

Article

Phenolic Profile of *Acer tegmentosum* Sprouts and Its Potential Relevance to *In Vitro* Antioxidant Activity

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Abstract

Oxidative stress plays a central role in the development of chronic degenerative diseases, prompting growing interest in natural antioxidants, particularly phenolic compounds from early developmental plant tissues. This study investigated the chemical composition and antioxidant capacity of *Acer tegmentosum* sprouts at the cotyledon expansion stage using integrated metabolite profiling and targeted quantification approaches. *A. tegmentosum* sprout extracts (ASE) were characterized by liquid chromatography–tandem mass spectrometry (LC–MS/MS) for untargeted metabolite annotation and by high-performance liquid chromatography (HPLC) for the targeted quantification of selected phenolic acids and coumarins using authentic standards. Antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays. LC–MS/MS analysis identified twelve phenolic compounds, including gallic acid, protocatechuic acid, scopoletin, and their derivatives. HPLC results confirmed gallic acid (5.54 mg/g extract) as the predominant phenolic constituent, and the overall composition indicated a phenolic acid-enriched profile. ASE showed notable DPPH and ABTS radical scavenging activities, with IC₅₀ values of 376.40 and 311.00 µg/mL, respectively, although these activities were lower than those of ascorbic acid. Overall, these findings define the baseline chemical and antioxidant properties of ASE and identify analytically traceable marker compounds for standardization and functional material development. Further studies across different developmental stages and cultivation conditions are needed to verify generalizability and refine marker selection for quality control purposes.

Keywords: LC–MS/MS; HPLC; radical scavenging assay; phenolic acids



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1. Introduction

Aerobic metabolism continuously generates reactive oxygen and nitrogen species in living systems, and oxidative stress occurs when antioxidant defenses fail to adequately counteract these oxidants [1]. Oxidative stress is commonly defined as a shift in the oxidant–antioxidant balance toward pro-oxidants, resulting in disrupted redox regulation and molecular damage, and it has been implicated in the pathophysiology of numerous chronic and degenerative diseases [2,3]. These considerations have intensified interest in

natural antioxidant resources and have highlighted the practical importance of reporting both the contents of key constituents and quantitative measures of antioxidant capacity, thereby enabling objective characterization and comparison of samples [4].

Phenolic compounds remain prominent candidates as natural antioxidants because of their ability to quench free radicals through electron or hydrogen donation mechanisms [5,6]. Among these compounds, phenolic acids and coumarins are frequently reported as representative classes associated with antioxidant activity across a wide range of plant species [7–11]. In addition, sprouts and seedlings represent early developmental stages characterized by pronounced remodeling of secondary metabolism, and numerous studies have shown that germination can increase phenolic content and antioxidant capacity in a species- and condition-dependent manner [12,13]. Accordingly, metabolite profiling and targeted quantification focused on sprout tissues are scientifically justified for generating foundational evidence relevant to antioxidant resources and quality standardization [14].

Because sprouts can differ substantially in chemical composition from mature organs, it is necessary to report sprout-specific constituent profiles and antioxidant capacity rather than extrapolating from data obtained for mature tissues [13,15,16]. Given its history of traditional use and the presence of reported antioxidant constituents in non-sprout tissues, *Acer tegmentosum* represents an appropriate target species for such investigation [17]. Previous studies have shown that methanolic extracts of *A. tegmentosum* stem bark contain higher levels of (+)-catechin and scopoletin than heartwood and leaves based on high-performance liquid chromatography (HPLC) analysis, while gas chromatography–mass spectrometry (GC–MS) further supported the presence of phenolic constituents in this species [17].

In Korea, *A. tegmentosum* is a montane deciduous tree used as a forest medicinal resource and is commonly referred to as the Manchurian striped maple [18]. It has traditionally been used for the treatment of hepatic disorders, and intensive medicinal harvesting has been associated with habitat restriction and population fragmentation, underscoring the need for evidence-based and sustainable utilization supported by reproducible chemical data [18]. Public exposure to *A. tegmentosum* preparations has increased through dietary supplements and functional foods, and the Ministry of Food and Drug Safety of South Korea has approved *A. tegmentosum* as a food material (No. 2018-7), further emphasizing the importance of compositional characterization and quality standardization [19–21]. Phytochemical investigations of mature organs have identified abundant phenolic constituents, including salidroside as well as catechin- and coumarin-type metabolites, collectively supporting *A. tegmentosum* as a suitable source for analytically robust indicators applicable to forest-derived materials [17,22].

Despite these findings, previous studies on *A. tegmentosum* have primarily focused on mature tissues such as stem bark, heartwood, leaves, and twigs, while evidence specific to sprouts remains limited [17,23,24]. Comprehensive chemical profiling of *A. tegmentosum* sprouts and systematic evaluation of their antioxidant-related characteristics have been reported only to a limited extent. As a result, it remains unclear which bioactive constituents are present in sprout tissues and whether compounds reported from mature organs are conserved or altered during early developmental stages, leaving a gap in the baseline data required for accurate characterization and comparison of sprout materials [15].

In this study, we evaluated the antioxidant potential of *A. tegmentosum* sprouts in conjunction with quantitative chemical information by applying liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based metabolite profiling to characterize phenolic composition, HPLC to quantify selected phenolic acids and coumarins using authentic reference standards, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays to assess antioxidant capacity. By reporting constituent contents together with antioxidant activity, this

work aims to provide foundational chemical data for *A. tegmentosum* sprouts and to support the selection of practical analytical indicators for the future development of sprout-based functional materials.

2. Materials and Methods

2.1. Plant Materials and Extraction

Samaras (single-seeded fruits) of *Acer tegmentosum* Maxim. were collected in October 2023 from the conservation nursery of the Forest Bioresources Department, National Institute of Forest Science, Suwon, Republic of Korea (37°15'09" N, 126°57'31" E; 44.6 m a.s.l.). The samaras were air-dried at room temperature for 3 days. Plant material authentication was performed by Dr. J. Ku (National Institute of Forest Science, Republic of Korea), and a voucher specimen (No. KHB-1663453) was deposited at the Korea National Arboretum Herbarium, Pocheon, Republic of Korea. The dried samaras were stored at 4 °C with silica gel until use. Based on previous samaras germination studies, germination was conducted in a growth chamber (DS-14MCLHP; Dasol Scientific Co., Hwaseong, Republic of Korea) maintained at 15 °C under a 16 h light/8 h dark photoperiod [25]. Samaras were placed in 90 mm Petri dishes lined with two layers of filter paper moistened with distilled water. Seedlings were harvested at the cotyledon expansion stage, 21 days after radicle emergence, pooled by replicate, immediately frozen in liquid nitrogen, and stored at −80 °C until extraction (Figure 1). Sprouts of *A. tegmentosum* (5 g), finely ground to pass through an 80-mesh sieve (180 µm), were extracted in triplicate under reflux extraction with ethanol (EtOH) according to a previously reported method [26]. The combined extracts were concentrated under reduced pressure using a rotary vacuum evaporator (OSB-2100, Eyela Co., Tokyo, Japan). After concentration, 0.9 g of extract was obtained, corresponding to an extraction yield of 18% (*w/w*). The resulting *A. tegmentosum* sprout extract (ASE) was used for subsequent experiments.



Figure 1. *A. tegmentosum* sprouts at the cotyledon expansion stage.

2.2. Apparatus and Chemicals

LC–MS/MS profiling was performed using a Vanquish ultra-high-performance liquid chromatography (UHPLC) system (Thermo Fisher Scientific, Waltham, MA, USA) coupled to a Q Exactive™ Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Targeted HPLC quantification was conducted using an HPLC system (Agilent

1260 Infinity II Quaternary Pump, Santa Clara, CA, USA) equipped with a pump, autosampler, and variable-wavelength detector (Santa Clara, CA, USA). Antioxidant assays were measured using a microplate reader (Epoch; BioTek, Winooski, VT, USA). Formic acid and trifluoroacetic acid (TFA) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Acetonitrile and water (LC–MS grade) were obtained from J.T. Baker (Radnor, PA, USA). Methanol (MeOH, HPLC grade) was purchased from Samchun Co. (Pyeongtaek, Republic of Korea), and acetonitrile and water (HPLC grade) were also obtained from J.T. Baker. Analytical standards, including gallic acid (1), protocatechuic acid (2), esculetin (3), protocatechualdehyde (4), epigallocatechin gallate (5), taxifolin (6), *p*-coumaric acid (7), scopoletin (8), vicenin 2 (9), *o*-coumaric acid (10), myricetin (11), and herniarin (12), were provided by the Natural Product Institute of Science and Technology (www.nist.re.kr; accessed on 10 October 2025), Anseong, Republic of Korea (Figure 2).

