

Article

Phytochemical Profiling and Antioxidant Activity of True Leaves and Cotyledons of *Adenocaulon himalaicum*

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Abstract: *Adenocaulon himalaicum* is widely distributed across Asia. In its early growth stages, *A. himalaicum* is traditionally consumed as a food source in Korea. Although previous research has identified the presence of bioactive compounds in *A. himalaicum* extract, suggesting its potential as a medicinal resource, the phytochemical profile of *A. himalaicum* extract has not been extensively determined. This investigation aimed to identify the phytochemicals present in the true leaf and cotyledon of *A. himalaicum* (TLA and CLA, respectively) and evaluate their radical-scavenging activity. By performing LC-MS/MS and HPLC, varying amounts of isochlorogenic acid A, cryptochlorogenic acid, isochlorogenic acid B, rutin, chlorogenic acid, hyperin, and neochlorogenic acid were detected in the TLA and CLA extracts. Chlorogenic acid (9.002 mg/g DW), isochlorogenic acid A (28.512 mg/g DW), and isochlorogenic acid B (12.223 mg/g DW) were the most abundant in TLA. TLA exhibited higher phytochemical content (49.737 mg/g DW), total phenolic content (45.51 mg tannic acid equivalent/g extract), and total flavonoid content (16.24 mg quercetin equivalent/g extract) than CLA. Moreover, the radical-scavenging activity of TLA was two times higher than that of CLA. The young leaf of *A. himalaicum* has a rich phytochemical profile and robust antioxidant activity; hence, it has potential as natural antioxidant sources for human health and valuable pharmacognosy raw materials for pharmaceutical and functional food applications.

Keywords: *Adenocaulon himalaicum*; antioxidants; LC-MS/MS; HPLC



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1. Introduction

Adenocaulon himalaicum Edgew. is classified as a long-lived herbaceous plant (the Asteraceae family). It typically grows in moist and shady environments in forests and mountainous regions and is commonly referred to as the Asian trail plant. The species is widely distributed across Central Asia, East Asia, and Russia. Due to its strong environmental adaptability, *A. himalaicum* shows minimal differences in antioxidant activity between invasive and native populations, suggesting its potential for commercial cultivation and broader utilization [1].

Although *A. himalaicum* is a commonly found plant, its bioactive properties have not been extensively studied. Previous studies have demonstrated that *A. himalaicum* can mitigate skin damage and delay aging by suppressing inflammatory responses [2]. Furthermore, *A. himalaicum* has been reported to exhibit immunostimulatory and anti-obesity effects, contributing to immune system enhancement and metabolic disorder management [3]. Additionally, *A. himalaicum* has been studied for its anticancer properties [4].

However, these studies have primarily focused on whole-plant crude extracts, and a systematic investigation regarding the chemical constituents and antioxidative properties of its edible leaf remains unexplored. Notably, the early-stage leaves of *A. himalaicum* have traditionally been consumed as food sources in Korea [2]. Given this historical use, it is essential to characterize the specific bioactive compounds and evaluate the antioxidant capacity of its edible leaf.

This investigation aims to address this research gap by qualitatively and quantitatively analyzing key phytochemicals in the true leaf and cotyledon of *A. himalaicum* (TLA and CLA, respectively). Furthermore, we analyzed their radical-scavenging activity as an indicator of antioxidant potential. These findings will enhance the understanding of *A. himalaicum*'s potential as a natural antioxidant source for health-promoting applications and as a valuable pharmacognosy raw materials for pharmaceutical and functional food applications.

2. Materials and Methods

2.1. Plant Materials

The *A. himalaicum* seeds were acquired from Daehak mountain (37°40'50.26" N; 128°5'27.83" E), Hongcheon, Republic of Korea (October 2022). To break seed dormancy, the seeds were subjected to cold stratification at 3 °C for 3 weeks. The seeds were sown on the field of the Gyeonggi-do Forestry Environment Research Center (early April 2023), Osan, Republic of Korea, and the resulting seedlings of TLA and CLA were analyzed (Figure 1). The cultivation climate conditions of sowing site were as follows: an average temperature of 25.65 °C, an average humidity of 88.86%, and an average soil moisture content of 31.65%.



Figure 1. TLA (a) and CLA (b) samples used in this study. The CLA represents the cotyledons, the initial leaf that develops during the germination process. In contrast, the TLA signifies the first true leaf to emerge after the cotyledon. The scale illustrates the difference in size between the two leaf types.

