

Selective Enhancement of Caffeoylquinic Acid Derivative via UV Irradiation and Validation of Analytical Method in the Aerial *Aster × chusanensis* Y. S. Lim

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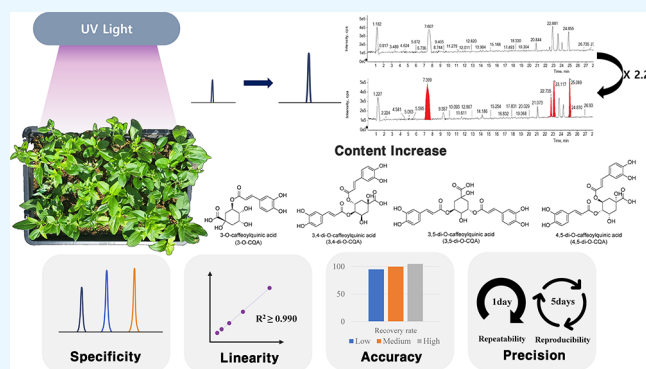
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ABSTRACT: *Aster × chusanensis* Y.S.Lim is equipped with mass plant growth strategies that can potentially develop into functional foods. *A. chusanensis* was grown on a vertical farm until it was budding. The plants were transported to a growth chamber equipped with ultraviolet (UV)-A and UV-B lights and irradiated for 48 h. The base peak intensity (BPI) of the *A. chusanensis* control displayed eight predominant metabolites, namely, 3-O-caffeoylquinic acid, rutin, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, biorobin, luteolin-7-O- β -glucoside, 4,5-di-O-caffeoylquinic acid, and luteolin, as identified using LC-Q-TOF/MS analysis. UV-A-irradiated *A. chusanensis* showed an increase in the content of caffeoylquinic acid (CQA) derivatives (peaks 1, 3, 4, and 7). Thus, CQA-enhanced *A. chusanensis*, treated with UV-A irradiation, was used to develop analytical validation methods using HPLC-DAD. Quantitative analysis of the CQA derivatives was conducted based on the developed analytical method. The requirements for specificity, linearity, accuracy, and precision were met in accordance with the Korea Food and Drug Administration (KFDA) and the Association of Official Agricultural Chemists (AOAC) guidelines. The total contents of CQA derivatives in *A. chusanensis* were improved by ~ 2.2 times (from 17,081 to 37,243 $\mu\text{g/g}$) following the UV-A irradiation treatment.



INTRODUCTION

Aster × chusanensis Y. S. Lim of the Asteraceae family is endemic to South Korea,¹ first discovered on Ullung Island in 2005 (Chusan-ri, Ullung-gun, Gyeongsangbuk-do, South Korea).² DNA analysis has revealed that *A. chusanensis* Y. S. Lim is a natural hybrid between *Aster glehnii* and *Aster spathulifolius*.³ It is a perennial plant with mauve-colored flowers that bloom from September to October and grows in the crevices of rocks along the seacoast, reaching a height of up to 80 cm.⁴ The shoots of *A. chusanensis* have been used locally as food ingredients; however, the germination rate of *A. chusanensis* was significantly lower, <10%.⁵ In 2022, the National Institute of Biological Resources (NIBR) in Incheon Metropolitan City, South Korea, developed a propagation method to enhance the productivity.⁶ A recent study has demonstrated the antioxidant and anti-inflammatory properties of *A. chusanensis*, indicating its potential as a nutraceutical.⁷ In plants, biological activities are closely linked to bioactive metabolites. To date, data on the metabolites in *A. chusanensis* remain lacking. To address this gap in the literature, this study evaluated the metabolites in the aerial parts of *A. chusanensis* including flavonoids and caffeoylquinic acid (CQA) derivatives.

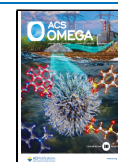
CQA exhibits various derivatives, such as mono-, di-, and tricafeoylquinic acid, depending on the number and location of the caffeic acids attached to quinic acid.⁸ CQA derivatives have demonstrated diverse biological effects depending on their structures. For example, 3-O-caffeoylquinic acid, also known as chlorogenic acid, comprises one caffeic acid attached to a hydroxy group on C-3 of quinic acid that contributes to its biological functions, including antioxidant, anti-inflammation, anticancer, and cardiovascular protective effects.⁹ Furthermore, di-O-CQA derivatives found in nature include 1,5-di-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA.¹⁰ The antioxidant, antidiabetes, cytoprotective, chondroprotective, and antinociceptive effects of di-O-CQA derivatives have also been well-documented.^{11–15}

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Therefore, CQA derivatives have been selected as key dietary supplements due to their specific biological effects, including liver protection, cholesterol metabolism, and weight management.^{16,17} Various supplements are currently being actively developed based on the functionality of CQAs, although the focus of these developments has been on utilizing coffee beans and artichoke leaves to mitigate issues with the CQA content in natural sources.^{18,19}

Various practical methods, including regulating environmental conditions, treating signaling molecules, and breeding a variety of plants, have been developed to enhance strategies for increasing active metabolite content in plants.²⁰ The regulation of environmental conditions can alter plant metabolism by changing factors such as light, temperature, water, salinity, and soil fertility.^{21–23} In particular, light sources for plant metabolism provide energy for photosynthesis, which, in turn, influences the synthesis of primary and secondary metabolites.²⁴ In industry settings, light intensity, spectrum, and exposure periods are utilized to enhance growth rates, germination rates, and metabolite contents in vertical farms.²⁵ Various studies have examined the effects of ultraviolet (UV) light on aromatic compounds in plants, particularly focusing on UV-A and UV-B light, part of the longer-wavelength spectrum.²⁶ UV light management can have beneficial effects on plant growth and metabolism.

Standardization and normalization of key compounds from these sources are essential for verifying the value of nutraceuticals.²⁷ Analyses of the standard compound contents from these sources must follow the guidelines of the Association of Official Agricultural Chemists (AOAC). The established guidelines for standard method performance requirements (SMPRs) for single or multiple analytes have been validated to meet the analytical parameters, including analytical range, limit of quantitation (LOQ), limit of detection (LOD), repeatability, and reproducibility.^{28,29}

The key analytical parameters are presented based on several evaluation criteria, including specificity, linearity, accuracy, and precision.