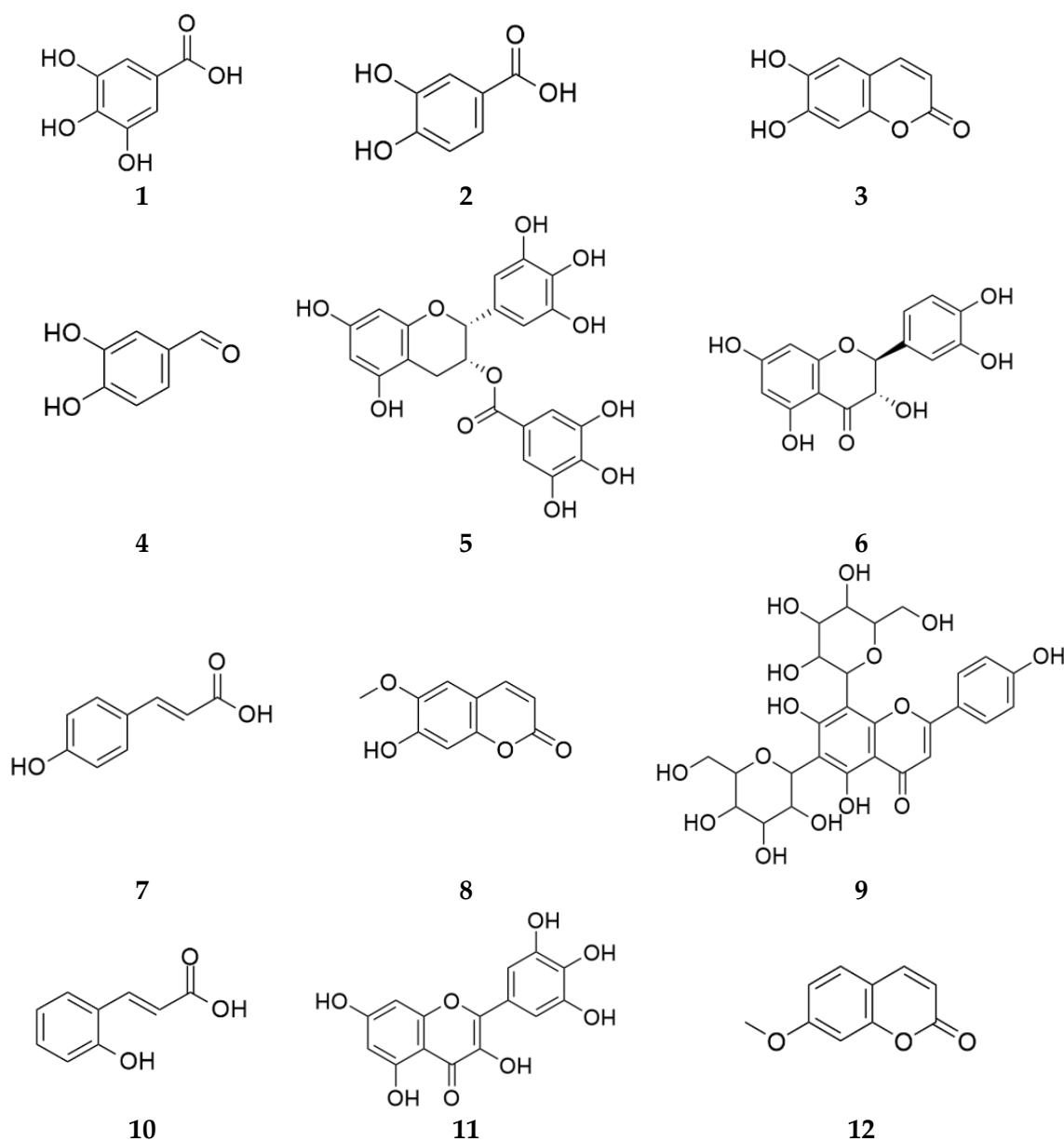


Figure 2. Chemical structures of gallic acid (1), protocatechuic acid (2), esculetin (3), protocatechualdehyde (4), epigallocatechin gallate (5), taxifolin (6), *p*-coumaric acid (7), scopoletin (8), vicenin 2 (9), *o*-coumaric acid (10), myricetin (11), and herniarin (12).

The provided purities and the chromatographic purities assessed by HPLC peak area (%) were as follows: gallic acid (>95%, HPLC), protocatechuic acid (>99%, HPLC), esculetin (>99%, HPLC), protocatechualdehyde (>99%, HPLC), epigallocatechin gallate (>97%, HPLC), taxifolin (>95%, HPLC), *p*-coumaric acid (>99%, HPLC), scopoletin (>98%, HPLC), vicianin 2 (>99%, HPLC), *o*-coumaric acid (>96%, HPLC), myricetin (>99%, HPLC), and herniarin (>95%, HPLC). These analytical standards were used for HPLC quantification via external calibration and as reference compounds in the antioxidant assays.

2.3. LC–MS/MS Acquisition Conditions

Prior to LC–MS/MS analysis, the extract was diluted to 80% (*v/v*) EtOH to improve compatibility between the injection solvent and the initial mobile-phase composition and to minimize chromatographic peak distortion caused by solvent-strength and viscosity mismatches [27]. Chromatographic separation was achieved using a UHPLC system equipped with a Waters -Cortecs C₁₈ column (2.1 mm × 150 mm, 1.6 µm; Waters Co., Milford, MA, USA), maintained at 45 °C with a flow rate of 0.3 mL/min. The mobile phases consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The gradient elution program was as follows: 0–0.1 min, 95% A/5% B; 10 min, 90% A/10% B; 50–55 min, 5% A/95% B; and 55.1–60 min, re-equilibration to 95% A/5% B. Mass spectrometric detection was performed using an Orbitrap mass spectrometer equipped with a heated electrospray ionization (H-ESI) source. The spray voltage was set to 3.5 kV in positive ion mode and 3.0 kV in negative ion mode. The sheath gas, auxiliary gas, and sweep gas were set to 50, 10, and 1 (arbitrary units), respectively, and the ion transfer tube temperature was maintained at 320 °C. Full-scan MS (MS1) spectra were acquired at a resolution of 70,000 over an *m/z* range of 100–1500. Data-dependent MS/MS (MS2) spectra were acquired at a resolution of 17,500 (TopN = 10) using stepped normalized collision energy (NCE) values of 10, 30, and 50.

2.4. LC–MS/MS Data Processing and Compound Annotation

Raw LC–MS/MS data files (Thermo RAW format) were processed using Elements Viewer (v2.1.1) and converted to mz5 format with ProteoWizard (pwiz v3.0.18176). Feature detection was conducted across an *m/z* range of 30–2000 over the full retention-time range, using a noise threshold of 0.2% of the maximum signal intensity and a minimum delta scan time of 0.5 s. MS1 peak grouping within individual samples was performed using same-charge and cross-charge inclusion thresholds of 2.0 s. Retention-time alignment was not applied; instead, consensus MS1 peak groups were generated using a maximum allowable retention-time (*t_R*) deviation of 60.0 s. Cross-sample gap filling and feature re-extraction were enabled to improve feature completeness. Compound annotation was conducted through spectral library searching based on exact mass, using a precursor mass tolerance of 20.0 parts per million (ppm). When available, experimental MS2 spectra were matched against library spectra using a fragment mass tolerance of 0.5 Da. The following adducts were considered during library searching: $[M \pm H]^{\pm}$, $[M \pm Na]^{\pm}$, $[2M \pm H]^{\pm}$, $[M]^{\pm}$, and $[M \pm HCOO]^{\pm}$. Spectral matching was performed against multiple databases, including MoNA–MassBank of North America export libraries, the NIST MS/MS library, and in-house positive- and negative-ion libraries configured in Elements Viewer. Features lacking library matches or experimental MS2 spectra were excluded. Annotation confidence was assessed using the Elements Viewer identification scoring system, which integrates mass accuracy, isotopic pattern matching, and fragmentation similarity. Only annotations meeting the predefined confidence threshold (ID score ≥ 0.7) were retained.