2.2. Apparatus and Chemicals

Quantitative analysis was performed on an HPLC (Waters Alliance 2695, Milford, MA, USA) with a PDA detector (Waters 996, Milford, MA, USA), a pump, and an auto-sampler with an INNO C₁₈ column (25 cm × 4.6 mm; pore size 5 µm) (YMC, Kyoto, Japan). LC–MS/MS analysis was conducted using an UHPLC system (Ultimate 3000, Thermo Scientific, San Jose, CA, USA) and a high-resolution mass spectrometer (Triple TOF 5600⁺, AB Sciex, Framingham, MA, USA). Organic solvent was evaporated by a vacuum rotary evaporator (OSB-2100, Eyela, Tokyo, Japan). HPLC solvents including MeOH, trifluoroacetic acid (TFA), and water were procured from J. T. Baker (Center Valley, PA, USA). The Folin–Ciocalteu reagent, tannic acid, quercetin, and AlCl₃·6H₂O were purchased from Sigma-Aldrich (St. Louis, MO, USA). The absorbance was conducted by using a microplate reader (Epoch; Bio Tek, Winooski, VT, USA). Neochlorogenic acid (1),

chlorogenic acid (2), cryptochlorogenic acid (3), rutin (4), hyperin (5), isochlorogenic acid A (6), and isochlorogenic acid B (7) were supplied from the Natural Product Institute of Science and Technology (www.nist.re.kr; accessed on 6 June 2024), Anseong, Republic of Korea (Figure 2).

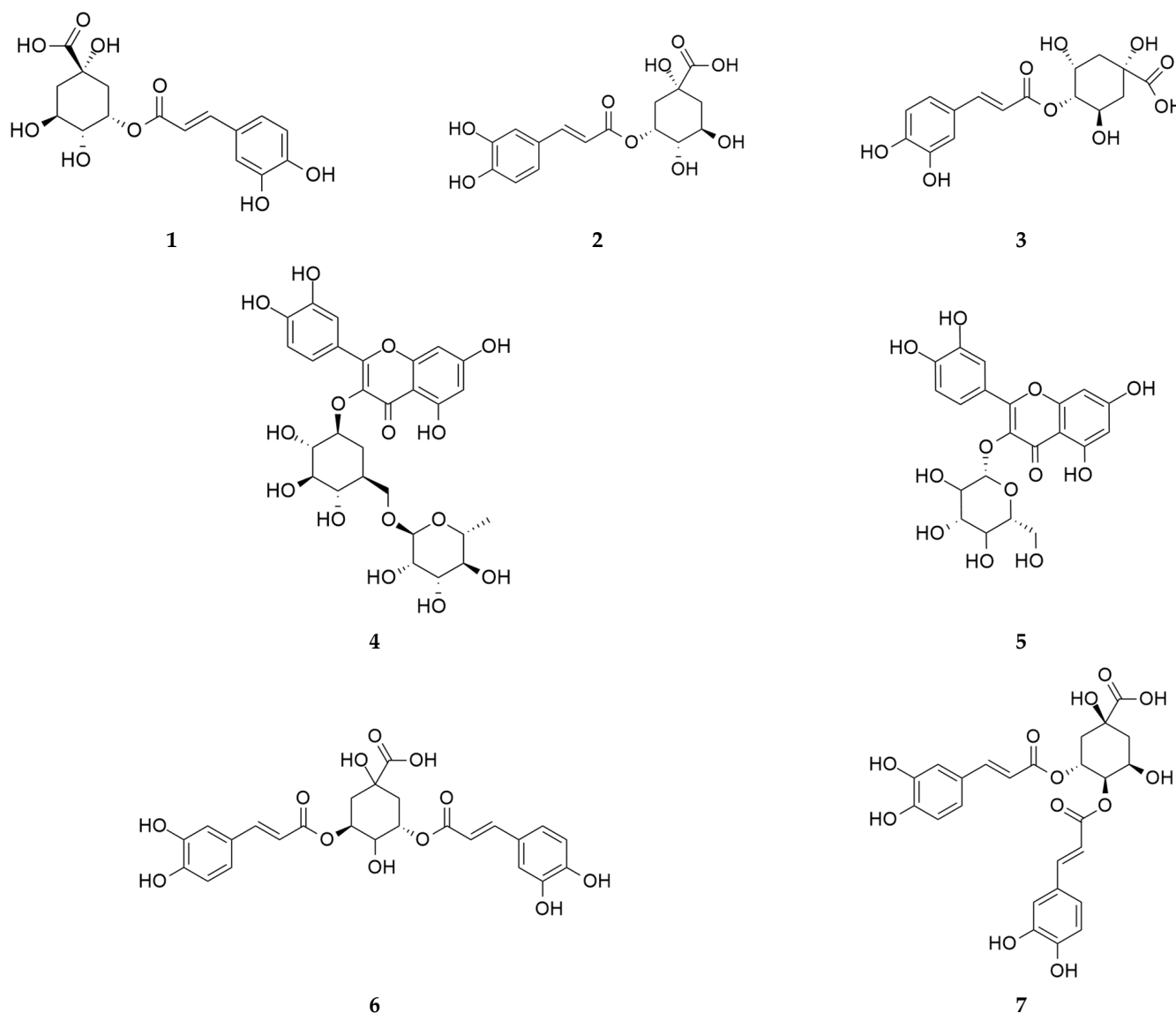


Figure 2. Chemical structures of compounds 1–7.

2.3. Sample Extraction

Dried TLA (10 g) and CLA (4 g) samples were finely crushed and refluxed with 30 volumes of EtOH for 3 h thrice. The EtOH extracts were concentrated by using a vacuum rotary evaporator. Subsequently, the extraction yield was calculated. The extraction method was performed according to a modified method from previous studies [5,6].