This study is the first to focus on the metabolomic analysis of *A. chusanensis*, particularly the flavonoids and CQA derivatives. Studies demonstrating the efficiency of this method remain lacking, as *A. chusanensis* was first discovered in South Korea in 2005. Furthermore, treating the plant chamber with UV-A and UV-B light selectively enhanced the content of the four CQA derivatives. The metabolites were annotated using LC-Q-TOF/MS by comparing their observed and theoretical masses. UV-A-treated *A. chusanensis* and effective practical methods were assessed through method validation, including specificity, linearity, accuracy, and precision, following the AOAC guidelines, considering the increased CQA derivatives 3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA.

MATERIALS AND METHODS

Plant Materials. *A. chusanensis* Y. S. Lim plants collected in Ulleungdo, South Korea, (37°31'44.4" N, 130°49'54.8" E and 37°32'031" N, 130°51'032" E.) were obtained from the Ministry of Environment, the Biological Resources Propagation Research Center of the NIBR (Sangnam-myeon, Milyang-si, Gyeongsangnam-do, South Korea). Ten plants were transferred to pots and stabilized in a growth chamber. Leaf branches of *A. chusanensis* were cut approximately 15 cm from the stabilized plant sources and directly treated with a rooting

agent (1-naphthyl acetamide 0.4% ROOTON, Jiwoobiotech Ltd., Gwangju-si, Gyeonggi-do, South Korea).

The branches treated with the rooting agent were transplanted into a soil-filled plastic plot and cultivated in a smart farm cube for 50 days, during which the growth conditions were carefully adjusted to achieve a temperature of 20 ± 3 °C, a humidity of $70 \pm 10\%$, and a light intensity of 140 ± 10 $\mu\text{mol}\cdot\text{m}^{-2}/\text{s}$.

UV Treatment on *A. chusanensis*. A portion of the propagated *A. chusanensis* was transferred to a plant growth chamber equipped with UV-A and UV-B lamps. The UV-A and UV-B lamps were purchased from LG Innotek (Seoul, South Korea). The plants were exposed to UV-A (370 nm) and UV-B (300 nm) lights at 12.9 and 0.31 W/m² of intensity, respectively, for 48 h, during which the plants were cultivated under consistent growth conditions, ensuring that the temperature (20 ± 3 °C) and humidity ($70 \pm 10\%$) remained stable. The aerial parts of the control and UV-irradiated *A. chusanensis* were randomly harvested and dried directly at 35 °C under dark conditions. The dried aerial parts of *A. chusanensis* were finely powdered using a grinder and used for qualitative and quantitative analyses. All *A. chusanensis* samples were measured based on their dry weight.

Identification of Metabolites Using LC-Q-TOF/MS. LC-Q-TOF/MS consisted of a liquid chromatograph (Shimadzu NEXERA, Kyoto, Japan) with an autosampler, quaternary pump, column oven, UV detector, and a quadrupole time-of-flight mass spectrometer (SCIEX XS500R Q-TOF, Framingham, MA, USA). Each 1 g sample of dried UV-A-treated, UV-B-treated, and untreated *A. chusanensis* was sonicated with 90% ethanol (50 mL) in a sonicator for 3 h. The extracts were filtered using 0.2 μm membrane filter to prepare samples for LC-Q-TOF/MS analysis.

The extracts (10 μL) were directly injected into an InfinityLab Poroshell 120 HILIC column (4.6×150 nm, 4 μm , Agilent Technologies, Santa Clara, CA, USA). A gradient solvent system consisting of mobile phases A (water with 0.1% acetic acid) and B (acetonitrile with 0.1% acetic acid) was used at a flow rate of 1 mL/min, with transitions from 100% A to 100% B for 50 min. The mass spectrometry (MS) settings were configured with a capillary voltage of 5.5 kV and a temperature of 450 °C in negative ionization mode. The collision energy was set to 10 V, and the desolvation gas flow was maintained at 800 L/h with a temperature of 400 °C. The mass scan range was 50–1000, based on the charge-to-mass ratio (m/z). The base peak intensity (BPI) grams and individual mass spectra were visualized by using SCIEX OS software.

Procedure of Analytical Method Validation. Method validation was applied to representative samples with the highest metabolite abundance of *A. chusanensis*, as the aerial parts of the UV-A-treated samples. Four CQA derivatives (3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA) from UV-A-irradiated *A. chusanensis* were evaluated according to the guidelines of the Korea Food and Drug Administration (KFDA) for linearity, specificity, accuracy, and precision (both intraday and interday) according to the guidelines of the KFDA. The standard 3-O-CQA was obtained from Alfa Aesar (Ward Hill, MA, USA). 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA were purchased from MedChem Express (Monmouth Junction, NJ, USA).

Sample Preparation. The dried powder of the aerial parts of the UV-irradiated *A. chusanensis* was weighed at 1.0, 1.5, 2.0, 2.5, and 3.0 g. These samples were extracted by using 50 mL of