2.5. HPLC Sample Preparation and Conditions

ASE (20 mg) was dissolved in 1 mL of MeOH. Individual reference standards (compounds 1–12) were prepared in MeOH at a concentration of 1 mg/mL. Sample and standard solutions were sonicated for 15 min and filtered through a 0.2 µm PVDF membrane filter (Cat. No. 6779, Piscataway, NJ, USA). Standard solutions were subsequently diluted with MeOH to appropriate concentrations for quantitative analysis. HPLC analysis was performed using an INNO C₁₈ column (250 mm × 4.6 mm, 5 µm; Young Jin Biochrom Co., Seongnam, Republic of Korea) maintained at 30 °C. The mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water (solvent A) and acetonitrile (solvent B). Gradient elution was applied as follows: 0–8 min, 90% A; 13 min, 80% A; 15–24 min, 79% A; 50 min, 50% A; 60–67 min, 0% A; and 68–75 min, re-equilibration to 90% A. Detection was carried out at 270 nm, with an injection volume of 10 µL and a flow rate of 0.5 mL/min.

2.6. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined based on calibration curve statistics derived from linear regression analysis [28]. Specifically, the standard deviation term (σ) was estimated from the residual standard deviation of the calibration regression line, consistent with recommended approaches for calibration-based determination of detection and quantification limits [28,29].

2.7. Calibration Curve

Calibration curves were constructed using five concentration levels for each reference standard. Compounds 1–12 were serially diluted to yield five concentrations ranging from 7.8, 15.6, 31.3, 62.5, and 125.0 µg/mL. Linearity was assessed by calculating the correlation coefficient (R value) for each calibration curve. Compounds in ASE were quantified using the corresponding calibration curve equations. Calibration curves were generated by plotting analyte concentration (µg/mL) on the x -axis against peak area on the y -axis.

2.8. DPPH and ABTS Radical Scavenging Assays

The DPPH radical scavenging activity of ASE was evaluated using a modified method described by Kedare and Singh [30]. Aliquots of extract solutions prepared at defined concentrations were mixed with 200 µL of a 0.2 mM DPPH stock solution, vortex-mixed, and incubated for 30 min at room temperature in the dark. Absorbance was then measured at 514 nm using a microplate reader. ABTS radical scavenging activity was determined following a previously reported method [31]. The ABTS radical cation was generated by preparing a 7.4 mM ABTS solution in distilled water, which was mixed at a 1:1 (v/v) ratio with 2.6 mM potassium persulfate and incubated at 4 °C in the dark for at least 24 h. For the assay, 10 µL of extract solution was combined with 200 µL of the ABTS working solution, vortex-mixed, and incubated for 30 min at room temperature in the dark. Absorbance was measured at 734 nm using a microplate reader. MeOH and distilled water were used as negative controls for the DPPH and ABTS assays, respectively, and ascorbic acid served as the positive control. Selected reference standards, specifically gallic acid (1), protocatechuic acid (2), protocatechualdehyde (4), p -coumaric acid (7), and scopoletin (8), were evaluated in parallel under the same DPPH and ABTS assay conditions to provide reference IC₅₀ values for interpreting the extract-level activity of ASE. Radical scavenging activity was calculated according to the following equation:

$$\text{Radical scavenging activity (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

The IC₅₀ value was determined by linear regression analysis of radical scavenging activity versus extract concentration and was defined as the concentration required to achieve 50% radical scavenging activity.

2.9. Statistical Analysis

All measurements were conducted in triplicate across three independent experiments ($n = 3$), each consisting of triplicate technical replicates. Data are expressed as the mean $\pm$ standard deviation (SD) of independent experiments unless otherwise specified. Statistical analyses were performed using GraphPad Prism version 8.0.2 (GraphPad Software, Boston, MA, USA). Data normality was assessed using the Shapiro–Wilk test prior to parametric analysis. One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was applied to evaluate statistically significant differences among formulations. A significance threshold of $p < 0.05$ was adopted.

3. Results and Discussions

3.1. LC–MS/MS Profiling Analysis

Representative base peak chromatograms of ASE acquired in negative and positive ionization modes are shown in Figure 3. The LC–MS/MS dataset of ASE was processed and annotated using the workflow described in Section 2.4, and the annotations retained according to the predefined identification criteria are summarized in Tables 1 and 2. Overall, the detected major features exhibited strong concordance with high-resolution, exact mass-based annotation, supported by coherent precursor-ion information, consistent ion formation behavior under the applied acquisition conditions, and library-guided MS/MS spectral similarity [32,33]. Collectively, these lines of evidence support the internal plausibility of the tentative assignments, while maintaining the appropriate level of caution inherent to library-based compound annotation [34].

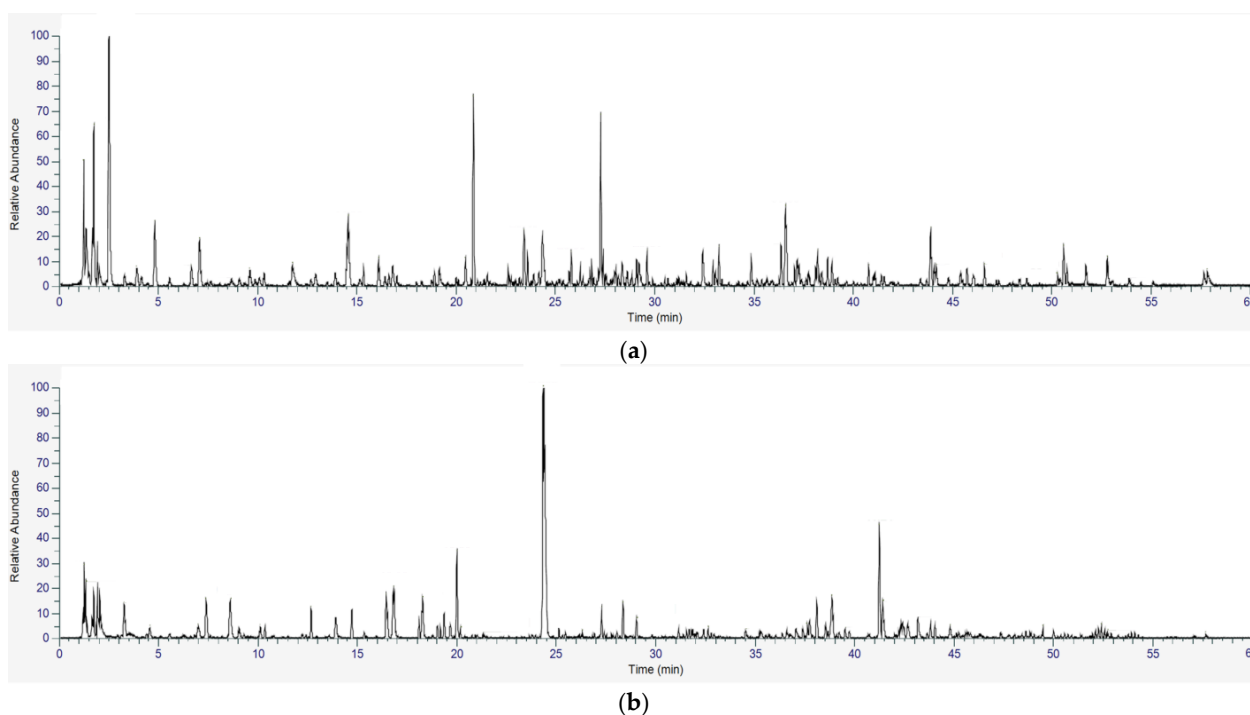


Figure 3. Base peak chromatograms of ASE acquired in negative (a) and positive (b) ionization modes.

From a chemical perspective, the observed profile is consistent with the extraction selectivity of EtOH toward polar to mid-polar secondary metabolites that are typically abundant in sprout tissues [35,36]. This compositional bias is analytically advantageous, as such metabolites generally exhibit efficient ionization under electrospray conditions and produce well-resolved, interpretable MS/MS fragmentation patterns that can be reliably matched against reference spectra [37]. However, EtOH extracts may also contain

structurally related analogs and positional isomers that share elemental compositions and generate partially overlapping fragmentation pathways, which should be carefully considered when interpreting putative compound assignments [38,39].

