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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*

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Abstract

Glehnia littoralis, a medicinal herb employed in traditional practices for alleviating fatigue, cough, and a dry throat, is recognized for its beneficial properties due to a diverse array of active compounds found in its extracts. For example, the *G. littoralis* roots (Radix Glehniae) mainly contain coumarins and phenolic acids, serving as the primary focus of this study. Despite the widespread use of the tools in various industries and the development of multiple analytical methods for their examination, the edible aerial parts have industrial potential, and there is currently no analytical method available to identify their key components. In this study, a high-performance liquid chromatography method combined with diode array detection (HPLC–DAD) was developed to simultaneously detect 16 phenolic compounds previously reported to be present in the edible aerial parts of *G. littoralis*. The proposed approach included using gradient elution to change the solvent system from water/acetonitrile to water/methanol. Furthermore, the method validation was conducted, assessing its linearity, limit of detection, limit of quantification, precision, accuracy, and recovery, all of which demonstrated satisfactory results. Subsequently, the developed method was applied to quantify the phenolic compounds in various *G. littoralis* samples obtained from different organs, solvent extraction processes, and processing methods. Moreover, the online HPLC-ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) assay was used to evaluate the antioxidant capacities of individual constituents, identifying four important antioxidants and estimate the overall antioxidant capacity of the *G. littoralis* extract.

Keywords High-performance liquid chromatography, Diode-array detection, Phenolic compound, *Glehnia littoralis*, Antioxidant activity

Introduction

Glehnia littoralis belongs to the Apiaceae family and is a perennial herb distributed in coastal dunes throughout East Asia [1]. The *G. littoralis* root, Radix Glehniae, has been traditionally used in medicine for diaphoretic, antipyretic, and analgesic purposes [2]. It is also listed in the Korean, Japanese, and Chinese pharmacopeia and is prescribed as a tonic and mucolytic for the treatment of gastrointestinal and respiratory disorders [3]. Furthermore,

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the dried roots are valuable as food ingredients and medicinal materials for healthy foods. For examination, the roots are often added to soups, porridge, medicinal wines, and teas [1]. To date, 186 chemical constituents have been identified for *G. littoralis*, including terpenoids, organic acids, phenylpropanoids, coumarins, lignans, flavonoids, steroids, volatile oils, polysaccharides, and polyols [1, 4]. As a result, this herb exhibits a range of pharmaceutical properties, including antibacterial, antifungal, anti-inflammatory, antioxidant, antitumor, analgesic, hepatoprotective, and immunoregulatory effects [4, 5]. Among the identified compounds present in *G. littoralis*, 69 major components were identified in the root, including the coumarins psoralen, imperatorin, isoimperatorin, and bergapten. Research into the pharmacological effects of these coumarin components has revealed their anti-carcinogenic effects on liver and gastric cancer cells, as well as anti-inflammatory properties [4]. In addition, flavonoids, including quercetin, isoquercetin, rutin, chlorogenic acid, and caffeic acid have been identified in the roots of *G. littoralis* as major antioxidants [6].

In several countries, the fresh leaves and stems of *G. littoralis* are commonly consumed and used in processed foods, such as rice cakes and tea [7]. Although the majority of phytochemicals in this plant are derived from the underground organs (i.e., the roots and rhizomes), a few studies have explored the phytochemical compositions of its aerial parts. For example, *G. littoralis* leaf extracts significantly inhibited tyrosinase and elastase, implying possible application in cosmetic products for skin whitening and anti-wrinkle qualities [5]. Moreover, extracts from the aerial parts of this plant have been shown to regulate humoral immunity and potently inhibit the development of human cancer cells. This also raises the possibility that the antiproliferative action of the crude extracts of *G. littoralis* may be partially attributed to the coumarins and polyacetylenes [8]. Moreover, the whole plant extract demonstrated a noteworthy inhibitory activity against nitric oxide production, suggesting that angular-type dihydropyranocoumarins possessing ester groups at the C-3′ and C-4′ positions hold considerable therapeutic promise as anti-inflammatory agents [9]. Despite ongoing studies into *G. littoralis* for application in pharmaceuticals, health-functional foods, and cosmetics, there have been very few studies on the edibility of the aerial parts, and research has predominantly focused on the root, which is used solely in traditional medicine [1]. As a result, there are no established methods to assess the quality of the different parts of *G. littoralis* and the quality of any processed foods containing this herb.

In this study, 16 phenolic compounds present in the *G. littoralis* aerial parts are identified using liquid chromatography–mass spectrometry (LC–MS). Additionally, an analytical method is developed by applying the optimal solvent conditions to an HPLC–DAD (diode array detection) system. To validate this method, the specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and quantification range are evaluated. Furthermore, the phenolic content is quantified based on the developed method, considering the various processing methods and extraction solvents used to obtain the *G. littoralis* aerial extract. Finally, the antioxidant properties of the individual phenolic compounds present in the aerial parts of the plant are determined using an online HPLC-ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) method.

Materials and methods

Chemicals and preparation of the standard solutions

Nicotiflorin (≥ 98.8%, CAS No. 17650-84-9), hyperoside (≥ 95.8%, CAS No. 482-36-0), and phellopterin (≥ 98.7%, CAS No. 2543-94-4) were purchased from Chromadex (Irvine, CA, USA). Chlorogenic acid (≥ 95.0%, CAS No. 327-97-9), caffeic acid (≥ 98.0%, CAS No. 331-39-5), scopoletin (≥ 99.0%, CAS No. 92-61-5), rutin (≥ 95.0%, CAS No. 153-18-4), isoquercetin (≥ 98.2%, CAS No. 482-35-9), psoralen (≥ 99.0%, CAS No. 66-97-7), xanthotoxin (≥ 98.0%, CAS No. 298-81-7), bergapten (≥ 99.0%, CAS No. 484-20-8), isopimpinellin (≥ 95.0%, CAS No. 482-27-9), oxypeucedanin (≥ 98.0%, CAS No. 737-52-0), imperatorin (≥ 98.0%, CAS No. 482-44-0), isoimperatorin (≥ 98.0%, CAS No. 482-45-1), bergamottin (≥ 98.0%, CAS No. 7380-40-7), ABTS (CAS No. 30931-67-0), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, CAS No. 53188-07-1), potassium persulfate (CAS No. 7727-21-1), and formic acid (CAS No. 64-18-6) were supplied by Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade water, methanol, and acetonitrile were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

The 16 standard compounds, including chlorogenic acid, caffeic acid, scopoletin, rutin, hyperoside, isoquercetin, nicotiflorin, psoralen, xanthotoxin, isopimpinellin, bergapten, oxypeucedanin, imperatorin, phellopterin, isoimperatorin, and bergamottin were individually weighed (1 mg each) and dissolved in pure methanol (1 mL) to prepare the standard stock solutions. Subsequently, working solutions were produced from the standard stock solutions by dilution with water until reaching the appropriate concentrations for construction of the calibration curves. All solutions were stored at − 20 °C until required for further use.