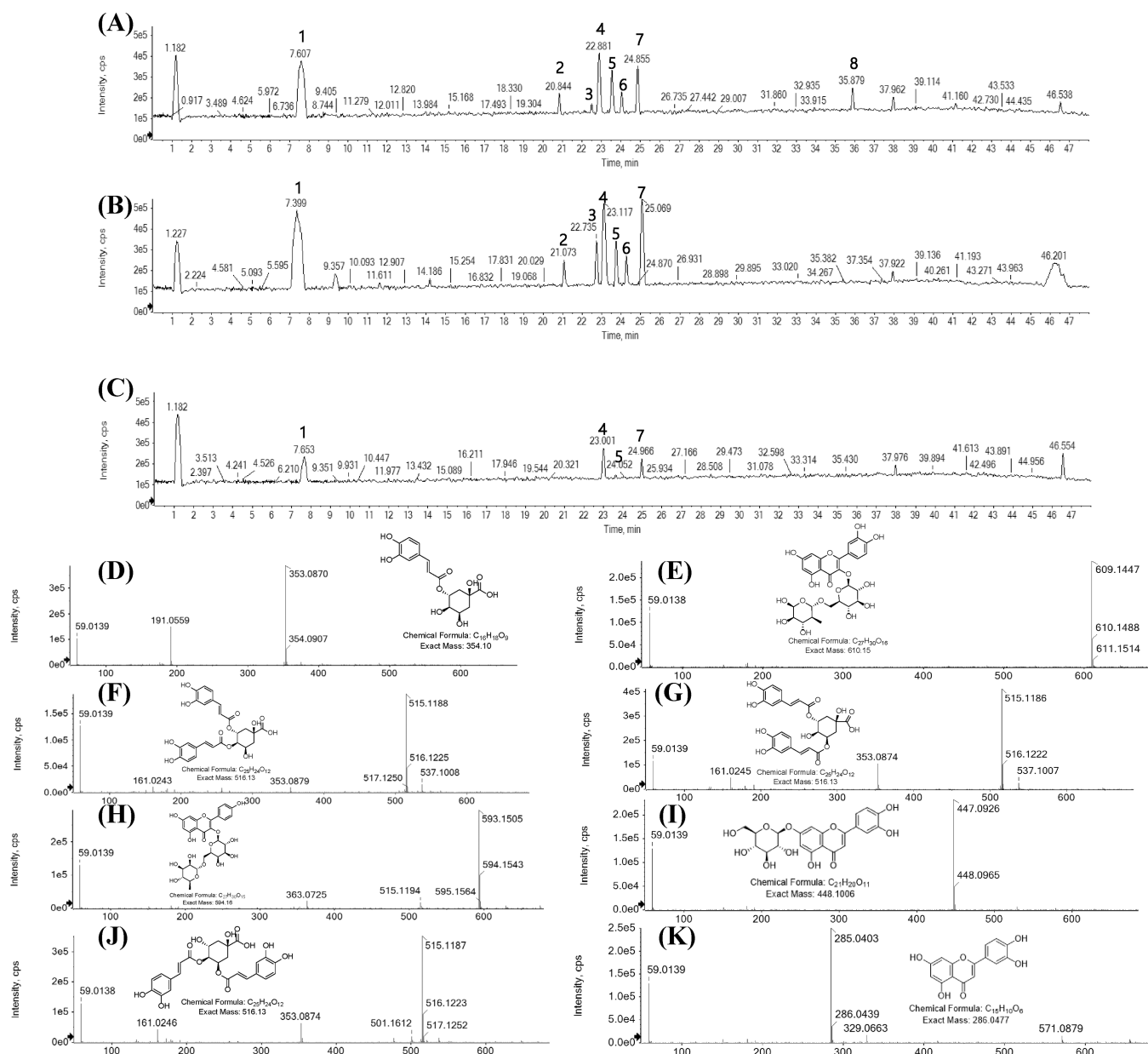


Figure 1. BPI chromatogram of (A) control, (B) UV-A, and (C) UV-B light irradiated *A. chusanensis* extract using LC-Q-TOF/MS analysis. Individual mass gram of (D) peak 1, 3-O-CQA; (E) peak 2, rutin; (F) peak 3, 3,4-di-O-CQA; (G) peak 4, 3,5-di-O-CQA; (H) peak 5, biorobin; (I) peak 6, luteolin-7-O- β -D-glucoside; (J) peak 7, 4,5-di-O-CQA; and (K) peak 8, luteolin.

90% ethanol and a sonicator for 3 h, followed by filtration through a 0.2 μ m syringe filter. The extracts were prepared for validation using HPLC-DAD. Ten mg of chemical standards (CQA derivatives) was dissolved in 10 mL of 90% ethanol as a stock solution. The stock solutions were diluted to match the requirements of each validation criterion.

HPLC-DAD Conditions. Method validation by HPLC-DAD analysis was performed using an Infinity 1260 HPLC (Agilent Technologies, USA) with a diode array detector. UV-A irradiated *A. chusanensis* extracts and four CQA derivatives solutions (injection volume: 10 μ L) were injected into the column (Supersil 120 ODS-I, 4.6 mm \times 250 mm, 5 μ m, Dalian Elite Analytical Instruments, China) at an oven temperature of 30 $^{\circ}$ C. The pump operated as a gradient solvent system, using mobile phases A (0.1% acetic acid in water) and B (0.1% acetic acid in acetonitrile) for 35 min. LC analysis was conducted by

gradually increasing the proportion of solvent B as follows: 0–13 min, 0–23% solvent B; 13–30 min, 23% solvent B; 30–35 min, 23–100% solvent B. The flow rate was 0.8 mL/min, and the UV wavelength was 254 nm.

Specificity. To determine specificity, an extract solution was prepared by dissolving 1 g of dried aerial *A. chusanensis* powder in 90% ethanol (50 mL). The four CQA derivatives 3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA were dissolved in 90% ethanol at concentrations of 500, 125, 125, and 31.25 μ g/mL, respectively, and analyzed using the established HPLC-DAD conditions by distinguishing the retention times of the individual peaks in the UV chromatogram at 254 nm.

Linearity. The linearity of CQA derivatives was confirmed by measuring the peak area at each concentration using HPLC-DAD analysis. A stock solution of 3-O-CQA was diluted to

Table 1. Annotated Metabolites in *A. chusanensis* Extract by LC-Q-TOF/MS Analysis

peaks	t_R (min)	detected ion (m/z)	calculated ion (m/z)	error (ppm)	chemical formula	compounds	classification
1	7.619	353.0870	353.0873	−0.85	$C_{16}H_{18}O_9$	3-O-CQA	CQA derivatives
2	20.86	609.1447	609.1456	−1.48	$C_{27}H_{30}O_{16}$	rutin	flavonoids
3	22.502	515.1188	515.1190	−0.39	$C_{25}H_{24}O_{12}$	3,4-di-O-CQA	CQA derivatives
4	22.908	515.1186	515.1190	−0.78	$C_{25}H_{24}O_{12}$	3,5-di-O-CQA	CQA derivatives
5	23.563	593.1505	593.1585	−0.25	$C_{27}H_{30}O_{15}$	birobin	flavonoids
6	24.036	447.0926	447.0927	−0.22	$C_{21}H_{20}O_{11}$	luteolin-7-O- β -glucoside	flavonoids
7	24.873	515.1187	515.1190	−0.58	$C_{25}H_{24}O_{12}$	4,5-di-O-CQA	CQA derivatives
8	35.853	285.0403	285.0399	+1.40	$C_{15}H_{10}O_6$	luteolin	flavonoids

concentrations of 31.25, 62.5, 125, 250, 500, and 1000 $\mu\text{g/mL}$. The stock solution of 3,4-di-O-CQA was diluted to concentrations of 3.9, 7.8, 15.6, 31.25, 62.5, and 125 $\mu\text{g/mL}$. Additionally, 3,5-di-O-CQA and 4,5-di-O-CQA stock solutions were prepared at 15.625, 31.25, 62.5, 125, 250, and 500 $\mu\text{g/mL}$. The peak area for each sample concentration was measured in triplicate to generate a calibration curve. The calibration equation and coefficient of determination (R^2) were derived from the calibration curves. The average of slope (S) and the standard deviation as the y-intercept (σ) in the calculation formula were utilized to determine the LOD and LOQ as follows: $\text{LOD} = 3.3 \times \sigma/S$ and $\text{LOQ} = 10 \times \sigma/S$.

