Influence of Harvesting Time on Phenolic and Mineral Profiles and Their Association with the Antioxidant and Cytotoxic Effects of *Atractylodes japonica* Koidz

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**Abstract:** Plant phytochemical accumulation is influenced by various external factors that change with the seasons (e.g., harvesting time). *Atractylodes japonica*, an important medicinal plant rich in bioactive compounds, is used to treat several human diseases. We analyzed the influence of harvesting time on phenolic compound concentration and antioxidant activity of *A. japonica* roots. We investigated the correlation between phenolic compound and minerals contents and antioxidant activity in different harvests. Total phenolic and flavonoid contents varied significantly with the harvesting time. Liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) analysis revealed significant changes in the concentration of various phenolics between harvests. The content of different types of phenolics were significantly higher in the samples collected in October. Among them, chlorogenic acids (133,833.30 µg/g dry weight of root extract) were the most dominant phytochemical compounds detected. Samples harvested in October had higher concentrations of flavonoids, including rutin, orientin, vitexin, and apigenin. Roots harvested in October had a significantly higher (p < 0.05) antioxidant activity than that of those harvested later. Root mineral concentrations also varied with the harvest time. The analysis revealed that macro elements such as Ca ad Mg contents were significantly increased with delaying harvesting time, whereas a different trend was observed for the microelements including Fe, Cu, Al, and As contents in the October harvest. We also found a significant relationship between antioxidant activity and phenolic compound content. The most abundant minerals (Ca, Mg, Mn, Fe, and Al) correlated positively with the antioxidant activity indicating that these elements and compounds may be associated with the *A. japonica* antioxidant potential. Furthermore, *A. japonica* root extracts inhibited NIH/3T3 cellular proliferation in a season- and dose-dependent manner. Hence, harvesting time influenced the antioxidant properties and phenolic compound accumulation of *A. japonica* roots. These results indicate that the harvesting time is essential for obtaining the specific phytochemicals.

**Keywords:** phenolic compounds; harvesting time; antioxidant activity; minerals; *Atractylodes japonica*

1. Introduction

*Atractylodes japonica* Koidz. (Asteraceae) is a perennial plant native to North Asia, including Korea, China, and Japan [1–3]. In traditional medicine, this plant is widely used in the treatment of several human conditions, including pain, bronchitis [4], arthritis, digestive disorders, and water retention [1]. *A. japonica* extracts possess pharmaceutical properties, including antioxidant anti-inflammatory [5,6], analgesic [5], antimicrobial [7], diuretic (5; 6), and antivirus effects [8]. Additionally, they inhibit gastric ulcers [9] and
promote distal colonic contraction in rats [10], and modulate the intestinal immune system [11]. Chemotaxonomic analyses in *A. japonica* roots revealed the presence of essential oils including sesquiterpenes [12], atractylone, 3-b-hydroxyatratylon, selina-4, hinesol, and b-eudesmol—and sesquiterpenoid glycosides [9,13], constituents which have important functional therapeutic activities. *A. japonica* roots are also rich in sesquiterpenolides [13,14] and diacetylatractylodiol and its derivatives [9]. Recently, studies on *A. japonica* phytocchemistry and biological activity have intensified due to its abundance in bioactive components with biomedical interest, including atractylenolides I (AT-I) and III (AT-III), flavone C-glucosides, isoorientin, vitexin, and isovitexin. Furthermore, several studies have attributed health benefits to various secondary metabolites that have been isolated from *A. japonica* rhizomes, including monoterpen glycosides, aromatic glycosides, sucrose esters, and steroids [7,13,15].

Phenolic compounds are widely distributed among plant species. Previous studies showed that the consumption of polyphenol rich food contributes significantly to preventing health problems, including cancer and cardiovascular diseases [16,17], as they inhibit lipid peroxidation and lipoxygenase activity in vitro [18,19]. Additionally, they play an important role in neutralizing or sequestrating free radicals and chelating transition metals [18,19]. Minerals are the building blocks of tissues and are necessary for various metabolic processes [20]. They are also critical for maintaining the osmotic pressure and acid-base balance and muscle and nerve activities. Adequate Mn intake is vital because this element has an antioxidant activity that acts in ROS scavenging in cells [21]. Mg is present in chlorophyll and influences solar energy absorption by green plants [22]. Minerals, including Zn, Cu, Mn, and Fe, participate in the synthesis of secondary metabolites, which are responsible for antioxidant and antimicrobial activities in plants [22].

Several environmental factors, including harvesting time, influence bioactive compound and mineral production in plants [23,24]. The harvesting time of plant products directly affects the composition and concentration of bioactive compounds and minerals by changing growth and weather conditions to which plants are exposed [25]. Previous studies showed a close relationship between phytochemical composition and concentration and compound activity in plants. Plant samples of the same species collected at different seasons or with different harvesting times may have significant differences in their phytochemical and pharmacological properties [26–28]. To ensure high production and accumulation of desired bioactive compounds, it is necessary to identify the optimal harvesting season for different plant parts. However, the influence of harvest time on phenolic composition, antioxidant activity, and cytotoxic effects of *A. japonica* roots has not been studied. Furthermore, there are no reports of antioxidant activity and mineral composition and their relationship with seasonality and of phenolic compound metabolism.

Here, we investigated a possible correlation between harvesting time and phenolic compound and mineral contents in root with antioxidant activity. The objectives were to evaluate the changes in the phenolic compound and mineral contents, antioxidant activity, cytotoxicity, and their relationship in *A. japonica* root samples harvested at two different periods.