2.4. LC–MS/MS States

Chromatographic separation was performed utilizing an LC system that included a UHPLC and Waters Cortex C18 column (1.6 μ m; 2.1 mm $\times$ 150 mm) kept at 45 $^{\circ}$ C. The flow rate was fixed at 0.25 mL/min. The mobile phase employed for gradient elution consisted of formic acid (0.1%) in water (A) and formic acid (0.1%) in acetonitrile (ACN) (B). The conditions of elution were set as follows: 3% B from 0 to 15 min, 15% B at 15 min, 100%

B at 50 min, 100% B from 50 to 55 min, and 3% B again from 55.1 to 60 min. MS analysis was conducted using a Triple TOF⁺ mass spectrometer, which was equipped with an electrospray ion source operated under negative and positive ionization conditions. A full-scan MS spectrum (100–2000 m/z) and an MS/MS scan (30–2000 m/z) were acquired using Information-Dependent Acquisition scanning. The nebulizing and heating gases were set to 50 psi, and the curtain gas was maintained at 25 psi. The desolvation temperature was adjusted at 500 °C. The collision gas used in this study was nitrogen.

2.5. Sample Preparation and Standard Solutions for HPLC

TLA and CLA extracts (each 30 mg) were melted in 80% MeOH (4 mL). Compounds 1–7 (each 1 mg) were melted in 80% MeOH (1 mL). Afterwards, the samples were sonicated for 30 min and then filtered through a 0.20 µm membrane filter of PVDF. This procedure was adapted from a previously reported method with slight modifications [7].

2.6. HPLC/PDA Conditions

HPLC/PDA analysis using an RP HPLC system with an INNO C₁₈ column kept at 35 °C was performed on TLA and CLA samples. The mobile phase employed in gradient elution consisted of 0.1% TFA in water (A) and ACN (B). The elution states were set as follows: 10% B from 0 to 18 min, 20% B at 18 min, 50% B at 28 min, 100% B from 38 to 48 min, and 10% B from 53 to 63 min. The column temperature was kept at 35 °C. The flow rate, injection volume, and wavelength were set at 1.0 mL/min, 10 µL, and 254 nm, respectively.

2.7. Calibration Curves

The calibration curve was established using standard stock solutions prepared through serial dilution, with concentrations ranging from 31.25 to 1000 ppm. The calibration function was defined using parameters such as the peak area (Y, mAU), concentration (X, µg/mL), and mean value ($n = 3$) ± SD. Additionally, linearity was evaluated by determining the correlation coefficient (r^2), and the amounts of compounds 2, 6, and 7 in the extracts were quantified accordingly.

2.8. TPC Assay

The TPC was determined using the Folin–Ciocalteu method, following a previously described procedure [8]. The stock solutions of TLA and CLA samples were prepared through serial dilution, with concentrations ranging from 0.125 to 2.0 mg/mL. In a 96-well plate, 60 µL of each diluted sample was first added, followed by 40 µL of Folin–Ciocalteu (2N) agent, and then 100 µL of sodium carbonate (7.5%). This process was repeated thrice to ensure the accuracy and consistency of the experiment. The mixtures were mixed properly by a microplate shaker and allowed to react in the dark at room temperature for 30 min. After the reaction was completed, the absorbance of the samples was measured at 760 nm using a microplate reader. The standard solutions of tannic acid were prepared at concentrations of 100.0, 50.0, 25.0, 12.5, and 6.25 ppm.

2.9. TFC Assay

The TFC was assessed through an aluminum chloride colorimetric assay, as described in a previous study [8]. The stock solutions of TLA and CLA samples were prepared through serial dilution, with concentrations ranging from 0.125 to 2.0 mg/mL. First, 100 µL of each diluted sample was added to a 96-well plate. Next, 100 µL of AlCl₃·6H₂O was added. This process was repeated thrice to ensure the accuracy and consistency of the experiment. The mixtures were mixed properly by using a microplate shaker and allowed to react for 10 min in the dark at room temperature. Following the completion of the

reaction, sample absorbance was determined at 430 nm using a microplate reader. The standard solutions of quercetin were prepared at concentrations of 100.0, 50.0, 25.0, 12.5, and 6.25 ppm.

2.10. DPPH Assay

At the beginning of the assay, DPPH stock solution (2 mM) dissolved in MeOH was diluted to make a working solution. The DPPH working solution was diluted until it reached an absorbance of 0.8 at 514 nm. The stock solutions of the samples were serially diluted in various concentrations. First, 10 μ L of each diluted sample was added to a 96-well plate, and 200 μ L of the DPPH working solution was added to each sample. This process was repeated thrice to ensure the accuracy and consistency of the experiment. The mixtures were mixed properly by using a microplate shaker and allowed to react for 30 min at room temperature in the dark. Following the completion of the reaction, sample absorbance was determined at 514 nm using a microplate reader. Ascorbic acid (AA) was used as the standard. In the case of the blank test, 95% MeOH was used instead of the DPPH working solution.