Accuracy. The dried aerial parts of UV-irradiated *A. chusanensis* powder (1 g) were extracted with 50 mL of 90% ethanol using a sonicator at 35 °C. The extract was filtered by using a 0.2 μm syringe filter. The filtered extract was mixed with the four CQA derivative solutions at concentrations of 50%, 100%, and 150%. Specifically, 100 μL of 3-O-CQA solutions (58.84, 117.69, and 176.53 $\mu\text{g/mL}$), 3,4-di-O-CQA solutions (6.29, 12.59, and 18.88 $\mu\text{g/mL}$), 3,5-di-O-CQA solutions (52.46, 104.92, and 157.38 $\mu\text{g/mL}$), and 4,5-di-O-CQA solutions (22.34, 44.69, and 67.03 $\mu\text{g/mL}$) was mixed with the filtered extract (900 μL) to prepare the accuracy sample. The accuracy was confirmed by calculating the concentration difference between the blank (90% ethanol) and the accurate samples.

Intraday Precision (Repeatability). The dried aerial parts of UV-irradiated *A. chusanensis* powder were weighed (1.0, 2.0, and 3.0 g) and sonicated with 50 mL of 90% ethanol using a sonicator for 3 h. The extract was filtered through a 0.2 μm syringe filter and analyzed using HPLC. The contents were calculated by accounting for the dilution factor of the sample, converting the concentration of CQA derivatives to $\mu\text{g/g}$ based on 1.0 g of dried weight. An experiment to evaluate intraday precision was conducted five times.

Interday Precision (Reproducibility). The dried aerial parts of UV-irradiated *A. chusanensis* powder (1.5 and 2.5 g) were extracted with 50 mL of 90% ethanol by using a sonicator for 3 h and filtered for HPLC injection. The contents were determined by considering the dilution factor of the sample and converting the concentration of CQA derivatives to $\mu\text{g/g}$ based on a dried weight of 1.0 g. The interday precision was measured five times over 5 days.

Quantitative Analysis of CQA Derivatives in *A. chusanensis*. The dried aerial parts of UV-A-treated, UV-B-treated, and untreated *A. chusanensis* (each 1 g) were extracted by using 50 mL of 90% ethanol for 3 h. The extracts were filtered and analyzed using HPLC under the same conditions as those used for method validation. The four CQA derivatives in the *A. chusanensis* extract were verified by comparing the retention times (t_R) of each peak. The contents of the four

CQA derivatives in the UV-A-treated, UV-B-treated, and untreated *A. chusanensis* extracts were determined by using calibration curves and quantified as the amount per 1 g of dried weight.

RESULTS AND DISCUSSION

Identification of Metabolites in *A. chusanensis* Using LC-Q-TOF/MS. Changes in the metabolites of the aerial parts of *A. chusanensis* induced by UV irradiation were analyzed using LC-Q-TOF/MS. As shown in Figure 1A–C, the BPI chromatograms of UV-A, UV-B-irradiated, and unirradiated *A. chusanensis* aerial part extracts displayed well-separated metabolite peaks. The control *A. chusanensis* extract showed eight predominant peaks (peaks 1–8). In the UV-A-irradiated aerial part of *A. chusanensis* extract, seven peaks (peaks 1–7) were detected in a pattern similar to that of the control *A. chusanensis*. Moreover, the peak areas of the four peaks (peaks 1, 3, 4, and 7) were confirmed to have increased compared with the control. In contrast, the UV-B-irradiated aerial part *A. chusanensis* extract showed a reduction in the number of detected peaks. Additionally, the overall peak area decreased compared to that of the control group. Metabolites within the eight peaks were identified by analyzing the mass in grams at each peak (Figure 1D–K).

Peak 1 ($t_R = 7.6$ min) showed a molecular ion peak at $[M-H]^- = m/z$ 353.0870 and a fragment peak at m/z 191.0559, corresponding to CQA ($C_{16}H_{18}O_9$) and quinic acid ($C_7H_{12}O_6$), respectively. Therefore, peak 1 was identified as 3-O-CQA because it showed the same mass value as the quinic acid remaining after the caffeoyl group was removed from the structures.^{30,31} Peaks 3 ($t_R = 22.5$ min), 4 ($t_R = 22.9$ min), and 7 ($t_R = 24.9$ min) showed the same ion peaks at m/z 515, 353, and 161, respectively. The fragment ions at 353 and 161 m/z were derived from cleavage of the caffeoyl moiety. Based on this typical fragmentation pattern, peaks 3, 4, and 7 were identified as di-O-CQA. Di-O-CQAs were identified by comparing their retention times with those of the corresponding standards. Therefore, peaks 3, 4, and 7 were assigned to 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA, respectively.^{32,33} Peak 2 ($t_R = 20.9$ min) was detected as the molecular ion peak at 609.1447 m/z , compared with the calculated ion of 609.1456 m/z derived from the chemical formula $C_{27}H_{30}O_{16}$, resulting in an error value of −1.4 ppm. Thus, based on its mass value, peak 2 was confirmed to be rutin.³⁴ Peak 5 ($t_R = 23.6$ min) was identified as birobin, confirming the agreement between the detected ion values at 593.1505 m/z and theoretical mass values at 593.1585 m/z with an error value of −0.25 ppm.³⁵ Peak 6 ($t_R = 24.0$ min) was observed, with the detected ion peak at $[M-H]^- = 447.0926$ m/z , which corresponded to the chemical formula of $C_{21}H_{20}O_{11}$. Thus, peak 6 was assigned as luteolin 7-O- β -D-