2. Materials and Methods

2.1. Chemicals

The Folin–Ciocalteu reagent, quercetin, *butylated hydroxytoluene* (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and gallic acid (GA) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide (H₂O₂) and nitric acid (HNO₃) were purchased from Showa Chemical Industry Co. Ltd. (Tokyo, Japan). Ultrapure distilled water was obtained with a Zeneer power 1 system (Human Corporation, Seoul, Korea). The 19 multielement standards used in the analysis of micro- and macro elements were obtained from Perkin Elmer (Seoul, Korea). The multielement stock solutions were obtained from Quality Control Standard 26 (Inorganic Ventures, Christiansburg, VA, USA).
2.2. Cultivation of A. japonica

Seedlings of *A. japonica* were kindly provided by the Bio Herb Research Center, Kangwon National University and planted in the experimental field of Kangwon National University at Chuncheon, Kangwon-Do, South Korea located at 37°52′09.53″ N; 127°44′42.82″ E; 100 m altitude. There were eight seedlings of *A. japonica* assigned to each experimental plot. The experimental plots consisted of rows of 50 m in length, spaced 1.5 m apart with 1 m between the planted seedlings. The mean minimum and maximum field temperature during the cultivation period were 19 °C and 35 °C, respectively, with approximate rainfall rate of 200 mm. The sandy loam texture of the experimental field was maintained at pH of 6.1. The cultivated field was irrigated regularly once weekly by installing a drip-irrigation system. The recommended doses of compound chemical fertilizers were applied to the experimental field (Nitrogen: Phosphorus: Potassium = 15%: 15%: 15%) at a rate of 120 kg ha⁻¹ before planting of the seedlings. Weeds were manually removed regularly during the seedling growth.

Sample Collection and Preparation of Plant Extracts

To investigate the phenolic compounds composition in the roots of *A. japonica* during the month of October and November, approximately 400 g of fresh and fully developed roots were sampled at first week of October and November 2018, 2019 and 2020. Each of the samples were randomly collected from five healthy and matured plants, which were mixed to form a root sample. The harvested root samples were thoroughly washed with distilled water and freeze-dried for one day. Furthermore, the root sample was crushed to the size of particles passing through a sieve (1 mm diameter). Briefly, 2 g of the freeze-dried finely ground samples were mixed with 20 mL of 80% methanol at room temperature (25 °C) with occasional mixing. Then, the extracts were filtered through filter paper (Whatman No. 1) and evaporated at 40 °C in a rotary evaporator (Eyela, SB-1300, Shanghai Eyela Co. Ltd., Shanghai, China). The obtained extracts were dissolved in 80% methanol (10 mL) and to obtain a solution of 50 µg/m. The extraction was conducted in triplicate per each root sample replicate and then the solution was filtered in a 0.45 µm filter unit before injection into the HPLC-MS/MS system.

2.3. Determination of Total Phenolic Contents (TPC)

The TPC of the *A. japonica* root extracts was determined in triplicate using the Folin–Ciocalteau reagent, as described by [29]. The analyses were conducted in 10 mL test tubes, where 100 µL of diluted *A. japonica* root extract (1 mg mL⁻¹), 50 µL of Folin–Ciocalteau reagent (1 M), and 1.85 mL of distilled water were mixed and incubated at room temperature (25 °C) for 4 min. Subsequently, 1.0 mL of saturated sodium carbonate (20% w/v) was added, and the tubes were vortexed slowly for 10 s, followed by the addition of distilled water to a final volume of 4 mL. Absorbance was measured after 1 h using a spectrophotometer (Jasco V530 UV-VIS spectrophotometer, Tokyo, Japan) at a wavelength of 725 nm against the blank. The measurements were compared with a GA calibration curve, and the results were expressed as GA equivalent (GAE) per gram of dry sample (mgGAEg⁻¹).

2.4. Determination of Total Flavonoid Contents (TFC)

*A. japonica* root TFCs were quantified in triplicate using the aluminum chloride colorimetric method [30]. Briefly, 250 µL of diluted *A. japonica* root extract (1:3) was mixed with 100 µL of 10% aluminum nitrate and 100 µL of potassium acetate (KCH₃COO) (1 M) in a 10 mL test tube. Then, 4.3 mL of 80% ethanol was added to achieve a final volume of 5 mL. The solution was mixed well and allowed to react for 5 min. The absorbance was measured at a wavelength of 410 nm using a spectrophotometer (Jasco V530 UV-VIS spectrophotometer, Tokyo, Japan). The TFC was expressed as the quercetin equivalent (QE) per gram of dry sample (mgQEq⁻¹).
2.5. Quantification of Phenolic Compound Contents by Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LC–MS/MS)

The phenolic compound constituents were identified in *A. japonica* using an LC-MS/MS system following the method described by [31]. The HPLC system used in this study was from an Agilent HPLC System (Agilent Technologies, Santa Clara, CA, USA) Pumps (Agilent 1200, Agilent Technologies, Palo Alto, CA, USA) and an autosampler (G1313A) (Agilent 1100 series, Agilent Technologies, Palo Alto, CA, USA) coupled to an API 2000 mass spectrometer (Applied Biosystems, Ontario, Canada) were integrated to the LC system. A reversed-phase analytical column (C18, 4.6 × 150 mm, 5 µm) (Thermo Scientific™ Syncronis™, San Jose, CA, USA) was used for phenolic compound separation. The mobile phase consisted of 0.1% formic acid (v/v) in water (phase A) and 0.1% acetonitrile in water (95:5, v/v) (phase B). The following mobile phase gradient profile was set: 10–40% phase B for 0–10 min; 40–50% phase B for 10–20 min; 50–100% phase B for 20–25 min; 100–10% phase B for 25–26 min; and 10% phase B for 26–30 min. The column temperature was maintained at 25 °C. The mobile phase was programmed at the rate of 0.7 mL min⁻¹, with an injection volume of 10 µL. The electrospray ion source (ESI) was used in negative mode under the multiple reaction monitoring (MRM) mode, with curtain gas pressure and collision gas pressure of 20 psi and 2 psi, respectively, capillary voltage of 4500 V, drying gas (nitrogen) pressure of 70 psi, and nebulizing pressure of 40 psi. The analyses were performed in triplicate.

Identification of the compounds was performed in MRM mode by comparing the retention times and m/z (mass-to-charge ratio) values of the molecular and product ions (Q1 and Q3 values (Table S1, See Supplementary File)) with the corresponding standard solutions. The stock solutions of standards were prepared by dissolving in the appropriate solvents by following their physical/chemical properties. The obtained standard solutions were used to construct their corresponding calibration curves. A quantitative analysis of the data and the external calibration curve was performed using Analyst software (version 1.6.2; AB Sciex, Framingham, MA, USA). All the obtained calibration curves exhibited good linearity (r² = 0.99). The LOD and LOQ values were calculated with signal-to-noise (S/N) ratios of 3 and 10 (Table S2, See Supplementary File).