Plant materials and sample preparation

The aerial parts and roots of *G. littoralis* were purchased from Wild-Crafted Sources (Gangneung,

Gangwon-do, Korea), and the materials were harvested from three-year-old plants (Fig. 1A). The samples were lyophilized, ground into fine powders, and stored in a freezer until required for further use. The sample powder (100 mg) was then dissolved in 80% methanol (1 mL) and extracted for 90 min via ultrasonication at room temperature. Each extract solution was subsequently subjected to centrifugation at 3500 rpm for 20 min prior to filtration with a 0.45 µm syringe filter. The analytical method was optimized and the 16 phenolic compounds present in *G. littoralis* were quantified using these prepared samples.

LC–MS

LC–MS analysis was done by using Liquid Chromatography/Mass Spectrometer at Gyeongnam Bio and Anti-aging Core Facility Center. To identify the 16 phenolic compounds, a LC–MS in positive ESI mode was employed. The ESI parameters were set as follows: capillary temperature = 350 °C, nebulizer gas pressure = 35 psi, drying gas flow = 9 L min^{-1} (N_2), and capillary voltage = 4000 V. Full-scan MS spectra were acquired in the mass-to-charge (*m/z*) range of 150–1500. Data analysis was performed using the Agilent Chem Station software. A Luna 5U C_{18} column (150 mm × 4.60 mm, 5 µm, Phenomenex Inc., USA.) was used for the chromatographic

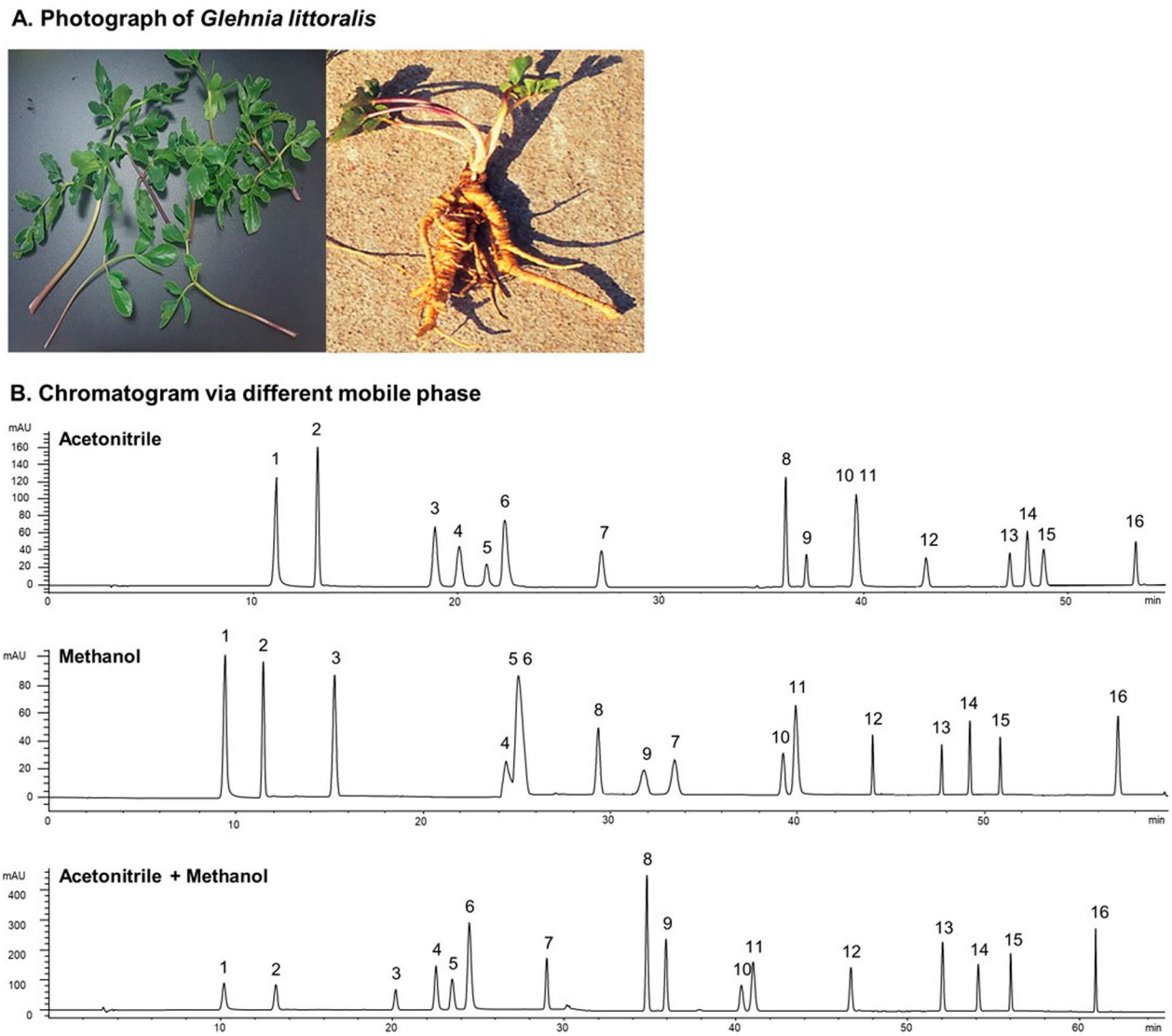


Fig. 1 **A** Photographic images of the aerial part (left) and root (right) from *G. littoralis*, and **B** representative chromatograms analyzed using different mobile phases. 1: Chlorogenic acid, 2: caffeic acid, 3: scopoletin, 4: rutin, 5: hyperoside, 6: isoquercetin, 7: nicotiflorin, 8: psoralen, 9: xanthotoxin, 10: isopimpinellin, 11: bergapten, 12: oxypeucedanin, 13: imperatorin, 14: phellopterin, 15: isoimperatorin, and 16: bergamottin

separation. Gradient elution was performed using mobile phase A (0.3% formic acid in water) and B (0.3% formic acid in acetonitrile). The gradient conditions were as follows: 15% B, increasing to 23% B at 5 min, 25% B at 12 min, 46% B at 15 min, 48% B at 25 min, 50% B at 35 min, 65% B at 45 min, and 90% B at 55 min. The flow rate was adjusted to 0.7 mL min^{-1}, maintaining a column temperature of 35 °C, and a 10 µL injection volume was employed.