2.11. ABTS⁺ Assay

At the beginning of the assay, the ABTS⁺ stock solution was made by dissolving ABTS⁺ (7.4 mM) and K₂S₂O₈ (2.6 mM) in deionized water. The ABTS⁺ stock solution was diluted until it reached an absorbance of 1.0 at 734 nm. The stock solutions of the samples were serially diluted in various concentrations. First, 10 μ L of each diluted sample was added to a 96-well plate, and 200 μ L of the ABTS⁺ working solution was added to each sample. This process was repeated thrice to make sure of the accuracy and consistency of the experiment. The mixtures were mixed properly by a microplate shaker and allowed to react for 30 min at room temperature in the dark. Following the completion of the reaction, sample absorbance was determined at 734 nm using a microplate reader. AA was used as the standard. For the blank test, deionized water was used instead of the ABTS⁺ working solution.

2.12. Statistical Analysis

All experiments were performed in triplicate to reduce statistical errors and increase the reproducibility and reliability of the results. The results were expressed as the mean $\pm$ SD. The data were analyzed by Student's *t*-test and one-way analysis of variance, followed by Tukey's post hoc test. The normality of the data was tested prior to employing the statistical tests described above. The statistical tests were conducted in Graphpad Prism 8.0.2 (Graphpad Software, Boston, MA, USA). A *p*-value of less than 0.05 was regarded as an indicator of statistical significance, suggesting that the observed differences were unlikely to have occurred by chance.

3. Results and Discussion

3.1. Sample Extraction Yield

The extraction yields of 95% EtOH extracts derived from TLA and CLA samples were 38% and 40%, respectively (Table 1). Previous studies on *A. himalaicum* reported that the extraction yields of MeOH, aqueous, and 30% EtOH extracts were 17.8%, 20.2%, and 22.3%, respectively [2,9]. Among various extraction solvents, EtOH demonstrated the highest extraction yield. Congruent with the results of previous research, the extraction yield of the 95% EtOH used in this study was consistently high; it was approximately twice as high as that of 30% EtOH.

Table 1. Extraction yields of TLA and CLA extracts.

Sample	Dry Sample (g)	Extract (g)	Yield (%)
TLA	10	3.8	38.0
CLA	4.0	1.6	40.0

3.2. LC–MS/MS Analysis

The compounds were analyzed by performing tandem MS, according to their mass-to-charge ratios. The primary peaks of the samples were characterized using a web-based database and multiple spectral libraries, leading to the identification of 14 compounds (Table 2).

Table 2. Proposed structures by performing LC-MS/MS using negative and positive modes.

t _R (min)	MW	Tentative Identification
7.63	354.1	Neochlorogenic acid ¹
10.89	354.1	Chlorogenic acid ^{1,2}
11.62	354.1	Cryptochlorogenic acid ¹
13.48	354.1	1-O-Caffeoylquinic acid ¹
13.91	338.1	4-O-Coumaroylquinic acid ^{1,2}
15.84	368.1	5-O-Feruloylquinic acid ^{1,2}
16.38	338.1	1-O-Coumaroylquinic acid ¹
19.68	610.2	Rutin ^{1,2}
19.70	564.1	Kaempferol 3-arabinofuranoside 7-rhamnofuranoside ¹
19.99	464.1	Hyperin ^{1,2}
20.88	516.1	Cynarin ¹
21.19	516.1	Isochlorogenic acid A ^{1,2}
21.96	516.1	Isochlorogenic acid B ^{1,2}
23.44	516.1	Dicaffeoylquinic acid ¹

¹ negative ion mode. ² positive ion mode.

In the negative ion mode, seven more compounds were detected compared to the positive ion mode. This discrepancy is probably due to the differential polarity of the compounds detectable in each ionization mode [10,11]. As EtOH was employed as the extraction solvent, it was expected that more polar substances would be extracted, which may have accounted for more compounds being identified in the negative mode.

LC–MS/MS profiling was conducted to investigate the phytochemical constituents of TLA. The UV chromatogram recorded at 330 nm, along with the base peak chromatograms obtained in the negative and positive ion modes, is depicted in Figure 3.

3.3. HPLC/PDA Analysis

HPLC/PDA analysis was conducted in connection with the LC–MS/MS data to further identify and quantify the phytochemicals in the TLA and CLA extracts. In the HPLC analysis, compounds 1–7 were used as the standard compounds in the HPLC analysis. The chromatogram of the standard compounds displayed well-separated peaks within the retention time, ranging from 7.61 to 25.45 min (Figure 4).

While the other compounds were undetected or only present in trace amounts, compounds 2, 6, and 7 displayed a significant peak in both the TLA and CLA chromatograms. Based on the results, calibration equations for compounds 2, 6, and 7 were calculated (Table 3). The linearity of this method was validated by a correlation coefficient (r^2) exceeding 0.999.