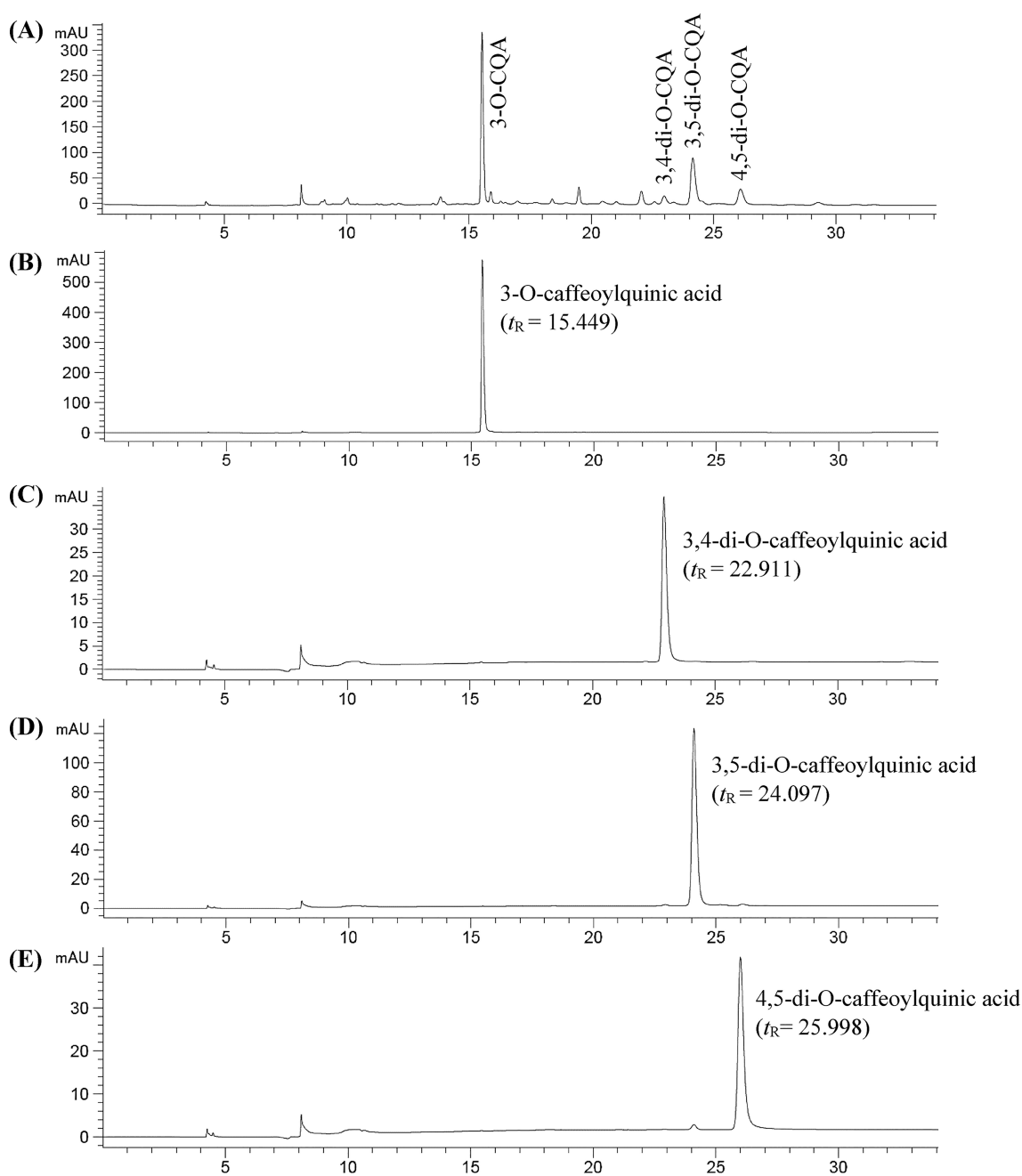


Figure 2. Chromatogram of (A) *A. chusanensis* extract irradiated by UV-A light, (B) 3-O-CQA, (C) 3,4-di-O-CQA, (D) 3,5-di-O-CQA, and (E) 4,5-di-O-CQA using HPLC at 254 nm.

glucose.³⁶ Peak 8 ($t_R = 35.9$ min) exhibited a molecular ion peak at 285.0403 m/z . The error value was calculated at +1.4 ppm, resulting from the comparison between the molecular ion and the calculated ion values at 285.0399 m/z . Peak 8 was confirmed as luteolin with the chemical formula $C_{15}H_{10}O_6$.³⁷

In conclusion, the detected eight peaks in the aerial part of the *A. chusanensis* extracts were annotated as 3-O-CQA (peak 1), rutin (peak 2), 3,4-di-O-CQA (peak 3), 3,5-di-O-CQA (peak 4), biorobin (peak 5), luteolin-7-O- β -D-glucose (peak 6), 4,5-di-O-CQA (peak 7), and luteolin (peak 8) (Table 1). Among these, peaks 1, 3, 4, and 7 were identified as derivatives of CQA, whereas peaks 2, 5, 6, and 8 corresponded to various flavonoid compounds.

Analytical Method Validation of CQA Derivatives Using HPLC-DAD Analysis. Analytical method validation of

the CQA derivatives was performed using UV-A-irradiated aerial parts of *A. chusanensis*. LC-Q-TOF/MS analysis confirmed that the levels of the four CQA derivatives selectively increased in *A. chusanensis* when exposed to UV-A irradiation. Thus, derivatives 3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA were selected as indicator components for the validation of the analytical method.

Specificity. As shown in Figure 2A, four CQA derivatives were detected in UV-A-irradiated *A. chusanensis* extracts using HPLC at 254 nm. They exhibited distinct peaks when retention times were compared as follows: 3-O-CQA ($t_R = 15.4$ min, Figure 2B); 3,4-di-O-CQA ($t_R = 22.9$ min, Figure 2C); 3,5-di-O-CQA ($t_R = 24.1$ min, Figure 2D); 4,5-di-O-CQA ($t_R = 26.0$ min, Figure 2E). Thus, the specificity was

Table 2. Evaluation of Linearity of 3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA Using HPLC Analysis

compounds	regression equation	coefficient of determination (R^2)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
3-O-CQA	$y = 16.948x + 35.87$	0.999	0.501	1.519
3,4-di-O-CQA	$y = 12.754x - 7.233$	0.999	1.187	3.596
3,5-di-O-CQA	$y = 10.547x - 44.61$	0.999	0.422	1.278
4,5-di-O-CQA	$y = 10.300x - 33.28$	0.999	1.636	4.957

confirmed by the well-separated CQA derivative peak in the UV chromatogram.