HPLC–DAD analysis

HPLC–DAD analysis was conducted using an Agilent 1200 series instrument (Agilent Technologies, USA) equipped with a quaternary pump, an ALS autosampler, a DADVL DAD, and a TTC column compartment. The column used was a Hydrosphere C_{18} column (4.6 × 250 mm, 5 µm) manufactured by YMC (Tokyo, Japan). The oven temperature was held constant at 35 °C with 10 µL injection volume, and the flow rate set to 1 mL min^{-1}. Mobile phase A was composed of water with 0.3% formic acid (v/v), while mobile phase B contained acetonitrile with 0.3% formic acid, and 100% methanol made up mobile phase C. The 16 phenolic compounds were separated via gradient elution as follows (time, %A/%B/%C): 0 min, 88/12/0%; 8 min, 88/12/0%; 12 min, 82/18/0%; 22 min, 82/18/0%; 26 min, 77/23/0%; 27 min, 50/0/50%; 37 min, 50/0/50%; 56 min, 9/0/91%; 65 min 9/0/91%. All compounds exhibited a maximum absorption band at 254 nm. DAD was employed for method development.

Method validation

The method used to quantify the 16 phenolic compounds was validated in terms of its linearity, LOD, LOQ, precision, and accuracy.

Linearity

An external standard calibration method was used. The stock solutions were serially diluted to prepare seven solutions with various concentrations of the phenolic compounds. The ranges of linearity were obtained for the 16 compounds as follows: chlorogenic acid and rutin, 0.5–1000 µg mL^{-1}; isoquercetin and nicotiflorin, 0.5–100 µg mL^{-1}; remaining 12 compounds, 0.25–50 µg mL^{-1}. Each standard was determined using the developed analytical method, and calibration curves were constructed using linear regression. The linearity of each standard was confirmed by evaluating the correlation coefficient of the calibration curve ($R^2 \geq 0.999$).

LOD and LOQ

The LOD and LOQ were calculated to assess the sensitivity of the method. The mean values from three replicated measurements of each standard solution at different concentrations were used to generate a calibration curve. The following equations were used to calculate the LOD and LOQ based on the standard deviation of the intercept (δ) and the slope of the calibration curve (C).

$$LOD = 3.3 \times \delta / C$$

$$LOQ = 10 \times \delta / C$$

Precision, accuracy, and recovery

The precision of the proposed technique was assessed based on the intra- and inter-day precisions. The intraday precision was assessed by calculating the retention time and peak area of each standard solution at three different concentrations within a single day, and is expressed as the relative standard deviation (RSD%). The RSD values of the standard solutions across a three-day period were used to evaluate the inter-day precision. Recovery tests were conducted by spiking the extracts with three different concentrations (50, 100, and 250 µg mL^{-1}) of the standard mixture. By calculating the recovery percentage from these tests that were run, the accuracy of the HPLC method was assessed.

$$Recovery(\%) = \frac{Detected\ concentration}{Nominal\ concentration} \times 100$$

Processing procedure for *G. littoralis*

In preparation for appropriate processing of the *G. littoralis* aerial part, the effects of thermal treatment and different solvent extraction processes were examined to compare the resulting contents of the various phenolic compounds.

Thermal treatment on the G. littoralis aerial parts

The fresh *G. littoralis* aerial parts were subjected to thermal treatment as follows. Shade drying was carried out under a natural airflow and at ~ 25 °C for 2–3 d. For oven drying, the aerial parts were dried in trays in a ventilated oven at 70 °C for 8 h. Roasting conditions were also examined by heating at 110 °C for 20 min without burning. To steam the materials, the aerial part was added into a steaming basket and placed in a large pot with an integral stainless-steel strainer for 40 min at 73–76 °C. After the completion of each thermal treatment, the materials were allowed to cool to room temperature for 30 min. Subsequently, they were frozen at − 80 °C for 24 h and then lyophilized at − 120 °C for 3 d. The freeze-dried samples were ground into fine powders, and the phenolic compounds present in each sample were quantified on a dry weight basis.

Optimization of the extraction solvent

Dried powders (60 g) from the *G. littoralis* aerial parts and roots were combined with the desired extraction solvent (95% ethanol or water, 600 mL). The mixtures were incubated in round-bottomed flasks at 80 °C for 3 h. Subsequently, each extract was filtered using No. 2 filter paper (8 μm, Whatman, Maidstone, UK) and concentrated at 40 °C for 3 h using a rotary evaporator. Subsequently, a sample of each extract (10 mg) was dissolved in 80% methanol (1 mL) and quantification of phenolic compounds was carried out.

On-line HPLC-ABTS radical scavenging activity

The antioxidant activities of the components representing the individual HPLC peaks were evaluated using an online HPLC antioxidant detector system employing the trolox equivalent antioxidant capacity (TEAC) assay, following the method outlined by Koleva et al. [10]. The separation conditions were identical to those described above for the HPLC method. The injection volume was 10 μL, and the detection wavelengths were set at 254 and 734 nm. The mobile phase was mixed with the ABTS solution (5.5 μM, prepared in a 10% solution of MeOH (v/v) in phosphate-buffered saline), which was delivered by a second pump at a flow rate of 0.5 mL min^{-1}. Using a multiwavelength detector (MWD), the reaction products were determined to show a negative peak at 734 nm.

Trolox was used as a standard compound to measure the radical scavenging activity, and the Trolox equivalent (TE) values were calculated using a calibration curve plotted from 0.25 to 62.58 μg mL^{-1} ($y = 53.101x + 3.9368$; $R^2 = 0.9988$). The sum of antioxidant activities of all relevant molecules was used to determine the total antioxidant activity (TAA). In this case, the antioxidant compounds were chlorogenic acid, caffeic acid, rutin, and isoquercetin. To quantify the radical scavenging capacity of each compound, the TEAC was used, and the results were expressed as the concentration of Trolox (μM) that exhibits the same activity as the compound of interest at a concentration of 1 μM.

$$TEAC = \frac{Compound(\mu M)}{Trolox(\mu M)}$$

Statistical analysis

All statistical analyses were performed using the SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA). Mean comparisons of the *G. littoralis* organs and extraction solvents were performed using the *t*-test at a level of $p < 0.05$. To evaluate any significant differences based on the different thermal treatment approaches, a one-way analysis of variance (ANOVA) was conducted, followed by Tukey's multiple range test at a significance level of $p < 0.05$. The results, obtained from three biological replicates, are presented as the mean ± standard deviation.