Table 1. LC–MS/MS profiling of ASE acquired in negative ionization mode.

t_R	Adducts	Observed m/z	Calculated m/z	$\Delta m/z$ ppm *	Accurate Mass	Molecular Formula	Tentative Identification
2.53	$[2M - H]^-$	339.0346	339.0357	± 3.3031	170.022	$C_7H_6O_5$	gallic acid
4.81	$[M - H]^-$	153.0177	153.0193	± 10.5327	154.027	$C_7H_6O_4$	protocatechuic acid
7.07	$[M - H]^-$	137.0228	137.0244	± 11.6261	138.032	$C_7H_6O_3$	protocatechualdehyde
10.43	$[M - H]^-$	177.0179	177.0193	± 7.8783	178.027	$C_9H_6O_4$	esculetin
13.92	$[M - H]^-$	457.0768	457.0776	± 1.6503	457.077	$C_{22}H_{18}O_{11}$	epigallocatechin gallate
14.56	$[M - H]^-$	163.0385	163.0400	± 9.2456	164.047	$C_9H_8O_3$	<i>p</i> -coumaric acid
15.33	$[M - H]^-$	593.1498	593.1511	± 2.2636	594.158	$C_{27}H_{30}O_{15}$	vicenin 2
16.60	$[M - H]^-$	163.0385	163.0400	± 9.2066	164.047	$C_9H_8O_3$	<i>o</i> -coumaric acid
16.81	$[M - H]^-$	191.0337	191.0349	± 6.3272	192.042	$C_{10}H_8O_4$	scopoletin
18.31	$[M - H]^-$	303.0504	303.0510	± 1.8056	304.058	$C_{15}H_{12}O_7$	taxifolin
21.55	$[M - H]^-$	317.0295	317.0302	± 2.2838	318.038	$C_{15}H_{12}O_8$	myricetin
23.43	$[M + HCOO]^-$	221.0444	221.0455	± 4.8354	176.047	$C_{10}H_8O_3$	herniarin

Only the predefined confidence threshold of ID score ≥ 0.7 were retained. * Δ ppm was calculated from unrounded observed m/z values using full software precision, whereas the m/z values reported in the table were rounded to three decimal places; Δ ppm was calculated as follows: Δ ppm = (observed m/z – calculated m/z)/calculated m/z $\times 10^6$.

Table 2. LC–MS/MS profiling of ASE acquired in positive ionization mode.

t_R	Adducts	Observed m/z	Calculated m/z	$\Delta m/z$ ppm *	Accurate Mass	Molecular Formula	Tentative Identification
15.33	$[M + H]^+$	595.1629	595.1657	± 4.6924	594.158	$C_{27}H_{30}O_{15}$	vicenin 2
16.81	$[M + H]^+$	193.0486	193.0495	± 4.5101	192.042	$C_{10}H_8O_4$	scopoletin

Only the predefined confidence threshold of ID score ≥ 0.7 were retained. * Δ ppm was calculated from unrounded observed m/z values using full software precision, whereas the m/z values reported in the table were rounded to three decimal places; Δ ppm was calculated as follows: Δ ppm = (observed m/z – calculated m/z)/calculated m/z $\times 10^6$.

The $\Delta m/z$ value, expressed as ppm, represents the relative mass measurement error between the observed and theoretical m/z values after normalization to the theoretical mass [40]. As shown in Tables 1 and 2, several features acquired in negative-ion mode exhibited larger $\Delta m/z$ values than those obtained in positive-ion mode. This trend is consistent with known polarity-dependent behavior in electrospray ionization, as negative-ion mode is more susceptible to corona discharge and spray instability [41,42]. These phenomena can reduce and destabilize ion flux while increasing chemical noise, thereby impairing ion-population control and peak definition and ultimately leading to greater apparent mass deviations, particularly for low-abundance ions [40,42,43]. Although a ± 5 ppm window is commonly applied as a conservative criterion in high-resolution mass spectrometry workflows, larger ppm deviations in negative-ion data may still be acceptable for formula-level reporting when supported by orthogonal constraints, including chemically plausible elemental composition filtering and corroborating MS/MS fragmentation evidence [40,44,45]. On this basis, all twelve detected features were conservatively reported at the molecular formula level.

3.2. HPLC Quantification

To translate the tentative LC–MS/MS annotations into practical quantitative indices, a targeted HPLC method was employed using authentic reference standards selected from the major phenolic acids and coumarins detected in ASE [46]. This strategy introduces an orthogonal analytical layer based on chromatographic retention behavior and UV detector response, thereby enabling consistent and reproducible quantification across samples and batches and reducing dependence on ion formation efficiency inherent to mass spectrometric analyses.

Targeted HPLC quantification using authentic standards established an independent, retention-based quantitative framework that complements LC–MS/MS-based metabolite annotation. The calibration performance, sensitivity parameters, and quantitative results are summarized in Table 3, and representative chromatograms are presented in Figure 4.

Table 3. HPLC quantification of compounds 1–12.

Compound	t_R	Calibration Equation	R^2	LOD (mg/mL)	LOQ (mg/mL)	RSD (%)	ASE (mg/g Extract)
1	9.548	$y = 69,622.87x + 77.23$	0.9989	0.006	0.017	0.490	5.54 ± 0.08
2	17.128	$y = 49,691.24x + 42.16$	0.9992	0.005	0.015	0.408	1.13 ± 0.01
3	20.823	$y = 15,065.80x - 4.10$	0.9995	0.003	0.011	0.403	ND
4	21.639	$y = 93,199.42x + 89.35$	0.9991	0.005	0.016	0.285	0.20 ± 0.01
5	28.237	$y = 88,749.20x - 166.90$	0.9982	0.007	0.022	0.491	ND
6	30.351	$y = 262,731.50x + 54.53$	0.9992	0.004	0.014	0.635	ND
7	31.349	$y = 74,041.95x + 65.73$	0.9992	0.005	0.015	0.381	0.76 ± 0.02
8	34.018	$y = 15,646.14x + 12.77$	0.9992	0.005	0.015	0.351	0.63 ± 0.04
9	36.542	$y = 121,611.00x - 205.60$	0.9983	0.006	0.021	0.482	ND
10	37.125	$y = 306,885.20x - 77.90$	0.9995	0.003	0.011	0.652	ND
11	44.118	$y = 135,721.10x - 255.20$	0.9980	0.007	0.023	0.516	ND
12	50.653	$y = 36,295.60x - 21.60$	0.9997	0.002	0.008	0.552	ND

ND: not detected. Compounds: gallic acid (1), protocatechuic acid (2), esculetin (3), protocatechualdehyde (4), epigallocatechin gallate (5), taxifolin (6), *p*-coumaric acid (7), scopoletin (8), vicianin 2 (9), *o*-coumaric acid (10), myricetin (11), and herniarin (12). Values are expressed as the mean $\pm$ SD ($n = 3$). RSD (%) denotes the relative standard deviation of peak areas from replicate injections for each standard and was calculated as (standard deviation/mean peak area) $\times$ 100.