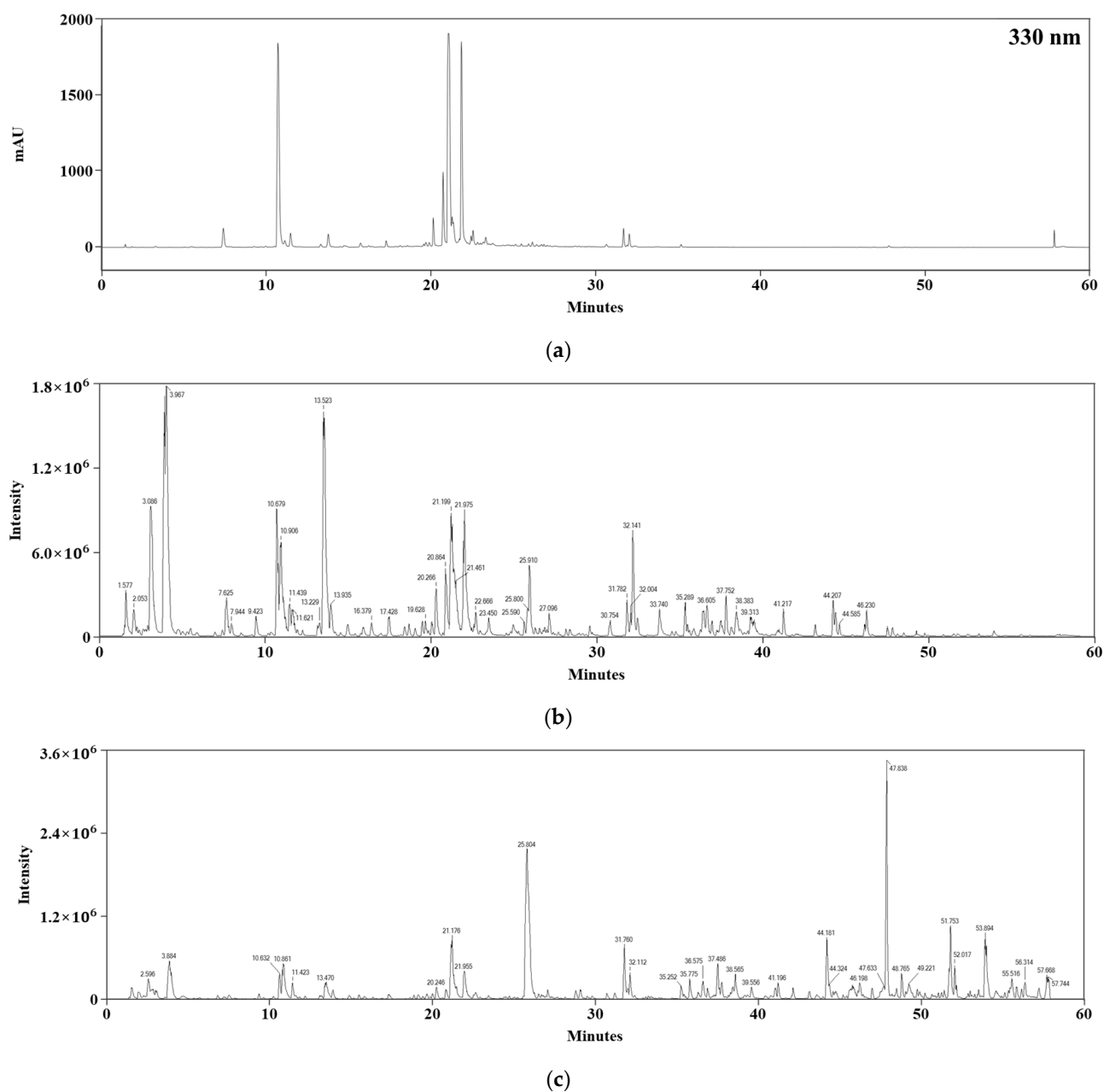


Figure 3. Ultraviolet chromatogram (a) of TLA extracts at 330 nm; the base peak chromatograms of the TLA extract analyzed in the negative (b) and the positive (c) ion modes.

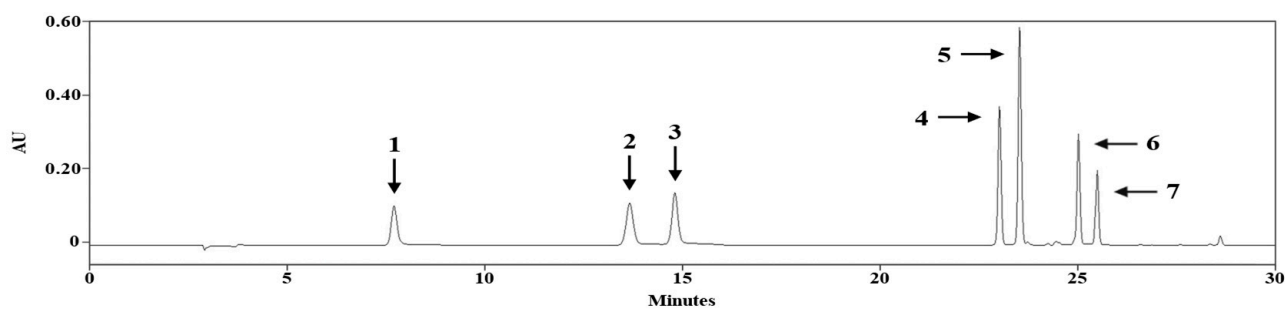


Figure 4. HPLC/PDA chromatogram of compounds 1–7. Neochlorogenic acid (1), chlorogenic acid (2), cryptochlorogenic acid (3), rutin (4), hyperin (5), isochlorogenic acid A (6), and isochlorogenic acid B (7).