Results and discussion

Optimization of the chromatographic separation process and identification of the phenolic compounds present in the aerial parts of *G. littoralis*

Based on previous literature [1], a total of 16 phenolic compounds, including ten coumarins [scopoletin (**3**), psoralen (**8**), xanthotoxin (**9**), isopimpinellin (**10**), bergapten (**11**), oxypeucedanin (**12**), imperatorin (**13**), phellopterin (**14**), isoimperatorin (**15**), and bergamottin (**16**)], four flavonoids [rutin (**4**), hyperoside (**5**), isoquercetin (**6**), and nicotiflorin (**7**)], and two hydroxycinnamic acids [chlorogenic acid (**1**) and caffeic acid (**2**)] were selected as the major phenolic compounds found in the *G. littoralis* aerial part. To establish the optimal HPLC conditions for these compounds, LC–MS analysis was conducted on the aerial part extract obtained using 80% methanol. As a result, 16 major peaks were obtained and subsequently analyzed using MS. For each detected compound, the mass value of the $[M+H]^+$ ion was confirmed, and the compound was identified by referencing the reported compounds present in *G. littoralis*. The absorption wavelengths and mass values of these compounds are listed in Additional file 1: Table S1, and their chemical structures are shown in Additional file 1: Fig. S1. It was confirmed that the major MS peaks of representative compounds from *G. littoralis* appeared with high intensity and exactly matched the $[M+H]^+$ values (Additional file 1: Fig. S2). The mobile phase elution conditions were then optimized to separate the 16 phenolic compounds from the 80% methanol extract. The use of acetonitrile and methanol individually did not provide efficient separation. For example, when acetonitrile was employed, peaks 10 and 11 co-eluted as a single peak. Furthermore, methanol alone did not adequately separate peaks 4, 5, and 6. As a result, the elution conditions were adjusted for the separation of peaks 1–6 using an acetonitrile/water system, and subsequently, a methanol/water system was used as the elution solvent for the separation of peaks 8–16. The chromatograms obtained under these elution conditions are shown in Fig. 1B, while the optimal analytical conditions are listed in Table 1.

Various bioactive compounds, including coumarins, flavonoids, lignanoids, polyacetylenes, and organic acids, have been identified in different organs of *G. littoralis*. Among these, the coumarins, which are characterized by a structure containing two fused six-membered rings (one benzene ring and the other containing an alkene functionality and an ester group) bearing a phenyl, alkoxyl, hydroxyl, or isopentenyl substituent, are prominent components of the *G. littoralis* root. In contrast,

Table 1 Proposed analytical method for determination of 16 phenolic compounds in *G. littoralis*

HPLC unit		**Agilent 1260 series**	
Column		YMC Hydrosphere C18 (4.6 × 250 mm, i.d., 5 μm particle size)	
Detector		Diode array detector (DAD)	
Detection wavelength (nm)		254 nm	
Flow rate (mL min^{-1})		1.0 mL min^{-1}	
Injection volume		10 μL	
Column temperature		35 ℃	
Mobile phase		A: 0.3% formic acid in Water B: 0.3% formic acid in Acetonitrile C: Methanol	
Time (min)	**Mobile phase A (%)**	**Mobile phase B (%)**	**Mobile phase C (%)**
HPLC gradient profile			
0	88	12	0
8	88	12	0
12	82	18	0
22	82	18	0
26	77	23	0
27	50	0	50
37	50	0	50
56	9	0	91
65	9	0	91

the flavonoids, which are typically present in the form of glycosides or carbohydrates, primarily accumulate in the aerial parts [4]. Growing knowledge of the biological and pharmacological effects of bioactive compounds has spurred the development of various analytical approaches for their qualitative and quantitative evaluation. Indeed, several chromatographic techniques have been used to analyze the phenolic compounds present in *G. littoralis*. To date, numerous analytical methods have been established for the quantitative examination of the bioactive compounds present in *G. littoralis* roots. As an example, 10 coumarins, four phenolic acids, and adenosine were detected in the root of *G. littoralis* using a combination of HPLC and tandem MS [11]. A method based on high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC–ESI–MS/MS) has been also established for the simultaneous identification of 15 bioactive compounds, including coumarins, phenolics, and adenosine, which are present in the *G. littoralis* roots [12]. This method employs a binary mobile phase consisting of acetonitrile and water in gradient mode. The detection and identification of peaks at 310 nm revealed the presence of bergapten, cnidilin, imperatorin, isoimperatorin, psoralen, and xanthotoxin. In addition, using HPLC coupled with photodiode array detection at 254 nm, a water/methanol mobile phase successfully separated seven specific bioactive compounds from the *G. littoralis* roots [13]. However, little research has been carried out into the edible potential of the aerial parts, and as a result, there are no defined procedures for assessing the quality of any samples obtained from these parts. In the simultaneous analysis of multiple analytes, the co-elution of isomers and analytes with similar physicochemical properties typically results in poor selectivities and/or longer analysis times when DAD or photodiode array detection are employed [14]. It is therefore essential to optimize and resolve these coelution problems, potentially through the use of three different mobile phases. However, to date, this has received little research attention for the development of unique separation methods. As one example, a method was established for the simultaneous separation of 12 impurities through gradient elution using three mobile phases, namely a disodium hydrogen phosphate solution, acetonitrile, and methanol [15]. With this in mind, a water/acetonitrile/methanol system was examined here in, and it was found that the good separation and precise quantification of 16 phenolic compounds could be achieved using extracts obtained from the *G. littoralis* aerial parts.