2.6. Antioxidant Activity

2.6.1. 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Assay

The antioxidant activity of *A. japonica* root extracts was determined using the DPPH radical scavenging assay following the method described before [32]. First, 200 µL of root extract (at concentration ranging from 0.05 to 10 mg mL⁻¹) was added to 4.5 mL of DPPH solution (0.004% in methanol). Then, the reaction mixture was mixed thoroughly and incubated at 25 °C for 40 min. Subsequently, the absorbance of the reaction mixture was recorded using a spectrophotometer (Jasco V530 UV-VIS spectrophotometer, Japan) at 517 nm. BHT was used as a positive control (standard antioxidant). Free radical scavenging activities were calculated with the following equation:

\[
\text{DPPH scavenging activity (IC50)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}}
\]

where Abs_{control} is the absorbance value of the reaction mixture without the root extract, and Abs_{sample} is the absorbance value of the reaction mixture with the root extract.

2.6.2. 2,2′-Azino-bis-3-Ethylbenzthiazoline-6-Sulphonic Acid (ABTS+) Assay

The ABTS radical scavenging activity assay was performed to determine the antioxidant properties of *A. japonica* root extracts following the method described previously [33]. Briefly, the ABTS solution was made by mixing 7.4 mM L⁻¹ ABTS and 2.6 mM L⁻¹ potassium persulphate in a 1:1 (v/v) ratio. Then, the reaction mixture was incubated at room temperature (25 °C) for 12 h. The mixture was then diluted with 80% methanol, and the absorbance of the solution was recorded at 734 nm using a spectrophotometer (Jasco V530
UV-VIS spectrophotometer, Japan). Trolox at various concentrations was used to construct the standard calibration curve. The ABTS radical scavenging results were expressed as micromoles of trolox equivalents (TE) per gram of dry weight (µmol TE g\(^{-1}\) DW). The ability to scavenge ABTS radicals was calculated with the following equation:

\[
\text{ABTS scavenging activity (IC50)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100
\]

where Abs\(_{\text{control}}\) is the absorbance of the ABTS solution without the plant sample, and Abs\(_{\text{sample}}\) is the absorbance of the ABTS solution with the plant sample. Inhibitory concentration (IC50) values indicate the amount of test sample needed to inhibit or scavenge 50% of the radicals present in the reaction mixture.

2.7. Sample Digestion

\(A. japonica\) root extracts were pre-digested in HNO\(_3\) by following the protocol described by the US Environmental Protection Agency, 2007. Briefly, 0.5 g of the ground root samples collected on two different months (October and November) were mixed with 7 mL of 70% HNO\(_3\) at room temperature (25°C) for 6 h. Then, 1 mL of 35% H\(_2\)O\(_2\) was added to the mixture; digestion was performed at 180°C for 20 min until samples became colorless. The digested samples were diluted with 50 mL of ultrapure distilled water. Blanks were made with the same method described above, excluding the sample.

2.8. Instrumentation and Quantification of Minerals

The digested samples were subjected to inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Optima 7300 DV, Perkin Elmer, Shelton, CT, USA) to quantify the mineral element content in the \(A. japonica\) root extracts. ICP-AES was operated under the following conditions: 0.2 L min\(^{-1}\) auxiliary; 1300 W RF powder; 15 L min\(^{-1}\) axial mode plasma, 0.65 L min\(^{-1}\) nebulizer; and 1.5 mL min\(^{-1}\) flow rate. The radial mode of the ICP-AES was used to quantify the mineral elements. The concentration ranges for the elemental standards are provided in Table 1. The limit of detection (LOD) and limit of quantitation (LOQ) for various elements were estimated with the following equations:

\[
\text{LOD} = 3 \times \text{SD}/S,
\]

\[
\text{LOQ} = 10 \times \text{SD}/S,
\]

where SD is the standard deviation of a response, and S is the slope of the calibration curve.

2.9. Assessment of Toxicity on Fibroblasts

The toxicity of \(A. japonica\) extracts was evaluated on NIH/3T3 fibroblasts according to the method previously described [34]. The cell line was provided by the School of Biomedical Science, Kangwon National University, South Korea. Briefly, the cells were grown in a 96-well microtitration plate at a density of 6 × 10\(^3\) per well with 100 mL of the medium. Cells were supplemented with 10 mL of culture medium composed of Dulbecco’s Modified Eagle’s Medium (DMEM) (Life Technologies, Carlsbad, CA, USA), 10% fetal bovine serum (v/v) (Life Technologies, USA), and 1% antibiotic (penicillin-streptomycin, Life Technologies, USA) (v/v), and inoculated in a humidified growth chamber (SANYO Electric cooperation, Osaka, Japan), with 5% CO\(_2\) at 37 °C (Ombredane, 2016) for 24 h. Then, the \(A. japonica\) root extracts were added to each well and transferred to the oven for 24 h. The supernatant was discarded, and the resultant formazan crystals were dissolved in DMSO (Sigma, USA). The absorbance value of each treated sample was assessed with a microplate reader (Thermofisher Scientific Instrument Co. Ltd., Shanghai, China) at 570 nm. Cell viability was determined with the following equation:

\[
\% \text{ cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100.
\]
Table 1. LC-MS/MS parameters of the phenolic compound quantitative analyses.