Linearity. A calibration curve was constructed using various concentration ranges of CQA derivatives in the sample. 3-O-CQA, the predominant compound in *A. chusanensis* extract, was set at 31.25, 62.5, 125, 250, 500, and 1000 $\mu\text{g/mL}$. The linearity of 3,4-di-O-CQA at the lowest concentration in the extract was confirmed by dilution from 125 to 3.9 $\mu\text{g/mL}$. The concentration levels of 3,5-di-O-CQA and 4,5-di-O-CQA in the extract were determined as 15.625, 31.25, 62.5, 125, 250, and 500 $\mu\text{g/mL}$. As shown in Table 2, the regression equations and coefficients of determination were derived from the calibration curves of the four CQA derivatives in the concentration range. The coefficient of determination (R^2) exceeded 0.999, indicating high linearity. LOD and LOQ were calculated as the slope (S) and the standard deviation as the y -intercept (σ) from a regression equation. The LODs of 3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA were 0.501, 1.187, 0.422, and 1.636, respectively, and the LOQs were 1.519, 3.596, 1.278, and 4.957, respectively.

Accuracy. Accuracy was determined by calculating the recovery rate of the four CQA derivatives at concentrations corresponding to 50, 100, and 150% in the *A. chusanensis* extract (Table 3). The concentrations of 3-O-CQA, 3,4-di-O-

Table 3. Recovery Rate for the Accuracy of 3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA in the Aerial Parts of *A. chusanensis* Powder

compounds	spiked conc ($\mu\text{g/mL}$)	measured amount ($\mu\text{g/mL}$)	average recovery (%)	RSD (%)
3-O-CQA	58.84	59.85	101.71	0.34
	117.69	119.81	101.80	2.17
	176.53	176.19	99.80	1.45
3,4-di-O-CQA	6.29	6.16	98.00	1.03
	12.59	12.29	97.71	1.46
	18.88	18.67	98.88	1.10
3,5-di-O-CQA	52.46	53.16	99.66	1.04
	104.92	103.12	100.28	1.19
	157.38	153.7	97.66	0.21
4,5-di-O-CQA	22.34	22.17	99.24	0.62
	44.69	45.58	102.00	0.58
	67.03	65.66	97.95	0.80

CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA in UV-A irradiated aerial *A. chusanensis* extract were approximately 117.69, 12.59, 104.92, and 44.69 $\mu\text{g/mL}$, respectively. Thus, the spike concentrations of 3-O-CQA corresponding to 50, 100, and 150% of the blank sample were 58.8, 117.7, and 176.5 $\mu\text{g/mL}$. We confirmed that it aligned well with the measured amount, with average recoveries of 101.7, 101.8, and 99.8%. The spike concentrations of 3,4-di-O-CQA in the 50, 100, and 150% blank samples were 6.29, 12.59, and 18.88 $\mu\text{g/mL}$, respectively. The results demonstrated strong alignment with the measured amounts (6.16, 12.29, and 18.67 $\mu\text{g/mL}$), achieving impressive average recoveries of 98.0, 97.7, and 98.9%, respectively.

Moreover, the comparison between the spike concentration and the measured amounts of 3,5-di-O-CQA and 4,5-di-O-CQA revealed average recovery rates of 97.7–100.3% and 97.9–102.0%, respectively. The relative standard deviation (RSD) of the average recovery rate was valid, ranging from 0.21 to 2.17%. All recovery rates of the four CQA derivatives in the UV-A-irradiated aerial part of the *A. chusanensis* extract were verified to comply with the accuracy guidelines, ranging from 97.7 to 102%.

Precision. The precision of the measurements was validated by evaluating the repeatability and reproducibility, specifically through intraday and interday precision assessments. For intraday precision, 1.0, 2.0, and 3.0 g of UV-A-irradiated aerial *A. chusanensis* powder were measured five times according to the change in the sample amount.

As shown in Table 4, the intraday precision of the four CQA derivatives was confirmed with a RSD range of 0.17–0.48% for

Table 4. Repeatability for Intraday Precision of 3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA Contents in the Aerial Parts of *A. chusanensis* Powder

compounds	<i>A. chusanensis</i> powder (g)	contents mean ($\mu\text{g/g}$)	SD	RSD (%)
3-O-CQA	1	6,665.21	11.62	0.17
	2	6,847.01	33.32	0.48
	3	6,828.46	15.71	0.23
3,4-di-O-CQA	1	682.14	2.65	0.39
	2	662.53	5.94	0.89
	3	632.96	5.32	0.84
3,5-di-O-CQA	1	6,476.84	58.72	0.91
	2	6,551.59	38.13	0.58
	3	6,407.01	82.19	1.28
4,5-di-O-CQA	1	2,582.31	29.99	1.16
	2	2,703.10	13.02	0.48
	3	2,623.03	21.35	0.80

3-O-CQA, 0.39–0.89% for 3,4-di-O-CQA, 0.58–1.28% for 3,5-di-O-CQA, and 0.48–1.16% for 4,5-di-O-CQA. For interday precision, 1.5 and 2.5 g of UV-A irradiated aerial *A. chusanensis* powder were extracted with 90% ethanol and diluted 10 times to prepare interday precision samples. In the 1.5 and 2.5 g of UV-A irradiated aerial *A. chusanensis* powder, the four CQA derivatives had similar contents for each compound per 1 g of dry weight as follows: 6,648 and 6,811 $\mu\text{g/g}$ of 3-O-CQA, 684 and 646 $\mu\text{g/g}$ of 3,4-di-O-CQA, 6,467 and 6,405 $\mu\text{g/g}$ of 3,5-di-O-CQA, and 2,372 and 2,541 $\mu\text{g/g}$ of 4,5-di-O-CQA (Table 5). For interday precision, the RSD values of all CQA derivatives ranged from 0.45 to 1.23%. These tests were repeated five times daily for five consecutive days. Intraday precision (RSD < 3.7%) and interday precision (RSD < 6%) were achieved following the Ministry of Food and Drug Safety's health functional food indicator substance validation guidelines.