Method validation

The established HPLC method was validated in terms of its linearity, specificity, precision, accuracy, LOD, and LOQ based on ICH guidelines [16]. Calibration curves

for the 16 phenolic compounds were obtained using five serial dilutions of mixed standard solutions, and plotting their peak areas (y) against their concentrations (x) to provide linear equations ($y = ax + b$). These equations, along with the corresponding correlation coefficients (R^2), LODs, and LOQs are summarized in Table 2. Excellent linearities were shown by all calibration curves, with correlation coefficients (R^2) > 0.999, thereby satisfying the criterion of $R^2 > 0.998$ for a satisfactory linearity. For the 16 phenolic compounds examined herein, the LOD ranged from 0.31 to 1.93 μg mL^{-1}, while the LOQ ranged from 0.95 to 5.86 μg mL^{-1}, indicating the high sensitivity of the method for detecting low concentrations of the analytes.

Method precision was evaluated through both intra- and inter-day experiments. The intraday precision was evaluated by performing three repetitive injections of the standard solutions containing 16 phenolic compounds in a single day, whereas the three consecutive days were used to evaluate inter-day accuracy. The intra- and inter-day precisions of the peak areas obtained for these 16 compounds were calculated and expressed as RSDs (Additional file 1: Table S2), giving values of 1.38–6.06% and 1.23–8.24%, respectively. The accuracy was determined by expressing the mean concentration of each quantified sample as a percentage of the nominal concentration, resulting in values of 90.35–116.40%. The precision and accuracy of the method were therefore clearly within the acceptable limits of a 15% RSD (20% close to the LOQ) [17]. The method recovery was determined by the addition of specific concentrations of the various standards, followed by subsequent sample recovery. For this purpose, 16 standard solutions were added to the *G. littoralis* extracts, and the average recoveries were calculated by comparing the detected quantity with the added quantity (Additional file 1: Table S3). As a result, the recoveries were determined to be 86.99–109.26%, with RSDs of 0.07–4.89%, thereby indicating that the developed method was accurate within the specified criteria. The above validation results confirm the reliability of the optimized analytical method for the simultaneous analysis of 16 phenolic compounds in *G. littoralis*.

Quantification of the different *G. littoralis* samples using the proposed HPLC method

The phenolic compounds present in the *G. littoralis* aerial parts and roots were quantified using the analytical method described herein. As shown in Table 3, the total phenolic contents were 18.32 and 0.19 mg g^{-1} dry weight (DW) in the *G. littoralis* aerial parts and roots, respectively. In the aerial parts, the majority of target coumarins detected were quantified in large amounts, whereas only four coumarins were detected in the roots, and these were present in very small quantities. Among the 10 coumarins of interest, psoralen (**8**) and bergamottin (**16**) were not detected in either the aerial parts or the roots. The highest flavonoid content was found in the aerial parts of the plant, and was determined to be 13.16 mg g^{-1} DW; among these, rutin (**4**) was the most abundant (i.e., 10.01 mg g^{-1} DW), accounting for more than half of total

Table 2 Results for the parameters of method validation of simultaneous determination for 16 phenolic compounds in *G. littoralis* by HPLC–DAD

Peak	Compound	Calibration range (μg mL^{-1})	Calibration curve	Correlation coefficient (R^2)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
1	Chlorogenic acid	5–1000	$y = 33.176x + 22.421$	0.9996	1.93	5.86
2	Caffeic acid	2.5–50	$y = 54.162x - 11.175$	0.9994	0.31	0.95
3	Scopoletin	2.5–50	$y = 39.628x - 5.7914$	0.9993	0.34	1.04
4	Rutin	5–1000	$y = 20.75x - 50.604$	0.9999	1.39	4.23
5	Hyperoside	2.5–50	$y = 28.517x - 5.9583$	0.9992	0.63	1.90
6	Isoquercetin	5–100	$y = 45.605x - 21.084$	0.9992	1.27	3.85
7	Nicotiflorin	5–100	$y = 16.772x - 4.5759$	0.9993	1.26	3.82
8	Psoralen	2.5–50	$y = 84.526x - 25.319$	0.9996	0.63	1.92
9	Xanthotoxin	2.5–50	$y = 46.756x - 7.2072$	0.9993	0.58	1.76
10	Isopimpinellin	2.5–50	$y = 23.361x - 3.2631$	0.9993	0.59	1.78
11	Bergapten	2.5–50	$y = 47.866x - 12.234$	0.9995	0.71	2.15
12	Oxypeucedanin	2.5–50	$y = 30.84x - 2.5168$	0.9993	0.69	2.08
13	Imperatorin	2.5–50	$y = 41.322x - 34.968$	0.9997	1.00	3.03
14	Phellopterin	2.5–50	$y = 25.084x - 1.9087$	0.9993	0.60	1.82
15	Isoimperatorin	2.5–50	$y = 24.793x - 3.4156$	0.9993	0.62	1.87
16	Bergamottin	2.5–50	$y = 29.773x - 5.1171$	0.9993	0.59	1.80

Table 3 Contents (mg g^{-1} dry weight) of 16 phenolic compounds in the *G. littoralis* aerial parts and roots

Classification	Peak	Compound	Aerial	Root
Coumarin	**3**	Scopoletin	$0.01 \pm 0.00^{**}$	0.01 ± 0.00
	8	Psoralen	n.d.[1]	n.d
	9	Xanthotoxin	$0.05 \pm 0.00^{*}$	0.04 ± 0.00
	10	Isopimpinellin	$0.16 \pm 0.02^{**}$	n.d
	11	Bergapten	$0.14 \pm 0.02^{**}$	n.d
	12	Oxypeucedanin	$0.02 \pm 0.00^{*}$	n.d
	13	Imperatorin	$0.41 \pm 0.01^{***}$	0.02 ± 0.00
	14	Phellopterin	$0.03 \pm 0.01^{***}$	n.d
	15	Isoimperatorin	$0.09 \pm 0.00^{***}$	0.02 ± 0.00
	16	Bergamottin	n.d	n.d
		Total	$0.93 \pm 0.04^{***}$	0.09 ± 0.01
Flavonoid	**4**	Rutin	$10.01 \pm 0.09^{***}$	n.d
	5	Hyperoside	$0.02 \pm 0.01^{*}$	n.d
	6	Isoquercetin	$0.94 \pm 0.01^{***}$	n.d
	7	Nicotiflorin	$2.19 \pm 0.04^{***}$	n.d
		Total	13.16 ± 0.11	n.d
Hydroxycinnamic acid	**1**	Chlorogenic acid	$4.23 \pm 0.02^{***}$	0.10 ± 0.01
	2	Caffeic acid	$0.02 \pm 0.00^{***}$	n.d
		Total	$4.25 \pm 0.02^{***}$	0.10 ± 0.01