| Compound                  | RT   | Q1 (m/z) 1   | Q3-1 (m/z) 2 | DP (V) 3 | EP (V) 4 | CEP (V) 5 | CE (eV) 6 | CXP (V) 7 | µg g⁻¹ (Dry Weight of Root Extract) |
|---------------------------|------|-------------|-------------|---------|---------|---------|---------|---------|----------------------------------|
| Protocatechuic acid       | 9.61 | 152.89      | 108.90      | -31.00 | -9.00  | -8.00  | -18.00 | -22.00 | 1167.60 ± 20.90 ± 636.54 ± 87.72 |
| p-Hydroxybenzoic acid     | 11.71| 136.88      | 92.90       | -16.00 | -9.50  | -14.00 | -18.00 | -18.00 | LOD | LOD |
| Chlorogenic acid          | 9.95 | 352.84      | 191.00      | -16.00 | -9.50  | -18.00 | -18.00 | -36.00 | 133833.30 ± 50.00 ± 5298.23 ± 38.82 |
| p-Coumaric acid           | 13.95| 162.86      | 118.90      | -16.00 | -5.00  | -8.00  | -20.00 | -22.00 | 36.80 ± 2.00 ± 170.33 ± 26.52 |
| Ferulic acid              | 14.27| 192.88      | 133.90      | -6.000  | -6.50  | -12.00 | -22.00 | -26.00 | LOD | LOD |
| Rutin                     | 11.90| 509.03      | 299.70      | -96.00  | -8.00  | -26.00 | -48.00 | -52.00 | 639.20 ± 10.00 ± 564.57 ± 13.0 |
| Quercetin                 | 18.16| 300.88      | 150.80      | -31.00  | -10.50 | -18.00 | -28.00 | -32.00 | LOD | LOD |
| 5′-Sulfosalicylic acid     | 6.68 | 216.77      | 198.80      | -21.00  | -7.50  | -10.00 | -18.00 | -44.00 | LOD | LOD |
| Homogentisic acid         | 8.62 | 166.92      | 122.90      | -21.00  | -7.00  | -14.00 | -28.00 | LOD | LOD |
| Salicylic acid            | 18.66| 136.87      | 92.90       | -16.00  | -6.00  | -12.00 | -20.00 | -20.00 | 536.70 ± 12.00 ± 354.40 ± 5.00 |
| Caffeic acid              | 10.85| 178.86      | 134.80      | -11.00  | -10.00 | -12.00 | -20.00 | -30.00 | 583.66 ± 15.00 ± 146.30 ± 7.00 |
| Orientin                  | 10.10| 447.09      | 327.00      | -61.00  | -11.00 | -20.00 | -54.00 | LOD | LOD |
| Vitexin                   | 14.60| 430.82      | 310.80      | -56.00  | -9.00  | -28.00 | -50.00 | 592.00 ± 11.90 ± 517.66 ± 13.0 |
| Apigenin                  | 21.00| 266.94      | 117.00      | -56.00  | -8.50  | -16.00 | -48.00 | -22.00 | 1325.00 ± 20.00 ± 67.30 ± 3.00 |
| Luteolin                  | 17.66| 284.96      | 133.20      | -66.00  | -10.00 | -16.00 | -46.00 | -30.00 | LOD | LOD |
| L-Phenylalanine           | 7.00 | 163.88      | 146.80      | -51.00  | -10.00 | -12.61 | -16.00 | -30.00 | LOD | LOD |

1 Precursor ion (Q1, m/z); 2 fragment ion (Q3, m/z); 3 DP: declustering potential; 4 EP: entrance potential; 5 CEP: cell entrance potential; 6 CE: collision energy; 7 CXP: collision cell exit potential; LOD: limit of detection, LOQ: limit of quantitation (LOQ). 8 Data with the same letter in a column did not differ significantly according to Tukey’s multiple comparison test (p < 0.05). Mean values within a column with the same lowercase letters were not significantly different (p < 0.05) according to Tukey’s multiple comparison test.

2.10. Assessment of Irritation Potential of A. japonica Root Extracts in Fertile Chicken Egg

The antiallergic properties of A. japonica root extracts were assessed with the HET-CAM test. Fertile chicken eggs were purchased from a chicken farm. Then, they were incubated at 37 °C with 60% relative humidity and allowed for the development of embryos. On the ninth day, the incubated fertile chicken eggs were opened to expose the CAM. Subsequently, root extracts at a concentration of 1000 ppm in a volume of 0.4 mL were added to the CAM. The irritant effect of A. japonica root extracts on capillaries and albumin was immediately recorded and scored between 0 and 21 on each treated egg on the basis of coagulation, hemorrhage, and lysis for 300 s. The ocular irritation index (OII) was then obtained by the following equation:

\[ OH = \left( \frac{301 - h}{3} \right) \times \frac{5}{300} + \left( \frac{301 - 1}{2} \right) \times \frac{7}{300} + \left( \frac{301 - c}{3} \right) \times \frac{9}{300} \]

where h represents the time to initiation of hemorrhage, l the time to lysis, and c the time to coagulation. The following classification was used: OII ≤ 0.9, slightly irritating; 0.9 < OII ≤ 4.9, moderately irritating; 4.9 < OII ≤ 8.9, irritating; and 8.9 < OII ≤ 21, severely irritating.

2.11. Statistical Analysis

All experiments were performed in triplicate. Statistical analysis was performed calculating the mean ± standard deviation using the one way analysis of variance (ANOVA). Significant differences between the parameters were determined with Tukey’s multiple comparison test at p < 0.05 and p < 0.01. Principal component analysis was used to examine the interrelationship between phenolic compounds, antioxidant activity, and minerals. Calibration curves were obtained by linear regression using Excel 2010. The interrelationship between minerals and phenolic compounds and antioxidant and cytotoxic properties were assessed by Pearson’s correlation coefficient using SPSS version 20 (SPSS, 2011).
3. Results

3.1. Total Phenolic and Flavonoid Contents

Total phenolic content (TPC) was quantified using the Folin–Ciocalteu method. The TPC averages in *A. japonica* root extracts differed between the two harvesting periods (Figure 1A). Samples harvested in October had significantly higher phenolic contents ($8.001 \pm 0.28 \text{ mgg}^{-1}$) than those harvested in November ($4.62 \pm 0.18 \text{ mgg}^{-1}$).

![Figure 1](image)

**Figure 1.** (A) Total phenolic content, and (B) total flavonoid content of *A. japonica* roots.

The total flavonoid content (TFC) of *A. japonica* root extracts collected at two different harvesting seasons were also significantly different. The TFC was $0.068 \text{ mgGAEg}^{-1}$ and $0.035 \text{ mgQEg}^{-1}$ in the October and November extracts, respectively (Figure 1B).