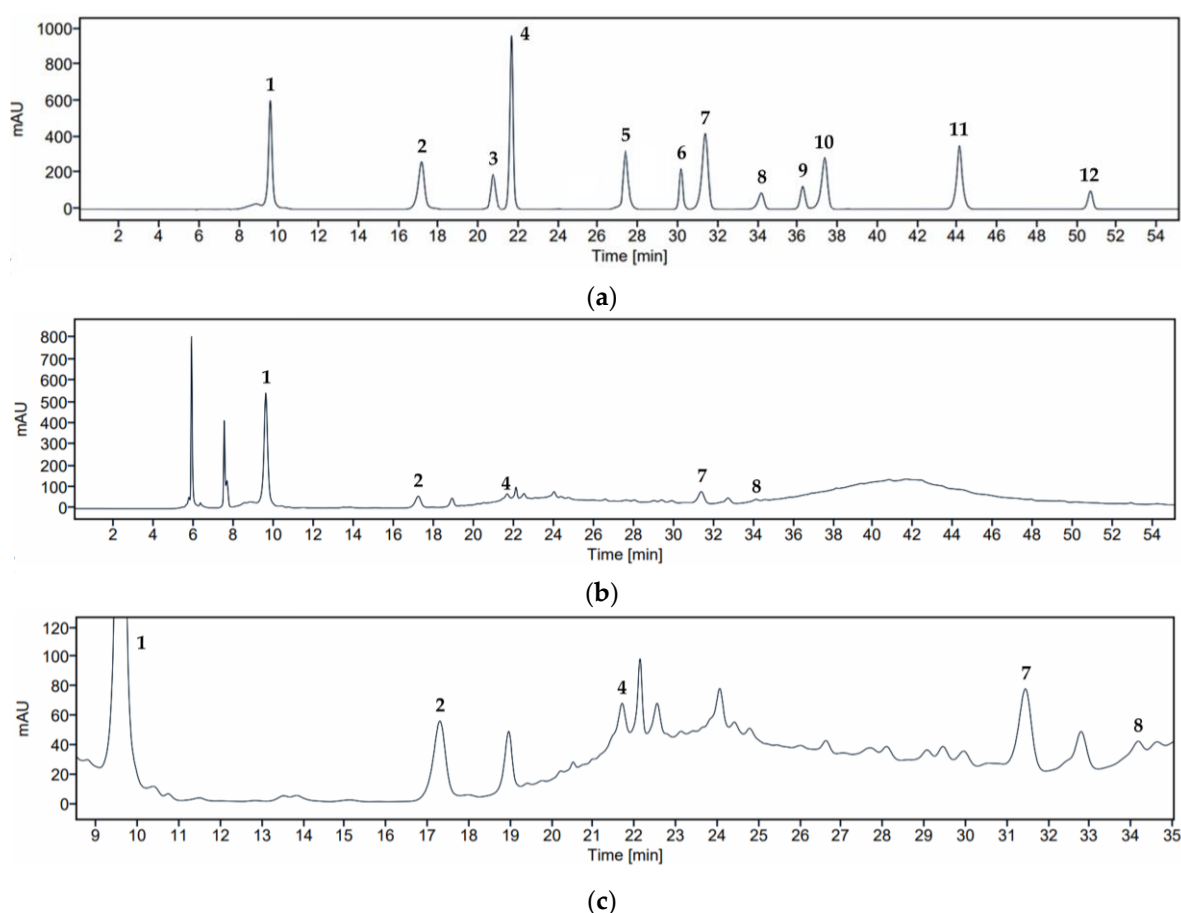


Figure 4. HPLC chromatograms of compounds 1–12 (a), ASE (b), and an expanded view of ASE (c). Compounds: gallic acid (1), protocatechuic acid (2), esculetin (3), protocatechualdehyde (4), epigallocatechin gallate (5), taxifolin (6), *p*-coumaric acid (7), scopoletin (8), vicianin 2 (9), *o*-coumaric acid (10), myricetin (11), and herniarin (12).

This integrated approach supports chemically defined, marker-based reporting of ASE and enhances cross-sample comparability by mitigating polarity-dependent ionization variability and matrix effects associated with mass spectrometric profiling. Furthermore, the quantified marker set provides a practical foundation for subsequent association analyses with antioxidant endpoints and for quality-oriented standardization of ASE using explicitly defined chemical constituents.

Overall, the HPLC results reinforce the chemical baseline established by LC–MS/MS by demonstrating that key phenolic constituents can be reliably tracked as quantitative markers in ASE. This quantitative characterization provides essential chemical context for interpreting the antioxidant potency reported in the subsequent section, while remaining appropriately cautious about attributing activity to any single compound. The phenolic acid-enriched profile of the present ASE, with gallic acid (1) as the predominant marker, differs markedly from phenolic compositions reported for other *Acer* species, underscoring the importance of genus-level contextualization in phytochemical comparisons [47]. Notably, *A. tegmentosum* extracts obtained using conventional aqueous extraction protocols have been reported to contain substantially higher concentrations of catechin and epicatechin derivatives than the phenolic acid-dominant profile observed here, whereas *A. saccharum* (sugar maple), a phylogenetically distinct species within the genus, is characterized primarily by lignans rather than phenolic acids. In *A. saccharum*, lignan-based quantification standards yield phenolic contents approximately threefold higher than gallic acid equivalents [47,48]. These compositional differences highlight that direct quantitative equivalence cannot be assumed across *Acer* species without parallel analyses conducted using identical marker panels. Accordingly, literature-based contextualization of the present data is most appropriately framed at the genus level, focusing on *Acer* species, rather than interpreted as species- and tissue-matched quantitative equivalence [49].

Collectively, Table 3 and Figure 4 illustrate a workflow in which tentative LC–MS/MS annotations are translated into reproducible quantitative indices using orthogonal chromatographic evidence, thereby strengthening cross-sample comparability and enabling marker-based standardization of ASE. The phenolic acid-enriched profile provides an appropriate chemical framework for interpreting subsequent extract-level antioxidant measurements, while maintaining caution against assigning biological activity to individual constituents in the absence of compound-specific bioassays. Given the scarcity of directly comparable quantitative datasets based on identical marker panels for ASE, literature-based contextualization should continue to be approached at the genus level rather than assumed to represent species- or tissue-matched quantitative equivalence.

3.3. Antioxidant Activity

ASE exhibited low IC₅₀ values in both the DPPH and ABTS radical scavenging assays, indicating a strong radical-quenching capacity (Table 4). Although ascorbic acid showed lower IC₅₀ values under the same experimental conditions, reflecting its higher antioxidant potency, ASE consistently demonstrated substantial antioxidant activity across both assays. These results highlight the pronounced antioxidant potential of ASE at the crude extract level.

Germination and early seedling growth involve pronounced metabolic reprogramming, including the activation of phenylpropanoid pathways and enzymatic remodeling processes that expand the pool of extractable phenolics and other redox-active metabolites in many sprout systems [50]. Empirical studies across diverse edible sprouts have consistently reported increases in total phenolic content and radical scavenging capacity during sprouting, although both the magnitude and direction of these changes depend strongly on species, sprouting conditions, and analytical protocols [14]. Within this framework, the

antioxidant activity observed for ASE is consistent with sprouts representing a transient developmental stage characterized by the accumulation of phenolic acids and coumarins associated with redox homeostasis and defense-related metabolism. This provides a reasonable biochemical basis for focusing on sprout tissues when linking chemical markers to antioxidant endpoints [51–54].

Table 4. Antioxidant activities of ASE and compounds 1, 2, 4, 7, and 8.

Sample	DPPH (IC ₅₀ , µg/mL)	ABTS (IC ₅₀ , µg/mL)
ASE	376.40 ± 14.34 ^a	311.00 ± 7.70 ^a
1	51.68 ± 0.97 ^d	31.32 ± 1.28 ^b
2	84.81 ± 1.55 ^c	113.32 ± 1.33 ^d
4	47.17 ± 2.55 ^d	33.65 ± 0.91 ^b
7	>1000	52.45 ± 2.52 ^c
8	>1000	189.09 ± 5.73 ^e
AA *	111.14 ± 1.00 ^b	115.08 ± 1.42 ^d

* Ascorbic acid: positive control. Compounds: gallic acid (1), protocatechuic acid (2), protocatechualdehyde (4), *p*-coumaric acid (7), and scopoletin (8). IC₅₀ values reported as >1000 µg/mL were not reached within the tested concentration range. Data normality was assessed using the Shapiro–Wilk test prior to parametric statistical analyses. Values are presented as means ± SD (*n* = 3). Within each column, different superscript letters (a–e) indicate significant differences (*p* < 0.05) as determined by one-way ANOVA followed by Tukey’s post hoc test.

In other studies, IC₅₀ values reported for DPPH and ABTS assays using crude plant extracts commonly fall within the tens to hundreds of micrograms per milliliter range, with absolute values being highly sensitive to assay conditions and calculation conventions, including radical concentration, reaction time, solvent composition, and matrix effects [55,56]. In this broader context, the IC₅₀ values in the hundreds of micrograms per milliliter observed for ASE can be regarded as comparatively strong. Nevertheless, numerical comparisons across studies should be interpreted cautiously and considered contextual rather than strictly quantitative unless experimental conditions are closely matched [57].

Although both DPPH and ABTS assays are widely employed to assess radical scavenging capacity, they are based on distinct radical systems and reaction environments, which can yield different sensitivities and relative sample ranking [58]. Mechanistically, both assays are predominantly governed by electron-transfer reactions, although hydrogen atom transfer may also contribute depending on the chemical structure of the antioxidant and the reaction medium [59]. The DPPH assay has traditionally been used as a simple, single-radical method, whereas the ABTS decolorization assay was developed to accommodate both hydrophilic and lipophilic antioxidants and has been applied broadly across diverse matrices [31,60]. Comparative evaluations have demonstrated that responses in these assays can diverge substantially depending on sample composition, underscoring the value of employing multiple complementary assays rather than relying on a single metric [61,62]. Accordingly, DPPH and ABTS were selected in the present study as rapid and complementary radical scavenging assays applicable to complex plant extracts across different reaction environments. The consistent observation of strong activity for ASE in both assays therefore enhances confidence in the present interpretation.

Further investigation is warranted beyond the single developmental stage and cultivation condition represented by the present ASE batch. Comparative analyses encompassing sprouts, intermediate growth stages, and mature tissues should be conducted under standardized extraction and assay conditions, as both developmental stage and plant part are known to influence the accumulation and composition of antioxidant constituents [63,64]. Integrating stage-specific IC₅₀ profiles with LC-based chemical profiling and global indices such as total phenolic and total flavonoid contents would help clarify whether the observed

activity is driven primarily by a limited number of high-abundance constituents or by broader compositional shifts within the extract [63].