Table 3. Calibration curves for compounds 1–7.

Compound	t _R (min)	Calibration Equation	Correlation Factor, r^2
1	7.61	-	-
2	13.51	$Y = 10,830X - 14,226$	0.9996
3	14.73	-	-
4	23.04	-	-
5	23.50	-	-
6	24.96	$Y = 10,251X + 53,292$	0.9998
7	25.45	$Y = 6733.6X + 99,840$	0.9991

Y, peak area; X, concentration of the standard ($\mu\text{g/mL}$); r^2 , correlation coefficient for six calibration data points ($n = 3$). Neochlorogenic acid (1), chlorogenic acid (2), cryptochlorogenic acid (3), rutin (4), hyperin (5), isochlorogenic acid A (6), and isochlorogenic acid B (7).

The HPLC results were consistent with those from LC–MS/MS, revealing that compounds 1, 2, 3, 6, and 7 were detected in both TLA and CLA extracts (Figure 5). However, compound 4 was only detected in the TLA extract, while compound 5 was only detected in the CLA extract. Rutin is a flavonol that serves as an essential nutritional compound in various dietary sources [12,13]. According to previous studies, rutin has cytoprotective, antioxidant, anticarcinogenic, neuroprotective, cardioprotective, and vasoprotective activities [14–21]. To synthesize rutin, two successive glycosylation processes from quercetin are required.

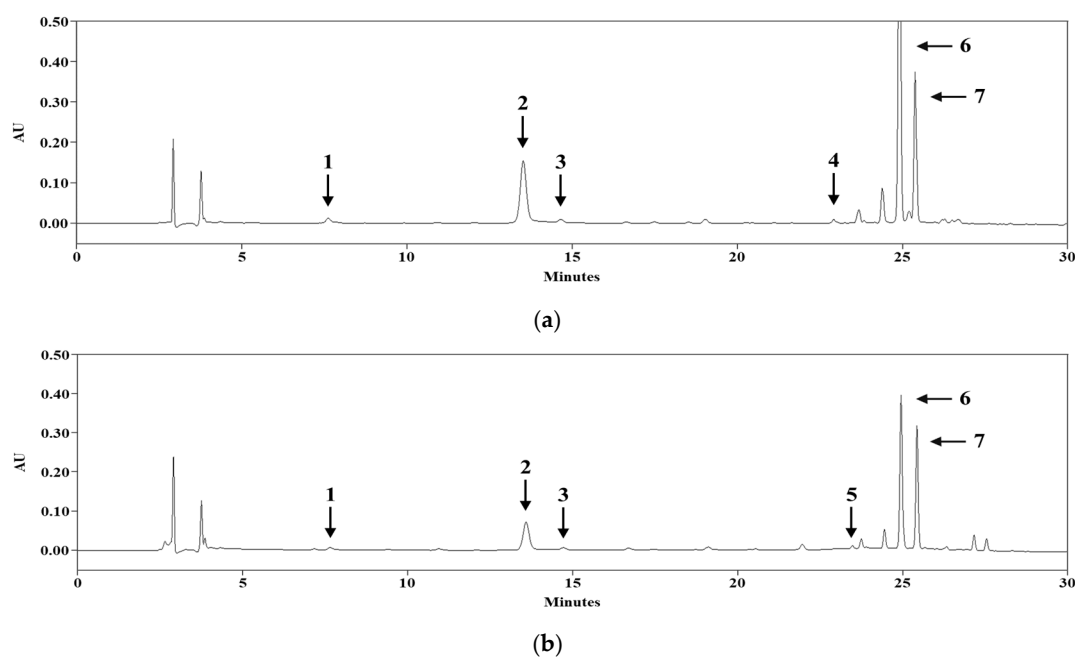


Figure 5. HPLC/PDA chromatograms of TLA (a) and CLA (b) extracts. Neochlorogenic acid (1), chlorogenic acid (2), cryptochlorogenic acid (3), rutin (4), hyperin (5), isochlorogenic acid A (6), and isochlorogenic acid B (7).

Ohgami et al. reported that the enzyme responsible for glycosylation is expressed at a higher level in mature leaf than in the young leaf of *Camellia sinensis* [22]. This aligns with another study indicating that the rutin content in mature tea leaf is 2.15 times higher than in young tea leaf [23]. These previous studies can be referred to for interpreting the difference in rutin content between the TLA and CLA extracts. Rutin is well known for its bitter taste [24], and this may have contributed to the utilization of young *A. himalaicum* leaf as food. On the contrary, hyperoside was only detected in the CLA extract. Hyperoside has various pharmacological effects such as anticancer, neuroprotective, and renoprotective

effects, among others. Moreover, hyperoside demonstrates a more superior antioxidant performance than rutin as the additional sugar group in rutin increases steric hindrance, which reduces its ability to interact effectively with free radicals [25,26]. The fact that *A. himalaicum* is used for dietary purposes rather than medicinal purposes suggests that the detection of bioactive compounds with strong antioxidant properties alone indicates sufficient utility value.