3.2. Phenolic Compound Contents

The seasonal fluctuation of phenolic compound accumulation in *A. japonica* roots is shown in Table 1. Ten phenolic and flavonoid compounds were detected and quantified in root samples (Figure 2A,B). The concentration of phenolic compounds including Protocatechuic acid, Chlorogenic acid, *p*-Coumaric acid, Rutin, Salicylic acid, Caffeic acid, Orientin, Vitexin, Apigenin, Luteolin were varied significantly in the two harvest. The content of phenolics such as Protocatechuic acid ($1166.70 \pm 20.90 \mu \text{gg}^{-1}$ DW), Chlorogenic acid ($133,833.30 \pm 50.00 \mu \text{gg}^{-1}$ DW), Rutin ($639.20 \pm 10.00 \mu \text{g/g DW}$), Salicylic acid ($536.70 \pm 12.00 \mu \text{gg}^{-1}$ DW), Caffeic acid ($583.66 \pm 15.00 \mu \text{gg}^{-1}$ DW), Orientin ($585.80 \pm 17.00 \mu \text{gg}^{-1}$ DW), Vitexin ($591.70 \pm 11.90 \mu \text{gg}^{-1}$ DW), Apigenin ($1325.00 \pm 20.00 \mu \text{gg}^{-1}$ DW) were significantly higher in the samples collected in October compared to those harvested in November ($1 \text{ DW}$ and $5298.90 \mu \text{gg}^{-1}$ DW, respectively). The samples harvested in November had a lower content of total phenolic compounds, with values ranging from $67.30$ to $5298.90 \mu \text{gg}^{-1}$.

| Phenolic Compound | October | November |
|-------------------|---------|----------|
| Protocatechuic acid | $1166.70 \pm 20.90 \mu \text{gg}^{-1}$ | $1166.70 \pm 20.90 \mu \text{gg}^{-1}$ |
| Chlorogenic acid | $133,833.30 \pm 50.00 \mu \text{gg}^{-1}$ | $100,200.00 \pm 20.00 \mu \text{gg}^{-1}$ |
| *p*-Coumaric acid | $639.20 \pm 10.00 \mu \text{g/g DW}$ | $425.00 \pm 10.00 \mu \text{g/g DW}$ |
| Rutin | $536.70 \pm 12.00 \mu \text{gg}^{-1}$ | $385.00 \pm 12.00 \mu \text{gg}^{-1}$ |
| Salicylic acid | $583.66 \pm 15.00 \mu \text{gg}^{-1}$ | $425.00 \pm 15.00 \mu \text{gg}^{-1}$ |
| Caffeic acid | $585.80 \pm 17.00 \mu \text{gg}^{-1}$ | $425.00 \pm 17.00 \mu \text{gg}^{-1}$ |
| Orientin | $591.70 \pm 11.90 \mu \text{gg}^{-1}$ | $425.00 \pm 11.90 \mu \text{gg}^{-1}$ |
| Vitexin | $1325.00 \pm 20.00 \mu \text{gg}^{-1}$ | $1000.00 \pm 20.00 \mu \text{gg}^{-1}$ |
| Apigenin | $133,833.30 \pm 20.00 \mu \text{gg}^{-1}$ | $1000.00 \pm 20.00 \mu \text{gg}^{-1}$ |
| Luteolin | $170.17 \pm 26.52 \mu \text{gg}^{-1}$ | $170.17 \pm 26.52 \mu \text{gg}^{-1}$ |
| Total flavonoid content | $0.068 \text{ mgGAEg}^{-1}$ | $0.035 \text{ mgQEg}^{-1}$ |

3.3. Antioxidant Activity

The antioxidant capacity of *A. japonica* samples collected in two different seasons was investigated using the 1,1-diphenyl–2-picrylhydrazyl (DPPH) and 2,2’-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays (Figure 3). The inhibitory concentration (IC$_{50}$) values were determined from plotted graphs and indicated the amount of test
The antioxidant activity measured using ABTS was significantly higher in October-collected root samples (932.71 ± 0.35 µg mL⁻¹) than in those collected in November (985.69 ± 0.35 µg mL⁻¹). The Pearson’s correlation between phenolic compound content and antioxidant potential (1/IC₅₀) varied greatly among samples collected in different months (Table 1). The DPPH and ABTS assay conducted in the sample collected in the month of October had a higher positive correlation with Caffeic acid (r = 0.817, p < 0.05; and r = 0.916, p < 0.05, respectively). Similarly, the DPPH and ABTS assay values showed a higher positive correlation with Orientin (r = 0.810, p < 0.05; and r = 0.910, p < 0.05, respectively). While, a moderate and significant positive correlation was observed between antioxidant activity with other phenolic compounds including protocatechuic acid, chlorogenic acid, p-coumaric acid, rutin and salicylic acid (Table 2). A moderate and significant positive correlation was observed between antioxidant activity in the sample collected in the month of November with phenolic compounds including protocatechuic acid, chloro-
genic acid, p-coumaric acid, rutin and salicylic acid, indicating that these compounds may be associated with the A. japonica antioxidant potential.

![Graph showing antioxidant activity of A. japonica roots](image_url)

**Figure 3.** Antioxidant activity of A. japonica roots.

**Table 2.** Pearson correlation coefficients between the phenolic compounds and antioxidant activity of A. japonica roots extracts.

| Assay         | TPC   | TFC   | Protocatechuic Acid | Chlorogenic Acid | p-Coumaric Acid | Rutin   | Salicylic Acid | Caffeic Acid | Orientin | Vitexin | Apigenin | Luteolin |
|---------------|-------|-------|---------------------|------------------|-----------------|---------|----------------|--------------|-----------|---------|----------|----------|
| DPPH (October)| 0.992 * | 0.961 * | 0.500 *             | 0.655 *          | 0.550 **        | 0.590 * | 0.500 *        | 0.817 *      | 0.810 **  | 0.693   | 0.721    | 0.655    |
| ABTS (October)| 0.997 * | 0.997 * | 0.663 **            | 0.792 *          | 0.663 *         | 0.663 **| 0.663 **       | 0.916 **     | 0.910 *   | 0.823   | 0.845    | 0.792    |
| DPPH (November)| 0.999 * | 0.993 * | 0.311               | 0.717 *          | 0.632 *         | 0.767 * | 0.632 **       | 0.539 *      | 0.622 *   | 0.539   | 0.767    | 0.632    |
| ABTS (November)| 0.965 | 0.911 * | 0.013               | 0.475            | 0.371           | 0.540 * | 0.371          | 0.262 *      | 0.614     | 0.262   | 0.540    | 0.371    |

