targeted isolation and identification of bioactive compounds lowering cholesterol in the crude extracts of crabapples using UPLC-DAD-MS-SPE/NMR based on pharmacology-guided PLS-DA. *J. Pharm. Biomed. Anal.* **2018**, *150*, 144−151.

(45) Xu, Y.; Li, Y.; Maffucci, K. G.; Huang, L.; Zeng, R. Analytical methods of phytochemicals from the genus Gentiana. *Molecules* **2017**, *22*, 2080.