Each data was represented as mean ± standard deviation (n = 3) and statistically analyzed using one-tailed *t*-test. Significant differences were assessed by ***($P < 0.001$), **($P < 0.01$), *($P < 0.05$)

[1] n.d., not detected

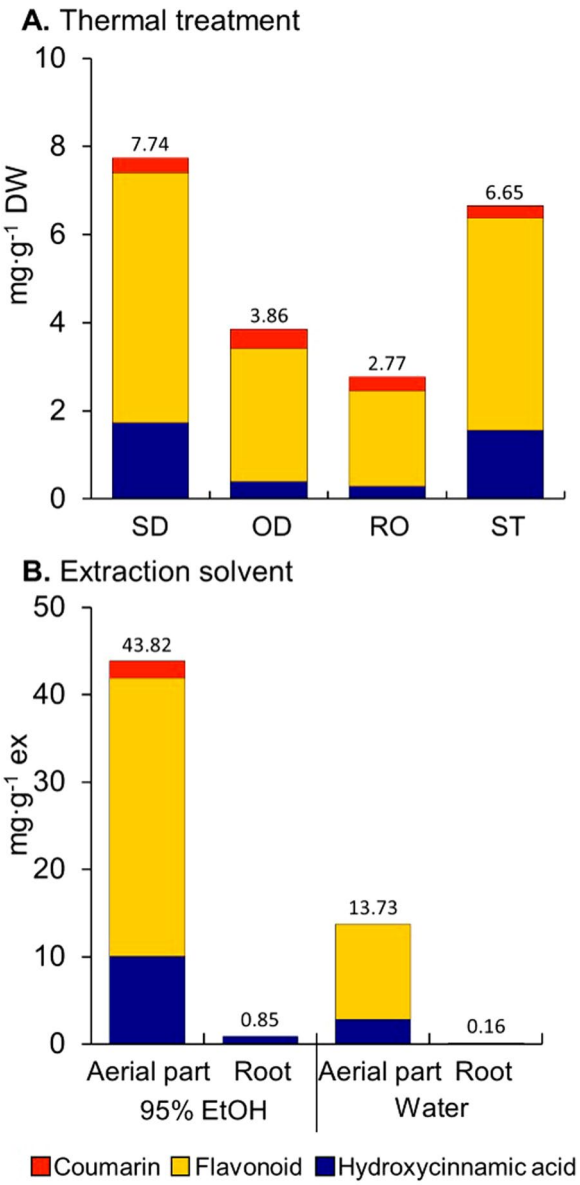


Fig. 2 Coumarin, flavonoid, and hydroxycinnamic acid contents following **A** different thermal treatment approaches, and **B** extraction using different solvents. Additional file 1: Tables S4 and S5 indicate the quantification results for the 16 individual phenolic compounds. SD, shade drying; OD, oven drying; RO, roasting; and ST, steaming

phenolic content. However, no flavonoids were detected in the roots. In terms of the hydroxycinnamic acids, chlorogenic acid (**1**) was detected at levels of 4.23 mg g^{-1} DW in the aerial parts, while the caffeic acid (**2**) content was 0.02 mg g^{-1} DW. In contrast, in the root, no caffeic acid (**2**) was detected, and the chlorogenic acid (**1**) levels were extremely low (i.e., 0.10 mg g^{-1} DW). Thus, among the 16 target compounds, only five were detected in the roots using the analytical method described herein, thereby indicating that this method is suitable for quantitative analysis of the *G. littoralis* aerial parts.

Given that the aerial parts (young leaves and stems) of *G. littoralis* are currently registered as food ingredients by the Korean Food and Drug Administration and hold potential for use as functional ingredients, it is crucial to employ appropriate processing procedures for the further utilization of this plant. Among the various processing procedures available for use with such specimens, thermal treatment was initially considered. Thus, the *G. littoralis* samples were subjected to a range of thermal conditions, including shade drying, oven drying, roasting, and steaming. Upon evaluation of the total phenolic contents after treatment, it was apparent that shade drying led to the highest content (7.74 mg g^{-1} DW), followed by steaming (6.65 mg g^{-1} DW), oven drying (3.86 mg g^{-1} DW), and roasting at (2.27 mg g^{-1} DW) (see Fig. 2A). Scopoletin (**3**), psoralen (**8**), bergamottin (**16**), and caffeic acid (**2**) were not detected after any thermal treatment, and the total coumarin levels remained consistent regardless of the thermal treatment approach employed (Additional file 1: Table S4). In contrast, the flavonoid and hydroxycinnamic acid contents varied, and with the exception of nicotiflorin (**7**), the contents of these compounds decreased upon increasing the harshness of the

treatment method (i.e., shade drying > steaming > oven drying > roasting). Thus, roasting led to the most significant reduction in the phenolic content, whereas shade drying was deemed most the suitable approach for further investigations.

It is well known that different thermal treatment conditions can significantly alter the phenolic contents of plant materials owing to the polymerization and degradation of these compounds [18]. More specifically, it has been reported that thermal treatment may lead to an increase in bound phenolic compounds, whilst promoting the degradation of free phenolic compounds [18, 19]. As indicated above, thermal treatments, such as oven drying, roasting, and steaming, negatively affected the levels of rutin (**4**) and chlorogenic acid (**1**) in the *G. littoralis* specimens. This aligns with previous research indicating that the levels of rutin and chlorogenic acid in avocado leaves decrease to varying degrees under different drying methods, including oven drying at 50 and 100 °C [20].