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

3.4. Mineral Content

The results of the mineral composition analysis of A. japonica root extracts are shown in Tables 3 and 4. Among the different minerals analyzed, macro-elements contents (Ca and Mg) was were significantly increased (92.770 ± 0.654 µg mL⁻¹ and 28.680 ± 0.155 µg mL⁻¹, respectively), with delaying harvesting time. The analysis revealed that micro-elements such as Mn, Fe, and Al were the most abundant in roots extracts. Among these, Cu and Fe, important components for chlorophyll synthesis in plants, were present in higher concentrations (0.057 ± 0.0004 µg mL⁻¹ and 0.953 ± 0.0063 µg mL⁻¹, respectively) in roots harvested in October. In this study, a seasonal fluctuation was observed in the concentration of micro-elements. Data revealed a higher accumulation of micro-elements such as Al, Cu, and Fe (0.701 ± 0.0073 µg mL⁻¹, 0.057 ± 0.0004 µg mL⁻¹, 0.953 ± 0.0063 µg mL⁻¹, respectively) in the sample collected in October. The statistical analysis indicated that the micro-elements such as Mn, Zn, Cd and Ba content was influenced by the harvesting time, reaching higher values (1.696 ± 0.0032 µg mL⁻¹, 0.303 ± 0.0047 µg mL⁻¹, 0.090 ± 0.0019 µg mL⁻¹, 0.269 ± 0.0035 µg mL⁻¹, respectively) in the samples harvested in November. The analysis revealed higher accumulation of trace elements such as As, Bi, and Si in the October harvest. The concentration of trace elements such as Co, Ni, and Pb increased with delaying harvesting time. The Ti, Cr, Li and Ag concentrations had a similar accumulation patterns in the two harvests.
Table 3. Calibration curves, limit of detection (LOD), and limit of quantitation (LOQ) of the 19 elements examined in this study.

| Element | Concentration (mg L\(^{-1}\)) | Linearity (r\(^2\)) | Slope (S) | Response SD | SD (%) | LOD \(^b\) | LOQ \(^b\) |
|---------|--------------------------------|---------------------|----------|-------------|--------|-----------|-----------|
| **Macro elements** | | | | | | | |
| Ca | 0.5–10 | 0.999576 | 8157 | 1103.17 | 1.15 | 0.405726 | 1.352421 |
| Mg | 0.5–10 | 0.999738 | 19,920 | 2106.85 | 0.94 | 0.317297 | 1.057656 |
| **Micro elements** | | | | | | | |
| Al | 0.5–10 | 0.999332 | 3730 | 358.88 | 0.96 | 0.288643 | 0.962145 |
| Ba | 0.5–10 | 0.999825 | 5071 | 505.09 | 0.99 | 0.298811 | 0.996036 |
| Cd | 0.5–10 | 0.999812 | 2007 | 156.53 | 0.78 | 0.233976 | 0.779920 |
| Cu | 0.5–10 | 0.999852 | 4031 | 352.03 | 0.87 | 0.261992 | 0.873307 |
| Fe | 0.5–10 | 0.999901 | 31,120 | 3071.07 | 0.98 | 0.296054 | 0.986848 |
| Mn | 0.5–10 | 0.999799 | 5112 | 378.98 | 0.74 | 0.222406 | 0.741354 |
| Zn | 0.5–10 | 0.999876 | 47.10 | 3.54 | 0.75 | 0.225478 | 0.751592 |
| **Trace elements** | | | | | | | |
| As | 0.5–10 | 0.999876 | 47.10 | 3.54 | 0.75 | 0.225478 | 0.751592 |
| Bi | 0.5–10 | 0.999907 | 286.5 | 38.39 | 1.34 | 0.401990 | 1.339965 |
| Co | 0.5–10 | 0.999131 | 1197 | 111.51 | 0.93 | 0.279474 | 0.931579 |
| Cr | 0.5–10 | 0.999841 | 4921 | 522.49 | 1.06 | 0.318527 | 1.061756 |
| Li | 0.5–10 | 0.999783 | 32,120 | 3995.40 | 1.24 | 0.373169 | 1.243898 |
| Ni | 0.5–10 | 0.999757 | 1951 | 174.43 | 0.89 | 0.268216 | 0.894054 |
| Pb | 0.5–10 | 0.999913 | 1197 | 111.51 | 0.93 | 0.279474 | 0.931579 |
| Se | 0.5–10 | 0.999901 | 31,120 | 3071.07 | 0.98 | 0.296054 | 0.986848 |
| Ag | 0.5–10 | 0.999799 | 5112 | 378.98 | 0.74 | 0.222406 | 0.741354 |
| Ti | 0.5–10 | 0.999876 | 47.10 | 3.54 | 0.75 | 0.225478 | 0.751592 |

\(^a\) The calibration curve was obtained using 3–7 different concentrations of a standard solution for each element. \(^b\) The LOD and LOQ were determined using each calibration curve as follows: LOD = 3 × SD/S and LOQ = 10 × SD/S, where SD is the standard deviation of the response and S is the slope of each calibration curve. \(^c\) SD is the standard deviation of a response and S is the slope of the calibration curve.

Table 4. Mineral content in the *A. japonica* root extracts.

| Elements | Concentration (µg mL\(^{-1}\)) | October | November |
|----------|--------------------------------|---------|----------|
| **Macro elements** | | | | |
| Ca | | 49.34 ± 0.838 | 92.770 ± 0.654 |
| Mg | | 19.66 ± 0.2750 | 28.680 ± 0.155 |
| **Micro elements** | | | | |
| Al | | 0.701 ± 0.0073 | 0.551 ± 0.027 |
| Ba | | 0.120 ± 0.0015 | 0.269 ± 0.0035 |
| Cd | | 0.089 ± 0.0013 | 0.090 ± 0.0019 |
| Cu | | 0.057 ± 0.0004 | 0.031 ± 0.0011 |
| Fe | | 0.953 ± 0.0063 | 0.875 ± 0.0115 |
| Mn | | 0.928 ± 0.0134 | 1.696 ± 0.0032 |
| Zn | | 0.218 ± 0.0018 | 0.303 ± 0.0004 |
| **Trace elements** | | | | |
| As | | 0.142 ± 0.0364 | 0.037 ± 0.0562 |
| Bi | | 0.076 ± 0.0442 | 0.071 ± 0.0215 |
| Co | | 0.131 ± 0.0029 | 0.134 ± 0.0022 |
| Cr | | 0.095 ± 0.0026 | 0.095 ± 0.0010 |
| Li | | 0.081 ± 0.0007 | 0.081 ± 0.0004 |
| Ni | | 0.113 ± 0.0017 | 0.116 ± 0.0017 |
| Pb | | 0.076 ± 0.0223 | 0.087 ± 0.0193 |
| Se | | 0.143 ± 0.0443 | 0.175 ± 0.0643 |
| Ag | | 0.060 ± 0.0010 | 0.060 ± 0.0007 |
| Ti | | 0.040 ± 0.0001 | 0.040 ± 0.0003 |