Among the tested reference compounds, gallic acid (1) and protocatechualdehyde (4) exhibited the strongest radical scavenging responses in both assays, whereas protocatechuic acid (2) showed weaker but clearly measurable activity. These findings support the notion that benzoic acid derivatives bearing multiple hydroxyl substituents can contribute substantially to extract-level radical quenching. In contrast, *p*-coumaric acid (7) and scopoletin (8) did not achieve 50% scavenging in the DPPH assay within the tested concentration range. In the ABTS assay, *p*-coumaric acid (7) retained measurable activity but was less potent than gallic acid (1) and protocatechualdehyde (4), while scopoletin (8) exhibited only weak activity, highlighting pronounced assay-dependent differences in apparent radical scavenging behavior. The higher IC₅₀ values observed for ASE relative to those of isolated standards indicate that the extract-level response reflects the combined effects of constituent abundance, extractability, and matrix interactions, rather than the intrinsic potency of any single compound. In complex mixtures, antagonistic interactions among co-occurring phenolics can lead to non-additive DPPH and ABTS radical scavenging responses, which may partially explain why crude extracts show weaker apparent activity than selected isolated standards [65].

Beyond direct radical scavenging, these marker compounds have been widely discussed for additional bioactivities mechanistically linked to oxidative stress and inflammation, which is relevant when contextualizing the potential functional significance of ASE constituents [66]. Gallic acid (1) has been reviewed as a phenolic acid with antioxidant and anti-inflammatory properties, including hepatoprotective effects in experimental models [67]. Protocatechuic acid (2) and protocatechualdehyde (4) have similarly been described as polyphenol-related metabolites exhibiting antioxidant and anti-inflammatory activities across multiple preclinical contexts [68–70]. *p*-Coumaric acid (7) has been reviewed not only for antioxidant and anti-inflammatory effects but also for tyrosinase-related anti-melanogenic activity, often discussed in relation to cosmetic applications [71]. Scopoletin (8) has been characterized as a bioactive coumarin with antimicrobial, anti-inflammatory, antidiabetic, and hepatoprotective properties in experimental studies [72]. Collectively, these literature-reported activities support the selection of phenolic acids and coumarins as chemically and biologically relevant markers, while the present study appropriately confines causal interpretation to extract-level radical scavenging supported by compositional profiling [73,74].

4. Conclusions

The evaluation of antioxidant activities using individual reference compounds provides additional support for the high antioxidant potential of ASE by supplying compound-level evidence that complements the extract-level radical scavenging results. This strategy enables a more direct assessment of relative activity contributions, strengthens structure–activity interpretation within a unified experimental framework, and facilitates the identification of robust chemical markers suitable for future comparative studies and quality control applications of ASE. The significance of this study lies in its integrated analytical approach, which bridges comprehensive metabolite profiling with practical quantitative standardization in a sprout tissue matrix. In doing so, it establishes a reproducible chemical baseline for ASE and supports its further development as an antioxidant-related functional resource. By integrating LC–MS/MS-based metabolite annotation with retention-based targeted HPLC quantification and extract-level bioactivity evaluation, this work provides a coherent framework for linking chemical composition to antioxidant-related endpoints and for defining analytically traceable markers that enhance cross-sample comparability. Nev-

ertheless, several limitations should be acknowledged. Particularly, the current evidence for antioxidant capacity is primarily derived from crude extract-level assays, and definitive mechanistic attribution to individual constituents remains to be established. In addition, the antioxidant evaluation is restricted to chemical radical scavenging assays, which do not capture factors such as cellular uptake, metabolism, or involvement in broader oxidative stress-related pathways. Finally, because metabolite abundance and apparent bioactivity may vary with developmental stage, cultivation conditions, and batch-to-batch variability, further validation across expanded biological replicates and growth stages will be necessary to confirm the generalizability of these findings and to strengthen the proposed marker set for future standardization efforts.

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References

1. Manful, C.F.; Fordjour, E.; Subramaniam, D.; Sey, A.A.; Abbey, L.; Thomas, R. Antioxidants and reactive oxygen species: Shaping human health and disease outcomes. *Int. J. Mol. Sci.* **2025**, *26*, 7520. [CrossRef]
2. Sies, H. Oxidative stress: Concept and some practical aspects. *Antioxidants* **2020**, *9*, 852. [CrossRef]
3. Ye, Z.W.; Zhang, J.; Townsend, D.M.; Tew, K.D. Oxidative stress, redox regulation and diseases of cellular differentiation. *Biochim. Biophys. Acta* **2014**, *1850*, 1607. [CrossRef] [PubMed]
4. Mendonça, J.d.S.; Guimarães, R.d.C.A.; Zorretto-Pinheiro, V.A.; Fernandes, C.D.P.; Marcelino, G.; Bogo, D.; Freitas, K.d.C.; Hiane, P.A.; Melo, E.S.d.P.; Vilela, M.L.B.; et al. Natural antioxidant evaluation: A review of detection methods. *Molecules* **2022**, *27*, 3563. [CrossRef]
5. Platzer, M.; Kiese, S.; Tybussek, T.; Herfellner, T.; Schneider, F.; Schweiggert-Weisz, U.; Eisner, P. Radical scavenging mechanisms of phenolic compounds: A quantitative structure-property relationship (QSPR) study. *Front. Nutr.* **2022**, *1*, 882458. [CrossRef]
6. Chen, J.; Yang, J.; Ma, L.; Li, J.; Shahzad, N.; Kim, C.K. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Sci. Rep.* **2020**, *10*, 2611. [CrossRef] [PubMed]
7. Todorov, L.; Saso, L.; Kostova, I. Antioxidant activity of coumarins and their metal complexes. *Pharmaceuticals* **2023**, *16*, 651. [CrossRef] [PubMed]
8. Cai, Y.; Luo, Q.; Sun, M.; Corke, H. Antioxidant activity and phenolic compounds of 112 traditional chinese medicinal plants associated with anticancer. *Life Sci.* **2004**, *74*, 2157. [CrossRef]
9. Doan, T.T.M.; Tran, G.H.; Nguyen, T.K.; Lim, J.H.; Lee, S. Antioxidant activity of different cultivars of *Chrysanthemum morifolium* and quantitative analysis of phenolic compounds by HPLC/UV. *Appl. Biol. Chem.* **2024**, *67*, 17. [CrossRef]
10. Park, Y.J.; Choi, Y.B.; Oh, S.B.; Moon, J.; Truong, T.Q.; Huynh, P.K.; Kim, S.M. Development and application of a high-performance liquid chromatography diode-array detection (HPLC–DAD) method for the simultaneous quantification of phenolic compounds in the aerial part of *Glehnia littoralis*. *Appl. Biol. Chem.* **2024**, *67*, 34. [CrossRef]
11. Kim, J.A.; Min, B.S.; Ha, M.T.; Le, T.T.; Tran, T.T.; Tran, T.H. *In vitro* and *in silico* analysis of PTP1B and α -glucosidase inhibitory activities of isoflavonoids isolated from *Belamcanda chinensis* roots. *Nat. Prod. Sci.* **2025**, *31*, 129–137.
12. Tarzi, B.G.; Gharachorloo, M.; Baharinia, M.; Mortazavi, A. The effect of germination on phenolic content and antioxidant activity of chickpea. *Iran. J. Pharm. Res.* **2012**, *11*, 1137.
13. Pająk, P.; Socha, R.; Gałkowska, D.; Rożnowski, J.; Fortuna, T. Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chem.* **2014**, *143*, 300–306. [CrossRef]