During the extraction of organic compounds from plant materials, the process is largely affected by the diverse compounds present in the plant matrix, the choice of extraction solvent, the active ingredient solubility, and the interactions of these compounds with other solutes. In addition, the concentrations of bioactive natural products in natural sources are typically low, and they are often embedded within the plant matrices. The effectiveness of the extraction process therefore also depends on the properties of the plant matrix, and the extraction solvent, temperature, pressure, and duration [21]. Thus, the phenolic compounds present in the extracts of the *G. littoralis* aerial parts and roots were extracted using the two most common extraction solvents, namely 95% ethanol and water (Fig. 2B). The extracts obtained from *G. littoralis* roots using these two extraction solvents did not contain coumarins or flavonoids, and only a small amount of hydroxycinnamic acid was detected. However, it appeared that 95% ethanol was more suitable as an extraction solvent, giving 43.82 mg g^{-1} extract (ex) compared to the 13.73 mg g^{-1} ex obtained using water for total phenolic compound contents. In addition, the 95% ethanol extract contained various coumarins, including isopimpinellin (**10**), bergapten (**11**), imperatorin (**13**), and isoimperatorin (**15**); no such coumarins were detected in the water extract (Additional file 1: Table S5). Furthermore, the majority of flavonoids and hydroxycinnamic acids were present in higher quantities in the 95% ethanol extract than in the water extract. Among these, rutin (**4**) represented the most abundant phenolic compound, followed by chlorogenic acid (**1**), nicotiflorin (**7**), and isoquercetin (**6**). These findings therefore demonstrate the successful enrichment of phenolic compounds in the 95% ethanol extract obtained from the aerial parts, and this is consistent with previous studies highlighting the significant impact of employing an ethanol/water-based solvent system for phenolic compound extraction from plant materials. For example, a higher the amount of total phenolic compounds was extracted from peanut skin using 80% ethanol instead of water [22].

Determination of the antioxidant activity using on-line HPLC-ABTS

Through the use of parallel chemical detection methods, online HPLC-ABTS analyses enable the simultaneous identification and quantification of active compounds [23]. Thus, using this approach, the analysis of a standard mixture of 16 phenolic compounds (Fig. 3A) revealed five components with ABTS radical-scavenging activity, namely chlorogenic acid (**1**), caffeic acid (**2**), rutin (**4**), hyperoside (**5**), and isoquercetin (**6**). A representative chromatogram of the 95% ethanol extract from the *G. littoralis* aerial parts is shown in Fig. 3B, wherein it can be seen that peaks of chlorogenic acid (**1**), rutin (**4**), and isoquercetin (**6**) were clearly visible at 734 nm, suggesting a significant ABTS radical-scavenging activity. Furthermore, a small peak was detected for caffeic acid (**2**). According to previous literature, the TEAC values for ascorbic acid, butylated hydroxyl anisole, and butylated hydroxyl toluene were found to be 1.08, 1.02, and 0.47 as representative antioxidant compounds, respectively [24]. In comparison, the TEAC values from *G. littoralis* was shown that among the four antioxidant compounds detected, rutin exhibited the highest TEAC (2.34), followed by isoquercetin, chlorogenic acid, and caffeic acid, with values of 1.56, 1.43, and 1.37, respectively (Table 4).

Furthermore, the radical-scavenging activities of the *G. littoralis* extracts from the aerial parts and the roots were measured. The concentration of each antioxidant compound was determined using an appropriate standard reference and the antioxidant activity was calculated based on the TE values. More specifically, for the four antioxidant compounds, the TE was highest in the aerial parts extracted with 95% ethanol (9.36 ± 1.20 mg TE g^{-1} ex), followed by the water extract of the aerial parts (3.39 ± 0.09 mg TE g^{-1} ex), the ethanol extract of the roots (0.65 ± 0.03 mg TE g^{-1} ex), and the water extract of the roots (0.15 ± 0.00 mg TE g^{-1} ex). The antioxidant effects of the total antioxidants obtained from each extract were also measured, and the highest TAA was found for the 95% ethanol extract of the aerial parts. These results suggest that the *G. littoralis* aerial parts exhibit a higher antioxidant activity than the roots, and that the ethanol extract exhibited a superior antioxidant potential than the water extract.

The online HPLC-ABTS approach is known to complement the conventional ABTS method, whilst also

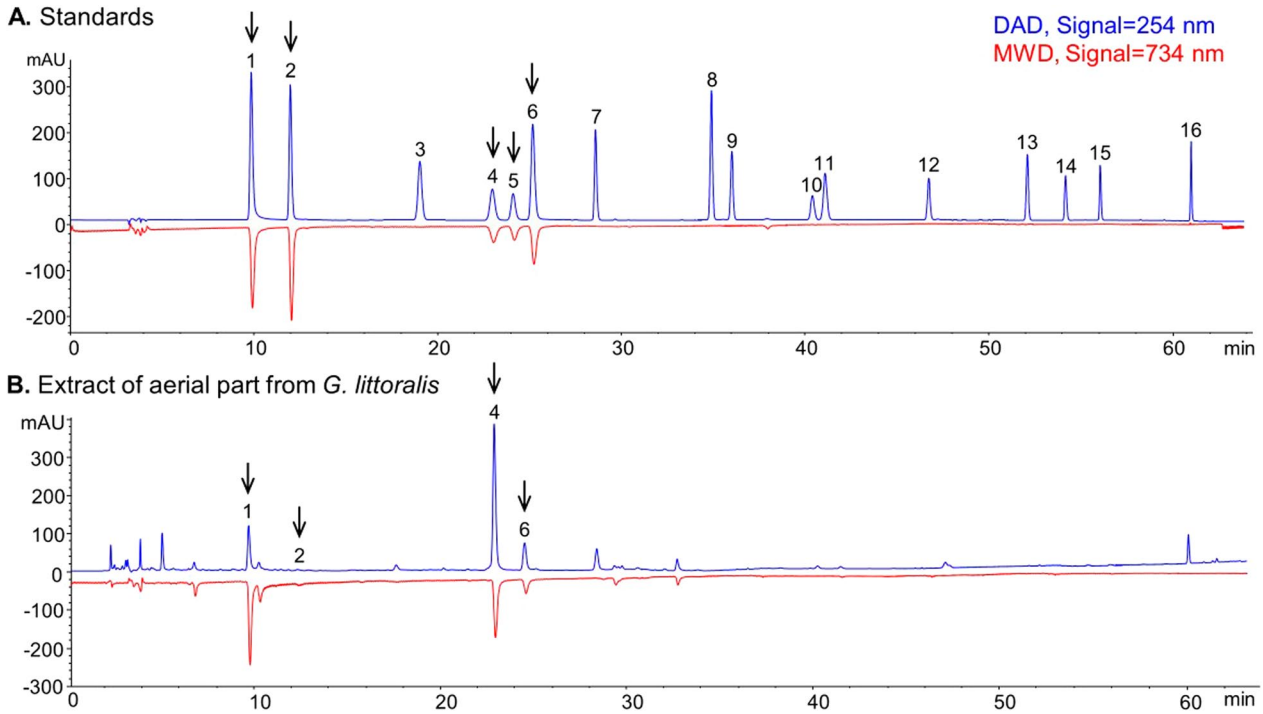


Fig. 3 Chromatographic fingerprints and ABTS inhibition profiles for **A** the standards containing the 16 phenolic compounds, and **B** the 95% ethanol extract of the *G. littoralis* aerial parts. Peak detection was carried out at 734 nm (red peak, negative) for the antioxidant activity and at 330 nm (blue, positive) for quantification. 1: Chlorogenic acid, 2: caffeic acid, 3: scopoletin, 4: rutin, 5: hyperoside, 6: isoquercetin, 7: nicotiflorin, 8: psoralen, 9: xanthotoxin, 10: isopimpinellin, 11: bergapten, 12: oxypeucedanin, 13: imperatorin, 14: phellopterin, 15: isoimperatorin, and 16: bergamottin. DAD, Diode array detector; MWD, Multiple wavelength detector