Quantitative Analysis of CQA Derivatives in *A. chusanensis*. The metabolite patterns of the aerial parts of

Table 5. Reproducibility for Interday Precision of 3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA Contents in the Aerial Parts of *A. chusanensis* Powder

compounds	<i>A. chusanensis</i> powder (g)	contents mean ($\mu\text{g/g}$)	SD	RSD (%)
3-O-CQA	1.5	6,648.36	54.94	0.82
	2.5	6,810.85	30.32	0.45
3,4-di-O-CQA	1.5	683.98	8.10	1.18
	2.5	645.72	5.92	0.91
3,5-di-O-CQA	1.5	6,467.43	79.84	1.23
	2.5	6,404.79	76.48	1.19
4,5-di-O-CQA	1.5	2,371.61	26.31	1.10
	2.5	2,540.67	26.40	1.03

A. chusanensis exposed to UV-A and UV-B were compared with those of unirradiated *A. chusanensis* (control). As shown in Figure 3, four peaks ($t_R = 15.4, 22.9, 24.1,$ and 26.0 min) in UV-A-treated *A. chusanensis* showed an increasing pattern, whereas the overall peaks in UV-B-treated *A. chusanensis* decreased compared to the control at an LC chromatogram of 254 nm. UV light was irradiated to *A. chusanensis* for 48 h in a plant growth chamber to maintain the temperature, humidity, and light intensity. In particular, UV-A irradiation of *A. chusanensis* selectively elevated the levels of 3-O-CQA, 3,4-di-O-CQA, 3,5-di-O-CQA, and 4,5-di-O-CQA (Figure 4 and Table 6). UV-A irradiation of aerial *A. chusanensis* increased total CQA content to $37,243 \mu\text{g/g}$, approximately 2.2 times higher than the control level of $17,081 \mu\text{g/g}$. Conversely, UV-B

irradiation of aerial *A. chusanensis* resulted in a decrease in total CQA contents, from $17,081$ to $10,839 \mu\text{g/g}$. In UV-A irradiated aerial *A. chusanensis*, the contents of 3-O-CQA ($1,886 \rightarrow 4,664 \mu\text{g/g}$), 3,4-di-O-CQA ($1,487 \rightarrow 3,162 \mu\text{g/g}$), 3,5-di-O-CQA ($10,760 \rightarrow 21,764 \mu\text{g/g}$), and 4,5-di-O-CQA ($2,948 \rightarrow 7,653 \mu\text{g/g}$) increased 2.47, 2.12, 2.02, and 2.59 times, respectively. This confirms that the content of the four CQA derivatives increased more than 2-fold. UV light is divided into UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm) wavelengths; the shorter the wavelength, the greater the energy associated with that light.³⁸ Consequently, the CQA derivative of *A. chusanensis* aerial parts was more effective in the long-wavelength than in the short-wavelength UV-B treatment. Moreover, they better enhanced the metabolite content under low-level than high-level light energy.

CONCLUSIONS

This study aimed to enhance metabolite levels in the aerial parts of *A. chusanensis* using light sources. UV-A and UV-B radiation were applied to *A. chusanensis* in the plant growth chamber equipped with a thermostat, humidity controller, and UV light. The metabolite levels increased with UV-A irradiation and decreased with UV-B irradiation of *A. chusanensis*. The predominant eight metabolites were 3-O-CQA, rutin, 3,4-di-O-CQA, 3,5-di-O-CQA, biorobin, luteolin-7-O- β -glucoside, 4,5-di-O-CQA, and luteolin identified using LC-Q-TOF/MS analysis. UV-A treatment of *A. chusanensis* resulted in a distinct increase in the CQA derivative content,

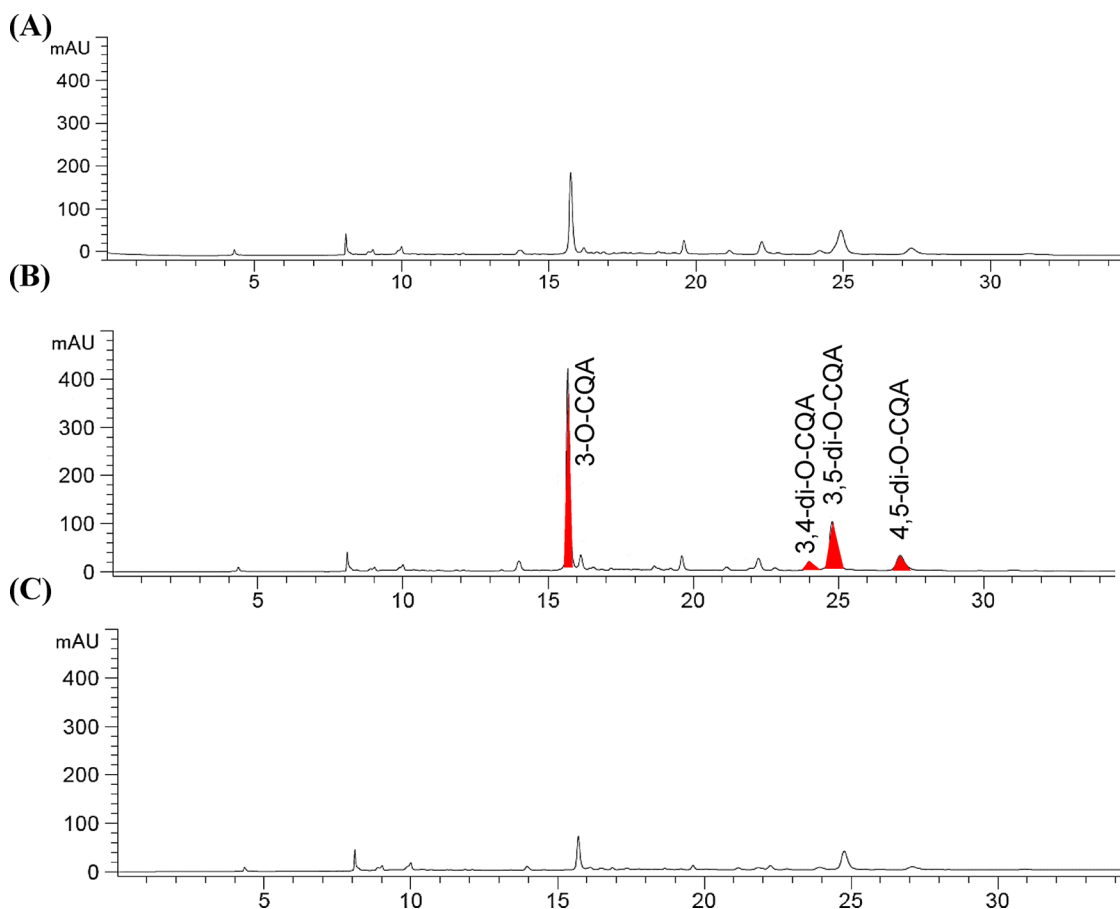


Figure 3. HPLC chromatogram of (A) control, (B) UV-A, and (C) UV-B irradiated *A. chusanensis* extract at 254 nm.