14. Liu, H.Y.; Liu, Y.; Li, M.Y.; Ge, Y.Y.; Geng, F.; He, X.Q.; Xia, Y.; Guo, B.L.; Gan, R.Y. Antioxidant capacity, phytochemical profiles, and phenolic metabolomics of selected edible seeds and their sprouts. *Front. Nutr.* **2022**, *9*, 1067597. [\[CrossRef\]](#)
15. Melegova, D.; Babelova, A.; Selc, M. Therapeutic potential of medicinal plant sprouts: Emerging opportunities and challenges in phytochemistry. *Planta* **2025**, *262*, 146. [\[CrossRef\]](#)
16. Lee, C.-D.; Lee, H.-D.; Lee, A.Y.; Jeong, M.-J.; Choi, K.; Kang, J.; Park, Y.-W.; Choi, D.; Lee, S. Tissue-specific accumulation of anti-inflammatory flavonoids in *Rhododendron micranthum* leaves. *J. Food Compos. Anal.* **2026**, *149*, 108714. [\[CrossRef\]](#)
17. Nugroho, A.; Song, B.M.; Park, H.J. HPLC and GC-MS analysis of phenolic substances in *Acer tegmentosum*. *Nat. Prod. Sci.* **2015**, *21*, 87–92.
18. Kim, S.; Ko, C.H.; Kwon, H.C.; Rhie, Y.H.; Lee, S.Y. Effect of temperature and covering structures in seed dormancy and germination traits of manchurian striped maple (*Acer tegmentosum* Maxim.) native to Northeast Asia. *Plants* **2025**, *14*, 767. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Park, H.S.; Jo, E.; Han, J.H.; Jung, S.H.; Lee, D.H.; Park, I.W.; Heo, K.S.; Na, M.K.; Myung, C.S. Hepatoprotective effects of an *Acer tegmentosum* Maxim. extract through antioxidant activity and the regulation of autophagy. *J. Ethnopharmacol.* **2019**, *239*, 111912. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Park, J.S.; Kwon, E.; Kim, Y.S.; Kim, S.M.; Kim, D.S.; Jang, J.J.; Yun, J.W.; Kang, B.C. Safety assessment of *Acer tegmentosum* Maxim. water extract: General toxicity studies in Sprague–Dawley rats and Beagle dogs with re-evaluation of genotoxic potentials. *Front. Pharmacol.* **2021**, *12*, 687261. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Nguyen, P.A.; Won, J.S.; Cho, M.K. *Acer tegmentosum* Maxim and *Bacillus Subtilis*-Fermented products inhibit TNF- α -induced endothelial inflammation and vascular dysfunction of the retina: The role of tyrosol moiety in active compounds targeting Glu²³⁰ in SIRT1. *Front. Pharmacol.* **2024**, *15*, 1392179. [\[CrossRef\]](#)
22. Lee, D.H.; Park, Y.; Hong, S.S.; Park, G.H.; Kim, H.-J. Study on the of the correlation between soil chemical properties and bioactive compounds of *Acer tegmentosum* Maxim. *Korean J. Plant Resour.* **2021**, *34*, 566–574.
23. Jin, J.; Yin, X.; Wu, N.; Sun, C.; Zhao, M.; Wang, S.; Jiang, Q.; Jin, L.; Zhang, C. Chemical constituents of *Acer tegmentosum* Maxim and their chemotaxonomic significance. *Biochem. Syst. Ecol.* **2024**, *116*, 104882. [\[CrossRef\]](#)
24. Ki, M.P.; Min, C.Y.; Kyu, H.L.; Kyung, R.K.; Sang, U.C.; Kang, R.L. Cytotoxic phenolic constituents of *Acer tegmentosum* Maxim. *Arch. Pharmacol. Res.* **2006**, *29*, 1086–1090. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Plitta-Michalak, B.P.; Ramos, A.A.; Trusiak, M.; Ratajczak, E.; Michalak, M. Impact of cryopreservation on DNA damage in *Acer platanoides* L. seeds evaluated by the comet assay. *Sci. Rep.* **2025**, *15*, 24081. [\[CrossRef\]](#)
26. Sultana, B.; Anwar, F.; Ashraf, M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* **2009**, *14*, 2167–2180. [\[CrossRef\]](#)
27. Keunchkarian, S.; Reta, M.; Romero, L.; Castells, C. Effect of sample solvent on the chromatographic peak shape of analytes eluted under reversed-phase liquid chromatographic conditions. *J. Chromatogr. A* **2006**, *1119*, 20–28. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Apostol, I.; Miller, K.J.; Ratto, J.; Kelner, D.N. Comparison of different approaches for evaluation of the detection and quantitation limits of a purity method: A case study using a capillary isoelectrofocusing method for a monoclonal antibody. *Anal. Biochem.* **2009**, *385*, 101–106. [\[CrossRef\]](#)
29. Phong, N.V.; Lee, Y.M.; Min, B.S.; Kim, J.A. Development and validation of an HPLC-DAD method for simultaneous quantitation of steppogenin and flavonoids from the stems of *Morus Alba*. *Nat. Prod. Sci.* **2024**, *30*, 65–71. [\[CrossRef\]](#)
30. Kedare, S.B.; Singh, R.P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* **2011**, *48*, 412. [\[CrossRef\]](#)
31. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [\[CrossRef\]](#)
32. Hoffmann, M.A.; Nothias, L.-F.; Ludwig, M.; Fleischauer, M.; Gentry, E.C.; Witting, M.; Dorrestein, P.C.; Dührkop, K.; Böcker, S. Assigning confidence to structural annotations from mass spectra with COSMIC. *bioRxiv* **2021**. [\[CrossRef\]](#)
33. Broeckling, C.D.; Ganna, A.; Layer, M.; Brown, K.; Sutton, B.; Ingelsson, E.; Peers, G.; Prenni, J.E. Enabling efficient and confident annotation of LC–MS metabolomics data through MS1 spectrum and time prediction. *Anal. Chem.* **2016**, *88*, 9226–9234. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Houriet, J.; Manwill, P.K.; Magaña, A.A.; Anderson, V.M.; Benididir, M.A.; Bertrand, S.; Choi, J.; Clark, T.N.; Foster, L.J.; Halabalaki, M.; et al. Multilaboratory untargeted mass spectrometry metabolomics collaboration to identify bottlenecks and comprehensively annotate a single dataset. *Anal. Chem.* **2025**, *97*, 16110–16122. [\[CrossRef\]](#)
35. Torres-Ortiz, D.; García-Alcocer, G.; Berumen-Segura, L.C.; Estévez, M. Green extraction of secondary metabolites from plants: Obstacles, current status, and trends. *Sustain. Chem. Environ.* **2024**, *8*, 100157. [\[CrossRef\]](#)
36. Atwi-Ghaddar, S.; Zerwette, L.; Destandau, E.; Lesellier, E. Supercritical fluid extraction (SFE) of polar compounds from *Camellia sinensis* leaves: Use of ethanol/water as a green polarity modifier. *Molecules* **2023**, *28*, 5485. [\[CrossRef\]](#) [\[PubMed\]](#)