Table 4 TE (mg g^{-1} ex) and TEAC values of major antioxidant compounds in *G. littoralis* extracts and their antioxidant capacities

Peak	Compound	TEAC[3]	TE from aerial part		TE from root	
			95% EtOH extract	Water extract	95% EtOH extract	Water extract
1	Chlorogenic acid	1.43	4.34 ± 0.91 *	1.44 ± 0.20	0.45 ± 0.01 *	0.01 ± 0.00
2	Caffeic acid	1.37	0.15 ± 0.01	0.14 ± 0.02	0.19 ± 0.03	0.14 ± 0.01
4	Rutin	2.34	3.86 ± 0.17 ***	1.51 ± 0.04	n.d.[4]	n.d
6	Isoquercetin	1.56	1.07 ± 0.08 **	0.31 ± 0.01	n.d	n.d
Total TE[1]			9.36 ± 1.20	3.39 ± 0.09	0.65 ± 0.03	0.15 ± 0.00
TAA (%)[2]			74.8	56.9	48.6	64.1

All value was calculated using Trolox calibration curve ($y = 53.101x + 3.9368$, $R^2 = 0.9988$) and represented as mean ± SD (n = 3). Each data was represented as mean ± standard deviation (n = 3) and statistically analyzed using one-tailed *t*-test. significant differences were assessed by ***($P < 0.001$), **($P < 0.01$), *($P < 0.05$)

[1] TE, Trolox equivalent

[2] TAA, Percentage of the total antioxidant activity of all identified compounds

[3] TEAC, Trolox equivalent antioxidant capacity, concentration of Trolox (μM) that exhibits the same activity as 1 μM each identified compound

[4] n.d., not detected

offering the advantage of real-time measurement of the antioxidant activity of a compound immediately after its constituent analysis [25]. Accordingly, the current findings confirmed that four compounds of the in *G. littoralis* specimen exhibited antioxidant activity, namely chlorogenic acid (**1**), caffeic acid (**2**), rutin (**4**), and isoquercetin (**6**). Additionally, the aerial parts have higher antioxidant activity than the roots. Similarly, a radical scavenging assay identified caffeic acid, chlorogenic acid, isoquercetin, quercetin and rutin as the principal antioxidant constituents of this plant [6]. It should be noted here that the ABTS radical-scavenging

potentials of various *G. littoralis* extracts, including the leaves, stems, fruits, whole plants, and roots, have been previously evaluated [26].

In conclusion, the establishment of an appropriate analytical method is required to set specific standards to facilitate the industrial application of the edible aerial parts of the *G. littoralis* plant. Using liquid chromatography–mass spectrometry (LC-MS), 16 major phenolic compounds were identified in the aerial parts of *G. littoralis*, namely 10 coumarins, 4 flavonoids, and 2 hydroxycinnamic acids. Subsequently, an optimal analytical method was established using a combination of three different mobile phases (water, acetonitrile, and methanol) to simultaneously separate the 16 phenolic compounds. The reliability of the developed method was validated using various parameters. Importantly, quantification of the aerial parts and roots of *G. littoralis* using the proposed analytical method showed that the majority of phenolic compounds were more abundant in the aerial parts than in the roots. Thus, to determine an appropriate processing procedure for the edible *G. littoralis* aerial parts to yield functional ingredients for foods, thermal treatment and solvent extraction were investigated. It was found that when the aerial parts were subjected to thermal treatment, roasting led to the most notable decline in phenolic compounds, whereas shade drying retained the greatest quantities of active compounds. Furthermore, extraction with 95% ethanol was found to be favorable, giving an extract enriched with phenolic compounds. The proposed analytical method was then applied to an online HPLC-ABTS assay to assess the antioxidant activities of the 16 phenolic compounds. As a result, four antioxidant components were determinded, namely chlorogenic acid, caffeic acid, rutin, and isoquercetin. Moreover, the 95% ethanol extract exhibited the highest total Trolox equivalent value and the highest total antioxidant ability, thereby indicating its superior antioxidant properties compared to the root and water extracts.

Abbreviations

ABTS	2.2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid
ANOVA	One-way analysis of variance
DAD	Diode-array detection
DW	Dry weight
ESI	Electrospray ionization
HPLC	High-performance liquid chromatograph
LC–MS	Liquid chromatography-mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MWD	Multi-wavelength detector
RSD	Relative standard deviations
TAA	Total antioxidant activity
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-024-00884-9.

Additional file 1: Fig. S1. Chemical structures of targeted compounds in *G. littoralis*. **Fig. S2.** Positive ion ESI/MS spectrum of representative compounds including chlorogenic acid (1), caffeic acid (2), rutin (4), nicotiflorin (7), isopimpinellin (10), and isoimperatorin (15) in *G. littoralis*. **Table S1.** ESI/MS data of 16 phenolic compounds in *G. littoralis*. **Table S2.** Results of accuracy and precision of 16 phenolic compounds in *G. littoralis* by HPLC–DAD. **Table S3.** Results of recovery test of 16 phenolic compounds in *G. littoralis* by HPLC–DAD method. **Table S4.** Contents (mg g^{-1} dry weight) of 16 phenolic compounds dissolved in different extraction solvents from aerial part and root of *G. littoralis*. **Table S5.** Contents (mg g^{-1} dry weight) of 16 phenolic compounds depending on different thermal treatments of *G. littoralis*.

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Author contributions

YJP and YBC visualized and analyzed data. SBO investigated experiments. YJP, YBC, JYM, TQT, and PKH wrote the original manuscript. YJP and SMK edited the manuscript. SMK conceptualized the research and supervised all processes. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests.

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