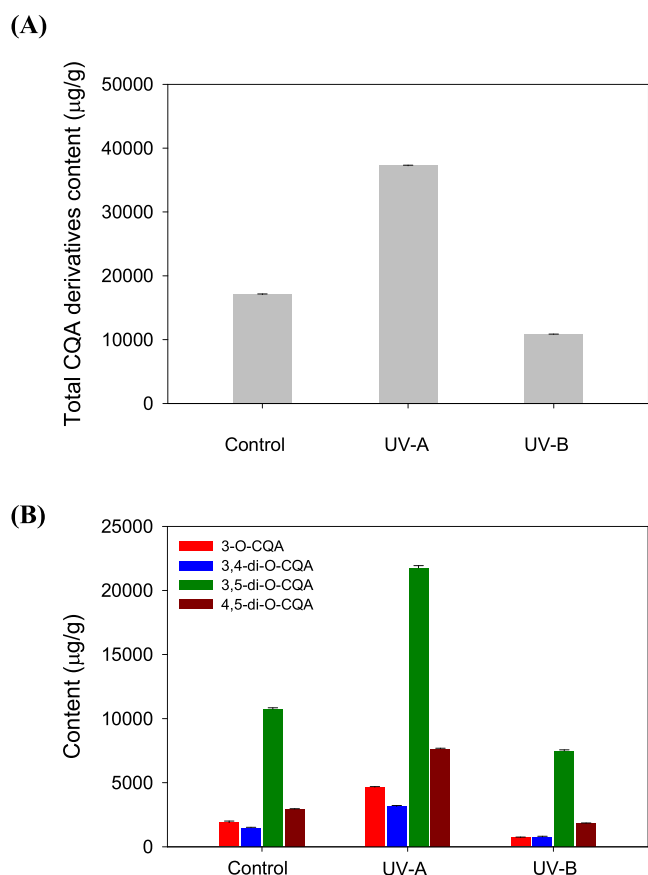


Figure 4. Comparison of (A) total CQA and (B) individual CQA content in the aerial parts of *A. chusanensis* by UV light irradiation.

whereas flavonoid levels remained unchanged. Therefore, four CQA derivatives were applied to validate the analytical method in representative UV-A-treated aerial parts of *A. chusanensis*, following the KFDA criteria, including specificity, linearity, accuracy, and precision. In the HPLC chromatogram, the peaks of the four CQA derivatives were well separated from those of UV-A-irradiated aerial *A. chusanensis*. An excellent coefficient of determination ($R^2 > 0.999$) was obtained using the calibration curve of the CQA derivatives at appropriate concentrations. The LOD and LOQ were calculated using regression equations as 0.501 and 1.519 for 3-O-CQA, 1.187 and 3.596 for 3,4-di-O-CQA, 0.422 and 1.278 for 3,5-di-O-CQA, and 1.636 and 4.957 for 4,5-di-O-CQA, respectively. The recovery rate for accuracy was 97.66–102%, consistent with the AOAC guidelines, which indicate a range of 95–105% at concentrations below 0.1% (1 mg/g). Intraday and interday precisions also displayed in the range of RSD as 0.17–1.28 and 0.45–1.23%, respectively, which complied with AOAC guidelines (repeatability RSD < 3.7% and reproducibility RSD < 6% at 0.1%). For the quantitative analysis, UV-A irradiated aerial *A. chusanensis* (37,243 μg/g) exhibited four CQA derivatives

that were selectively elevated by more than 2-fold than the control (17,081 μg/g). In this study, UV-A irradiation significantly increased the content of CQA derivatives within 48 h in the aerial parts of *A. chusanensis*. In this study, analytical method validation of increased CQA derivatives was implemented in aerial *A. chusanensis* for the first time.

■ ASSOCIATED CONTENT

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.5c05804>.

Linearity of CQA derivatives; calibration curve of CQA derivatives; and images of plant materials (*Aster × chusanensis*) (PDF)

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Table 6. Contents of Induced CQA Derivatives (μg/g) in the Aerial Parts of *A. chusanensis* by UV Irradiation

treatment	compounds				total CQA contents
	3-O-CQA	3,4-di-O-CQA	3,5-di-O-CQA	4,5-di-O-CQA	
control	1,886 ± 120	1,487 ± 35	10,760 ± 80	2,948 ± 24	17,081 ± 67
UV-A	4,664 ± 25	3,162 ± 62	21,764 ± 174	7,653 ± 33	37,243 ± 78
UV-B	721 ± 30	776 ± 42	7,493 ± 71	1,837 ± 16	10,839 ± 40

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Author Contributions

J.Y.K.: investigation, formal analysis, conceptualization, methodology, and validation; Y.-H.Y., J.E.P., and H.Y.B.: conceptualization, resources, and funding; M.-J.K., H.-S.S., and Y.G.S.: investigation and formal analysis; K.D.K.: writing—review and editing; K.-H.S.: writing—review and editing and resources; and J.Y.K.: writing—original draft and supervision

Notes

The authors declare no competing financial interest.

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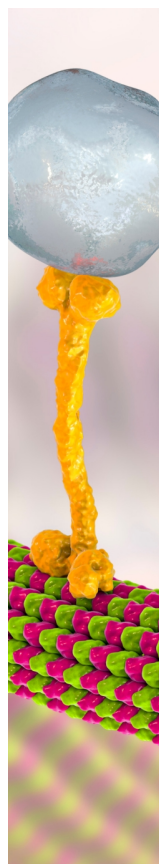
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