3.5. Cytotoxicity Activity

Cytotoxicity was determined with the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) assay using NIH/3T3 cells treated with the *A. japonica* 80% methanolic extracts or a positive control (Tamoxifen). As shown in Figure 4, there was a dose-dependent effect in the inhibition of NIH/3T3 cellular proliferation by the root extracts.
We recorded the cytotoxicity of root extracts at various concentrations. As shown in Figures 4 and 5, the viability of NIH/3T3 cells was not significantly affected when the plant extract concentration was between 15.63 and 62.5 ppm, and high cellular viability was maintained. At the lower concentrations (less than 62.5 ppm), the root extracts led to more than 90% cell viability after 24 h of exposure. A slight reduction in cell viability was observed at 125 ppm, indicating that there was still a toxicity effect that should be considered before medicinal use. Moreover, we observed a strong positive correlation between cytotoxicity and p-coumaric acid (r = 0.974, p < 0.05), salicylic acid (0.908, p < 0.05), chlorogenic acid (r = 0.978, p < 0.05), caffeic acid (r = 0.959, p < 0.05), and Protocatechuic acid (r = 0.987, p < 0.01) contents (Table 5).

![Figure 4](image_url) **Figure 4.** The effect of root extracts concentration on cell viability on NIH 3T3 cells lines.

![Figure 5](image_url) **Figure 5.** Cytotoxic effect of the root extracts (IC$_{50}$ concentrations) on the NIH 3T3 cell lines. (i) Bright field, (ii) AO/EB staining, (iii) ROS.

### 3.6. Assessment of Irritation Potential of Root extracts by the HET–CAM Assay

The Hen’s egg test–on the chorioallantoic membrane (HET–CAM) of chicken embryos is a reliable, rapid, and cost-effective approach to investigate the irritation potential of chemicals; it is routinely used in the cosmetic industry and in the development of cosmetics. We tested the irritation potential of different root extract concentrations using different parameters (coagulation, hemorrhage, and lysis). The analyses showed that lower extract concentrations did not exhibit embryonic toxicity (Figure 6 and Table 6).
Table 5. Pearson correlation coefficients between the phenolic compounds and cytotoxicity of the A. japonica root extracts.

| Phenolic Compounds     | Cytotoxicity |
|------------------------|-------------|
| Protocatechuic acid    | 0.987 **    |
| Rutin                  | 0.899       |
| Chlorogenic acid       | 0.978 *     |
| p-Coumaric acid       | 0.974 *     |
| Salicylic acid        | 0.908 *     |
| Caffeic acid          | 0.959 *     |
| Orientin              | 0.509       |
| Vitexin               | 0.350       |
| Apigenin              | 0.240       |
| Luteolin              | 0.150       |

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Table 6. Assessment of test sample irritation potential in the HET–CAM assay.

| Samples                  | Irritation Score | Irritation Assessment |
|--------------------------|------------------|-----------------------|
| Negative control         | 0                | Non-irritant          |
| NaOH (0.1 M)             | 18.00            | Strong irritant       |
| Root extracts (50 mg/mL) | 0                | Non-irritant          |

4. Discussion

This study was performed to assess the influence of harvesting time on the TPC, TFC, phenolic compound profile, mineral content, and cytotoxic effects of A. japonica root extracts harvested in October or November. The TPC and TFC were higher in samples harvested in October. Higher levels of total phenolics in samples harvested earlier were also observed in Vaccinium corymbosum [35] and Dioscorea cayenensis [36]. Similar results were also reported by [37]. Moreover, flavonoid biosynthesis is closely related to the plant development stage and is significantly affected by diverse ecological and developmental factors [38,39]. Other reports indicated that genotype, ontogenesis, analysis time, processing, environment, developmental stage, and storage conditions affect the plant phenolic compound contents [40,41]. Wide changes in the environmental conditions, including temperature, humidity, and precipitation, observed during A. japonica growth (Supplementary Figures S1 and S2). Flavonoids might be converted to other compounds–flavones, flavonols, flavanones, cell wall components, or oligo and polymeric compounds–with changes in the environment [42,43]. UV radiation also affects flavonoid biosynthesis [44–47]. Flavonoids decrease the photo-oxidative damage caused by UV absorption and inhibit ROS generation by inhibiting the enzymes involved in radical formation [48]. Therefore, the higher flavonoid accumulation in the October samples could derive from higher temperatures and irradiance. This change likely increased UV light...
absorption and reduced ROS generation in A. japonica plants. Previous studies reported increased flavonol contents in response to increased irradiance levels [49–51]. Similar results were obtained in Aloysia gratissima [52], Moringa oleifera [53], Fagus sylvatica [54], and Ginkgo biloba [55]. Moreover, a positive correlation between phenolic compound content and light availability has also been previously reported [51].

Phytochemicals have various biological activities, including biochemical and pharmacological properties and high antioxidant potential (e.g., polyphenols) [56]. Various studies have attributed the plant antioxidant potential to phenolic compounds, including protocatechuic acid, rutin, ferulic acid, naringenin, gentisic acid, and quercetin, which effectively act as reducing agents, hydrogen donors, and singlet oxygen quenchers [57]. We observed a strong correlation between TPC and DPPH and a moderate positive correlation between TPC and ABTS in A. japonica root extracts. There was also a positive significant correlation between antioxidant activity (DPPH assay) and the phenolic acids rutin, chlorogenic acid, and p-coumaric acid, indicating that these compounds are strongly associated with the A. japonica antioxidant potential. However, we did not observe a correlation between ABTS and phenolic compound contents. The differences between the two assays may derive from different responses of antioxidant and oxidant substances to different radicals. Our results showed significant differences in the antioxidant activity in samples with different harvesting times. The ferulic acid content was well correlated with the DPPH scavenging activity in A. japonica. Similarly, there was a strong positive correlation between the sum of the p-coumaric acid and gallic acid (GA) contents and the antioxidant activity of root extracts. Previous studies have also revealed a significant positive correlation between phenolic compounds and the antioxidant activity of leaf extracts [58,59]. Phenolic compounds act as antioxidants by neutralizing/scavenging free radicals produced during metabolic processes [56–60]. Chlorogenic acid and rutin have well-established antioxidant activity [61]. Our results corroborate the hypothesis that phenolic compounds contribute to the A. japonica antioxidant activity. Different phenolic compounds may interact and synergistically scavenge free radicals [62]. Therefore, these phytochemicals may be crucial to the plant’s antioxidant activity and highly influenced by the harvesting time.