By using the calibration equations in Table 3, the amounts of phytochemicals in the TLA and CLA extracts were determined (Table 4). The overall phytochemical content of the TLA extract (49.73 mg/g DW) was approximately two times higher than that of the CLA extract (25.73 mg/g DW), suggesting that cotyledons, with their relatively small leaf area and short duration, may contain fewer phytochemicals than true leaf. Phytochemicals are produced in response to biotic and abiotic stressors [27,28].

Table 4. The quantification of compounds 1–7 in the TLA and CLA extracts.

Sample	Content (mg/g DW)							
	1	2	3	4	5	6	7	Total
TLA	tr	9.002 ± 0.055 ****	tr	tr	ND	28.512 ± 0.217 ****	12.223 ± 0.074 ****	49.737 ****
CLA	tr	4.422 ± 0.032 ****	tr	ND	tr	10.062 ± 0.070 ****	11.255 ± 0.049 ****	25.739 ****

The statistical significance of the mean ± SD values in each bar is determined using a *t*-test, indicating significant differences. The asterisks indicate different levels of statistical significance between TLA and CLA extracts (****, *p* < 0.0001). Neochlorogenic acid (1), chlorogenic acid (2), cryptochlorogenic acid (3), rutin (4), hyperin (5), isochlorogenic acid A (6), and isochlorogenic acid B (7).

Compounds 1, 2, 3, 6, and 7 are phenolic acids belonging to the caffeoylquinic acid family and share a common biosynthetic pathway [29]. These compounds are characterized by having an ester bondage between a caffeoyl group and a quinic acid group and are derived from the phenylpropanoid pathway [30]. The caffeoylquinic acid family exhibits anticancer, antioxidant, antiviral, anti-Alzheimer's, antibacterial, and neuroprotective properties [31–42]. Compounds 1 and 3, from the caffeoylquinic acid series, were detected in relatively small quantities in the TLA and CLA extracts. According to Mullen et al. [43], the content of isomers may vary depending on the extraction method employed. It is possible that the 95% EtOH used in the experiment may have hydrolyzed the ester bonds during the extraction process, which might have affected the isomer formation of caffeoylquinic acid. Of note, significant amounts of compounds 2, 6, and 7 were commonly detected in both the TLA and CLA extracts. Compound 6 contained the largest amount of caffeoylquinic acid (28.512 mg/g), followed by compounds 7 and 2. The composition ratios for compounds 2, 6, and 7 in both the TLA and CLA extracts exhibited similar patterns. Compounds 6 and 7, which had the highest content of caffeoylquinic acid, are isomers of dicaffeoylquinic acid. According to Xu et al. [44], their antioxidant activity is superior to that of caffeoylquinic acid. In addition to their direct structural radical-scavenging capacity, these compounds contribute to indirect antioxidant activity by stimulating the transcription factor Nrf2 [4]. The quantitative abundance of these phytochemicals, in addition to their outstanding bioactivity, indicates that the leaves of *A. himalaicum* have significant potential for promoting human health.

3.4. TPC and TFC

The quantification of TPC and TFC was based on calibration curves established using tannic acid and quercetin as standard compounds, respectively. The calibration curve equations and correlation coefficients for each standard are presented (Table 5).

Table 5. Calibration curves for tannic acid and quercetin.

Standard	Calibration Equation	Correlation Factor, r^2
Tannic acid	$Y = 0.0147X + 0.0883$	0.9995
Quercetin	$Y = 0.0167X - 0.0329$	0.9999

Y, absorbance at the respective wavelength (760 nm for tannic acid, 430 nm for quercetin); X, concentration of the standard ($\mu\text{g/mL}$); r^2 , correlation coefficient for five calibration data points ($n = 3$).

The TPC and TFC assay results revealed a significant difference in phytochemical content between TLA and CLA. The TLA (45.51 mg tannic acid equivalent/g extract) was approximately 1.7 times higher than that of CLA (26.52 mg tannic acid equivalent/g extract). Similarly, the TLA (16.24 mg quercetin equivalent/g extract) was about 1.4 times higher than that of CLA (11.53 mg quercetin equivalent/g extract) (Figure 6).

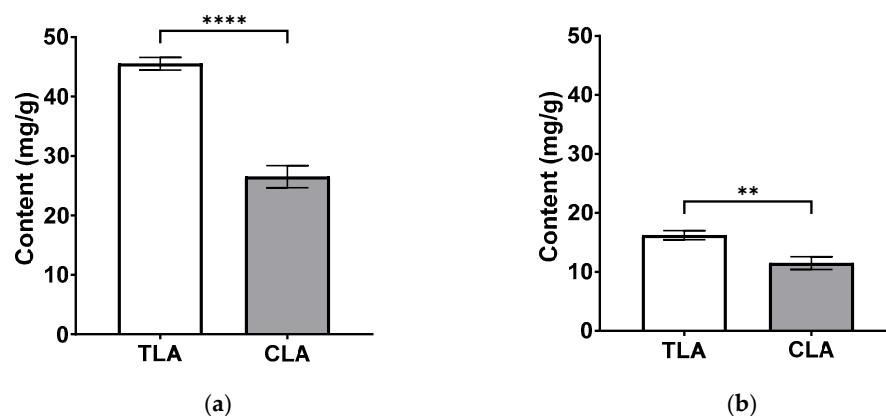


Figure 6. Comparison of TPC (a) and TFC (b) of TLA and CLA extracts. Statistical significance of mean $\pm$ SD values in each bar is determined using a t -test, indicating significant differences. The asterisks indicate different levels of statistical significance between TLA and CLA extracts (**, $p < 0.01$; ****, $p < 0.0001$).