37. Sinosaki, N.B.M.; Tonin, A.P.P.; Ribeiro, M.A.S.; Polisel, C.B.; Roberto, S.B.; Da Silveira, R.; Visentainer, J.V.; Santos, O.O.; Meurer, E.C. Structural study of phenolic acids by triple quadrupole mass spectrometry with electrospray ionization in negative mode and H/D isotopic exchange. *Artic. J. Braz. Chem. Soc.* **2020**, *31*, 402–408. [\[CrossRef\]](#)
38. Rojas-Chertó, M.; Kasper, P.T.; Willighagen, E.L.; Vreeken, R.J.; Hankemeier, T.; Reijmers, T.H. Elemental composition determination based on MSn. *Bioinformatics* **2011**, *27*, 2376–2383. [\[CrossRef\]](#)
39. van Tetering, L.; Spies, S.; Wildeman, Q.D.K.; Houthuijs, K.J.; van Outersterp, R.E.; Martens, J.; Wevers, R.A.; Wishart, D.S.; Berden, G.; Oomens, J. A spectroscopic test suggests that fragment ion structure annotations in MS/MS libraries are frequently incorrect. *Commun. Chem.* **2024**, *7*, 30. [\[CrossRef\]](#)
40. Phillips, A.L.; Williams, A.J.; Sobus, J.R.; Ulrich, E.M.; Gundersen, J.; Langlois-Miller, C.; Newton, S.R. A framework for utilizing high resolution mass spectrometry and non-targeted analysis (NTA) in rapid response and emergency situations. *Environ. Toxicol. Chem.* **2022**, *41*, 1117–1130. [\[CrossRef\]](#)
41. Li, C.; Attanayake, K.; Valentine, S.J.; Li, P. Facile improvement of negative ion mode electrospray ionization using capillary vibrating sharp-edge spray ionization. *Anal. Chem.* **2020**, *92*, 2492. [\[CrossRef\]](#)
42. McClory, P.J.; Håkansson, K. Corona discharge suppression in negative ion mode nanoelectrospray ionization via trifluoroethanol addition. *Anal. Chem.* **2017**, *89*, 10188. [\[CrossRef\]](#)
43. Inglese, P.; Huang, H.X.; Wu, V.; Lewis, M.R.; Takats, Z. Mass recalibration for desorption electrospray ionization mass spectrometry imaging using endogenous reference ions. *BMC Bioinform.* **2022**, *23*, 133. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Kind, T.; Fiehn, O. Metabolomic database annotations via query of elemental compositions: Mass accuracy is insufficient even at less than 1 ppm. *BMC Bioinform.* **2006**, *7*, 234. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Koch, B.P.; Dittmar, T.; Hertkorn, N.; Benner, R.; Frommberger, M.; Schmitt-Kopplin, P.; Witt, M.; Kaiser, K.; Kettrup, A.; Hedges, J.I.; et al. Fundamentals of molecular formula assignment to ultrahigh resolution mass data of natural organic matter. *Anal. Chem.* **2007**, *79*, 1758–1763. [\[CrossRef\]](#)
46. Neeraj, J.V.; Rajendra, U.K. Novel HPLC method for determination of polyphenols in AGRO-industrial waste of date fruits. *Int. J. Adv. Res.* **2020**, *8*, 626–634. [\[CrossRef\]](#)
47. Liu, Y.; Ma, H.; Seeram, N.P. Development and UFLC-MS/MS characterization of a product-specific standard for phenolic quantification of maple-derived foods. *J. Agric. Food Chem.* **2016**, *64*, 3311–3317. [\[CrossRef\]](#)
48. Park, S.Y.; Han, Y.K.; Vinh, L.B.; Lee, K.Y. Analysis of α -glucosidase inhibitory constituents from *Acer tegmentosum* using LC-QTOF MS/MS and molecular networking. *Nat. Prod. Sci.* **2023**, *29*, 242–250. [\[CrossRef\]](#)
49. Ren, H.; Feng, Z. Comparison and correlation analysis of flavonoids and chlorogenic acid contents in different strains of *Acer truncatum*. *Food Sci. Technol.* **2018**, *38*, 319–326. [\[CrossRef\]](#)
50. Zhang, Y.; Xia, Y. Quercetin enhances tomato seed germination via phenylpropanoid-dependent regulation of ROS, hormone signaling, and starch hydrolysis. *Plant Physiol. Biochem.* **2026**, *230*, 110590. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Gao, H.; Li, Q. The prediction of antioxidant Q-markers for *Angelica dahurica* based on the dynamics change in chemical compositions and network pharmacology. *Molecules* **2023**, *28*, 5248. [\[CrossRef\]](#)
52. González, M.C.; Roitsch, T.; Pandey, C. Antioxidant responses and redox regulation within plant-beneficial microbe interaction. *Antioxidants* **2024**, *13*, 1553. [\[CrossRef\]](#)
53. Parvin, K.; Hasanuzzaman, M.; Mohsin, S.M.; Nahar, K.; Fujita, M. Coumarin improves tomato plant tolerance to salinity by enhancing antioxidant defence, glyoxalase system and ion homeostasis. *Plant Biol.* **2021**, *23*, 181–192. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Wang, Y.; Guan, T.; Yue, X.; Yang, J.; Zhao, X.; Chang, A.; Yang, C.; Fan, Z.; Liu, K.; Li, Y. The biosynthetic pathway of coumarin and its genetic regulation in response to biotic and abiotic stresses. *Front. Plant Sci.* **2025**, *16*, 1599591. [\[CrossRef\]](#)
55. Wołoskiak, R.; Drużyńska, B.; Derewiaka, D.; Piecyk, M.; Majewska, E.; Ciecierska, M.; Worobiej, E.; Pakosz, P. Verification of the conditions for determination of antioxidant activity by ABTS and DPPH assays—A practical approach. *Molecules* **2021**, *27*, 50. [\[CrossRef\]](#)
56. Kalembe, M.R.K.; Makhuvele, R.; Njobeh, P.B. Phytochemical screening, antioxidant activity of selected methanolic plant extracts and their detoxification capabilities against AFB1 toxicity. *Heliyon* **2024**, *10*, e24435. [\[CrossRef\]](#)
57. Menezes, B.d.B.; Frescura, L.M.; Duarte, R.; Villetti, M.A.; da Rosa, M.B. A critical examination of the DPPH method: Mistakes and inconsistencies in stoichiometry and IC₅₀ determination by UV–Vis spectroscopy. *Anal. Chim. Acta* **2021**, *1157*, 338398. [\[CrossRef\]](#)
58. Platzer, M.; Kiese, S.; Herfellner, T.; Schweiggert-Weisz, U.; Miesbauer, O.; Eisner, P. Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. *Molecules* **2021**, *26*, 1244. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Apak, R.; Özyürek, M.; Güçlü, K.; Çapanoğlu, E. Antioxidant Activity/Capacity Measurement. 1. Classification, Physicochemical Principles, Mechanisms, and Electron Transfer (ET)-Based Assays. *J. Agric. Food Chem.* **2016**, *64*, 997–1027. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Food Sci. Technol.* **1995**, *28*, 25–30. [\[CrossRef\]](#)

61. Floegel, A.; Kim, D.O.; Chung, S.J.; Koo, S.I.; Chun, O.K. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food Compos. Anal.* **2011**, *24*, 1043–1048. [\[CrossRef\]](#)
62. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Hawkins Byrne, D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.* **2006**, *19*, 669–675. [\[CrossRef\]](#)
63. Jiang, X.; Liu, Y.; Li, W.; Zhao, L.; Meng, F.; Wang, Y.; Tan, H.; Yang, H.; Wei, C.; Wan, X.; et al. Tissue-specific, development-dependent phenolic compounds accumulation profile and gene expression pattern in tea plant [*Camellia sinensis*]. *PLoS ONE* **2013**, *8*, e62315. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Drozdowska, M.; Leszczyńska, T.; Koronowicz, A.; Piasna-Słupecka, E.; Dziadek, K. Comparative study of young shoots and the mature red headed cabbage as antioxidant food resources with antiproliferative effect on prostate cancer cells. *RSC Adv.* **2020**, *10*, 43021–43034. [\[CrossRef\]](#)
65. Olszowy-Tomczyk, M.; Wianowska, D. Antioxidant properties of selected flavonoids in binary mixtures—Considerations on myricetin, kaempferol and quercetin. *Int. J. Mol. Sci.* **2023**, *24*, 10070. [\[CrossRef\]](#)
66. Fakhri, S.; Abbaszadeh, F.; Moradi, S.Z.; Cao, H.; Khan, H.; Xiao, J. Effects of polyphenols on oxidative stress, inflammation, and interconnected pathways during spinal cord injury. *Oxid. Med. Cell. Longev.* **2022**, *2022*, 8100195. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Ojeaburu, S.I.; Oriakhi, K. Hepatoprotective, antioxidant and, anti-inflammatory potentials of gallic acid in carbon tetrachloride-induced hepatic damage in Wistar rats. *Toxicol. Rep.* **2021**, *8*, 177. [\[CrossRef\]](#) [\[PubMed\]](#)
68. Chang, Z.Q.; Gebru, E.; Lee, S.P.; Rhee, M.H.; Kim, J.C.; Cheng, H.; Park, S.C. *In vitro* antioxidant and anti-inflammatory activities of protocatechualdehyde isolated from *Phellinus gilvus*. *J. Nutr. Sci. Vitaminol.* **2011**, *57*, 118–122. [\[CrossRef\]](#)
69. Zhang, S.; Gai, Z.; Gui, T.; Chen, J.; Chen, Q.; Li, Y. Antioxidant effects of protocatechuic acid and protocatechuic aldehyde: Old wine in a new bottle. *Evid.-Based Complement. Altern. Med.* **2021**, *2021*, 6139308. [\[CrossRef\]](#)
70. Chook, C.Y.B.; Cheung, Y.M.; Ma, K.Y.; Leung, F.P.; Zhu, H.; Niu, Q.J.; Wong, W.T.; Chen, Z.Y. Physiological concentration of protocatechuic acid directly protects vascular endothelial function against inflammation in diabetes through Akt/ENOS pathway. *Front. Nutr.* **2023**, *10*, 1060226. [\[CrossRef\]](#)
71. Boo, Y.C. *p*-Coumaric acid as an active ingredient in cosmetics: A review focusing on its antimelanogenic effects. *Antioxidants* **2019**, *8*, 275. [\[CrossRef\]](#) [\[PubMed\]](#)
72. Gao, X.Y.; Li, X.Y.; Zhang, C.Y.; Bai, C.Y. Scopoletin: A review of its pharmacology, pharmacokinetics, and toxicity. *Front. Pharmacol.* **2024**, *15*, 1268464. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Mohsen Ali, S.S.; Robin, P. Comprehensive phytochemical profiling of bioactive compounds from *Barleria prattensis* for their antioxidant and cytotoxic capacity and its characterization using GC-MS. *Biochem. Biophys. Rep.* **2025**, *43*, 102083. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Cao, X.; Yang, L.; Xue, Q.; Yao, F.; Sun, J.; Yang, F.; Liu, Y. Antioxidant evaluation-guided chemical profiling and structure-activity analysis of leaf extracts from five trees in *Broussonetia* and *Morus* (Moraceae). *Sci. Rep.* **2020**, *10*, 4808. [\[CrossRef\]](#)

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