A. japonica grown in October received longer daily light periods and higher temperatures than plants grown in November; they might have had higher protection against UV light through increased accumulation of phenolic compounds. Samples harvested in October had higher phenolic compound concentrations, including ferulic acid, rutin, quercetin, kaempferol, and apigenin, indicating a greater need for protection against photo-oxidative stress caused by higher irradiance. Flavonoids, including kaempferol and quercetin, protect plant tissues against UV radiation [48]. Additionally, plants enhance their phenolic content in response to the oxidative pressure caused by higher light intensity [63]. However, the correlation between the phytochemical content and environmental conditions, such as precipitation, temperature, and harvesting time, remains controversial [64]. Higher flavonoid contents were observed in Lippia alba leaves harvested during the summer. Conversely, the TPC increased in the bark of Secondatia floribunda harvested during the dry season [65]. Future work should explore the correlation between these parameters in more detail. Taken together, the results suggest that irradiance, precipitation, and temperature influenced phenolic compound accumulation in A. japonica. Therefore, the harvesting period of A. japonica roots should be carefully selected when considering the intended plant applications. Overall, higher temperature and solar radiation intensity are the main factors that promote phenolic compound biosynthesis.

Harvesting time had a significant influence on the concentration of minerals in A. japonica root samples. Early harvesting (October) decreased Mg and Ca concentrations. The difference in the mineral content between the two harvesting times may result from differences in the mineral quantities absorbed from the soil. Mg is a very mobile element in the phloem [66] and actively associated with the in the processes of respiration, DNA and RNA synthesis, and involved in chlorophyll structure. Apart from this, magnesium plays an important role in photosynthesis by activating the enzymes related
to carbohydrate metabolism [67]. Higher Mg contents observed in the October root samplings may be related to the rapid mobility of Mg and occurrence of greater demand of Mg being related to higher carbohydrate metabolism due to rapid growth, flowering and green fruit production of A. japonica. In addition to the association with the mechanical strength of tissues, calcium is important for pollen germination and pollen tube growth during flowering period [68]. A notable increase of the calcium in the October sample could be due to increase its demand in the foliar region and higher mobility of calcium from root for potential use in the flowering and in the maturity of the fruit. Moreover, Moreno and Garra-Creus [69] observed reduction in the Ca content via transpiration stream in Olive tree during hot (higher temperature) and dry period, which also corroborates with present study. Depletion in the supply of Mg and Ca to the foliar region in the cold month of November could result in its higher accumulation in the A. japonica root. According to [70], this variation may be due to changes in the ecological conditions at different harvesting times. Fe is an important enzymatic constituent that plays significant roles in respiration, photosynthesis, and DNA synthesis [71]. Its concentration was higher in samples harvested in October, which could be explained by the higher photosynthesis and respiration rates caused by higher temperatures. Our study also corroborated the results obtained in Lablab lines [72] and in Phalaris arundinacea [73], in which the content of various minerals increased with early harvesting. Zn and Mn plant components that are critical for secondary metabolite synthesis, therefore influencing antioxidant activity and ROS scavenging in cells [22]. Mn cations influence phenylalanine ammonia-lyase (PAL) function, PAL is one of the key enzymes responsible for phenol biosynthesis [74]. We observed an increase in Mn, and Zn concentrations in the October harvest. These minerals could be associated with the antioxidant properties of A. japonica, as revealed by a positive correlation between those factors (Table S1). Hence, mineral availability changes with the season, as well as with the plant developmental stage.

The MTT assay is a rapid and reliable colorimetric method to assess cell viability and death [75]. A. japonica root extracts of collected in October exhibited cytotoxicity in a concentration-dependent manner. Cell viability was not significantly affected at low extract concentrations. Unnecessarily high bioactive compound concentrations may disrupt their function and cause cell death. Various studies attributed the cytotoxicity of plant extracts to the presence of specific phytochemicals [76–79]. For example, higher rutin, chlorogenic acid, and luteolin concentrations significantly reduced the growth of NIH/3T3 cells. Similarly, there was a strong positive correlation between cytotoxicity and the concentration of protocatechuic acid, chlorogenic acid, p-coumaric acid, salicylic acid, quercetin, luteolin, and quercetin derivatives [80–83]. Here, we observed a strong positive correlation between cytotoxicity and p-coumaric acid, salicylic acid, chlorogenic acid, salicylic acid, and rutin contents, suggesting that the higher concentration of these compounds is associated with the cytotoxicity effect of A. japonica. The root extracts of this plant contain diverse essential phytochemical and exhibits wide range of biological properties, but the potential toxic effects of the extract still remain unclear. Therefore, in this study, we performed HET-CAM assay, which is was a widely used to identify substances that can consider as irritant or non-irritant natural substances [84]. Our result indicate that a lower concentration of A. japonica roots extracts has no toxic effects.

5. Conclusions

In this study, different harvest times directly influenced the phenolic composition of A. japonica plants. Hence, we inferred that the seasonal variation in the composition and concentration in phenolic compounds and minerals in A. japonica plants resulted from changes in environmental factors, including temperature, humidity, precipitation, and harvesting time. The concentration of various phenolic compounds, including protocatechuic acid, chlorogenic acid, rutin, salicylic acid, caffeic acid, orientin, vitexin, and apigenin, was significantly changed between harvesting seasons, which directly influenced
the plant antioxidant activity. This study further elucidated that *A. japonica* harvesting time is essential for obtaining desired chemical constituents for specific applications.

**Supplementary Materials:** The following are available online at [https://www.mdpi.com/article/10.3390/agronomy11071327/s1](https://www.mdpi.com/article/10.3390/agronomy11071327/s1), Figure S1. Fluctuation in the (A) humidity, (B) precipitation in the month of October and November during growth of *A. japonica*. Figure S2. Fluctuation in the temperature in the month of October and November during growth of *A. japonica*. Table S1. Pearson’s correlation coefficients of the antioxidant properties and minerals. Table S2. Calibration curves of 16 phenolic compounds.

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