Trichomes are hair-like outgrowths present on various plant species' surfaces [45,46]. The presence of more well-developed trichomes in TLA may have led to the higher TPC and TFC of TLA than those of CLA. According to previous studies, various types of trichomes have been identified in the Asteraceae family [47], and web-like white trichomes are densely distributed on the stem of *A. himalaicum* and on the abaxial surface of TLA. Generally, trichomes serve as physical and chemical defense mechanisms against herbivores and pathogens [48]. In terms of chemical defense mechanisms, they produce or store various phenolic compounds [49]. Although there have been no studies on the trichomes of *A. himalaicum*, a previous study on *Helianthus annuus*, another member of the Asteraceae family, identified trichomes as reservoirs of flavonoids and sesquiterpenes [50–53]. Similarly, the well-developed trichomes in TLA are likely contributors to its enriched phytochemical profile.

Additionally, TLA performs photosynthetic activity and is exposed to environmental stress for a longer duration than CLA. Since TLA has a larger leaf surface area than CLA, it inevitably receives higher light exposure, including UV radiation, which is known to induce oxidative stress and trigger secondary metabolite production [54]. The biosynthesis of phenolic compounds and flavonoids, which function as protective agents against oxidative stress, were affected by UV radiation, which is a kind of major environmental factors [55]. Several studies have reported that leaves exposed to high UV radiation accumulate increased levels of flavonoids and phenolic compounds as part of their protective mechanisms [56,57]. Given that TLA remains exposed to natural light for a longer period

than CLA, and its larger surface area further enhances its susceptibility to UV radiation, it is reasonable to assume that climatic factors, particularly light quality and UV exposure, contribute to the higher TPC and TFC in TLA.

3.5. DPPH and ABTS⁺ Radical-Scavenging Assays

DPPH and ABTS⁺ assays were performed to evaluate the radical-scavenging properties of the TLA and CLA extracts, which were expressed as the IC₅₀ and were compared with AA—the representative positive control for these assays. Based on the IC₅₀ values in the DPPH and ABTS⁺ assays, the TLA extract demonstrated higher antioxidant ability than the CLA extract (Table 6). However, the IC₅₀ value of the TLA extract was much lower than the antioxidant level of AA. A positive correlation was observed between the relative content of compounds 1–7 and the DPPH and ABTS⁺ radical-scavenging activity of the TLA and CLA extracts, aligning with the findings from the quantitative analysis. The observed difference in DPPH and ABTS⁺ radical-scavenging activity between TLA and CLA may be attributed to the quantity of phytochemicals in each extract.

Table 6. DPPH and ABTS⁺ radical-scavenging activities of TLA and CLA extracts.

Sample	DPPH (IC ₅₀ , mg/mL)	ABTS ⁺ (IC ₅₀ , mg/mL)
TLA	1.59 ± 0.02 ^a	1.43 ± 0.04 ^a
CLA	2.82 ± 0.12 ^b	2.53 ± 0.05 ^b
AA	0.12 ± 0.00 ^c	0.13 ± 0.00 ^c

^{a–c} Different letters indicate statistically significant differences within the same column, determined by Tukey's test ($p < 0.05$).

According to previous studies, the antioxidant activity of dicaffeoylquinic acid is more superior than that of caffeoylquinic acid due to hydroxyl groups and additional ortho-hydroxyl groups linked to the aromatic ring [44,58–60]. The radical-scavenging capacity of TLA and CLA is considered to be significantly influenced by compounds 6 and 7 as a result of their high content in quantitative analysis and their highly effective structural radical-scavenging capacity.

4. Conclusions

This study comprehensively detected the phytochemical constituents and antioxidant activities of TLA and CLA. Utilizing LC-MS/MS and HPLC analyses, seven phytochemical constituents were identified and quantified. Significantly, TLA exhibited considerably higher total phytochemical content, as indicated by TPC and TFC, in comparison to CLA. This trend was further substantiated by the superior radical-scavenging activities observed in DPPH and ABTS⁺ assays. The results highlight that TLA serves as a richer source of biologically active compounds than CLA. This discrepancy is postulated to be a consequence of the prolonged exposure of the true leaf to biotic and abiotic stressors in conjunction with the presence of well-developed trichomes. Additionally, *A. himalaicum* leaves, especially TLA, are rich in dicaffeoylquinic acids, implying their strong antioxidant potential. These findings provide a basic understanding of the potential of *A. himalaicum* as a natural antioxidant source for use in the production of functional foods and natural therapeutic agents. This study successfully identified its phytochemical composition and confirmed its potential antioxidant activity. However, additional *in vivo* research is necessary to evaluate its actual physiological effects, bioavailability, and mechanism of action in biological systems. Future research should focus on validating these findings through *in vivo* models to fully explore its practical applications.

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