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Comparative Analysis of Caffeoylquinic Acids and Lignans in Roots and Seeds among Various Burdock (*Arctium lappa*) Genotypes with High Antioxidant Activity

Jingyi Liu,[†] Yi-Zhong Cai,[†,‡] Ricky Ngok Shun Wong,[§] Calvin Kai-Fai Lee,[∥] Sydney Chi Wai Tang,[⊥] Stephen Cho Wing Sze,[†] Yao Tong,[†] and Yanbo Zhang*,[†]

[†]School of Chinese Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 10 Sassoon Road, Pokfulam, Hong Kong, China

[‡]Life Science and Technology Center, China National Seed Group Company, Ltd., Wuhan 430075, China

[§]Department of Biology, Hong Kong Baptist University, 24 Waterloo Road, Kowloon, Hong Kong, China

[∥]Department of Obstetrics and Gynaecology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong, China

[⊥]Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 201 Pokfulam Road, Hong Kong, China

ABSTRACT: Caffeoylquinic acids and lignans in the crude extracts of both roots and seeds from different burdock (*Arctium lappa* L.) genotypes were simultaneously characterized and systematically compared by LC−MS and matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry (MALDI-QIT-TOF MS), and their antioxidant activities were also investigated. A total of 14 lignans were identified in burdock seeds and 12 caffeoylquinic acids in burdock roots. High levels of caffeoylquinic acids were also detected in burdock seeds, but only trace amounts of lignans were found in burdock roots. Burdock seeds contained higher concentrations of lignans and caffeoylquinic acids than burdock roots. Quantitative analysis of caffeoylquinic acids and lignans in roots and seeds of various burdock genotypes was reported for the first time. Great variations in contents of both individual and total phenolic compounds as well as antioxidant activities were found among different genotypes. Burdock as a root vegetable or medicinal plants possessed considerably stronger antioxidant activity than common vegetables and fruits.

KEYWORDS: *burdock, roots, seeds, caffeoylquinic acids, lignans, antioxidant activity, LC−MS, MALDI-QIT-TOF MS*

INTRODUCTION

Burdock (*Arctium lappa* L.), called niupang in Chinese and gobo in Japanese, also known as greater burdock, is a biennial of the genus *Arctium* in the family Asteraceae. It has been cultivated in Eastern Asian countries (particularly in China, Japan, and Korea) as a root vegetable or a traditional medicinal plant for centuries and remains popular.[1,2] Its slender roots (about 0.7−1 m long and 0.2−0.3 m across) are very crisp and have sweet, mild, and pungent flavor, which are usually cooked and eaten as different popular dishes (e.g., well-known kinpira gobo in Japan) and are also processed and consumed as an instant powder and a snack food similar to potato chips or as a popular heath tea/beverage in Taiwan.[2,3] In the past decades, burdock as a root vegetable gradually achieved international recognition for its culinary use due to its nutritional values, bioactivities, and healthy effects and because of the increasing popularity of the macrobiotic diet. Burdock roots contain a fair amount of dietary fiber and inulin and are rich in antioxidant polyphenols, which possess in vitro antioxidant activity, free radical scavenging activity, and anti-inflammatory effect.[3−6] Major antioxidant components in burdock roots were caffeoylquinic acids.[2,5]

Burdock seeds, called niupangzi in Chinese, have been long used in traditional Chinese/Korean medicine as anti-inflammatory, detoxifying, or diuretic agents for relieving sore throat, dispelling pathogenic wind-heat, promoting eruption, and removing toxic substances.[1,7] Major bioactive principles in burdock seeds are a category of phenolic compounds, for example, lignans (mainly arctigenin and arctiin). Arctigenin and its glucoside (arctiin), belonging to monolignans (lignanolides), mostly occur in burdock seeds[1] and are also distributed in its leaves.[8] Low levels of sesquilignans and dilignans are also detected in its seeds.[9−12] The lignans from burdock seeds had a variety of biological properties, such as in vitro and in vivo anticancer, antioxidant, antibacterial, antiviral, anti-inflammatory, and immunosuppressive activities.[13−15]

Separation and identification of phenolic compounds (caffeoylquinic acids and lignans) from burdock were mainly conducted by comparison of spectroscopic and chromatographic properties with authentic standards or literature data and by using traditional and modern analytical techniques, such as high performance liquid chromatography (HPLC), high-speed counter-current chromatography (HSCCC), mass spectrometry (MS), liquid chromatography−mass spectrometry (LC−MS), and nuclear magnetic resonance

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(NMR).[8,9,11,12,16] Although the methods for identification of many individual phenolic compounds in burdock were previously reported, direct simultaneous determination of all caffeoylquinic acids and lignans in crude extracts of both burdock roots and seeds has not yet been described systematically.

Previous studies[2,8,9,16] reported that the caffeoylquinic acids were identified only in burdock roots and lignans were detected just in burdock seeds or leaves, but there was little information about caffeoylquinic acids in its seeds and lignans in its roots.[17] In addition, quantitative analysis and comparison of caffeoylquinic acids and lignans in roots and seeds among various burdock genotypes have not yet been reported, and the relevant information is scarce. In this study, direct simultaneous identification and quantitative determination of all lignans and caffeoylquinic acids in methanolic crude extracts from both seeds and roots of burdock using LC−MS were carried out. A rapid MALDI-QIT-TOF MS technique was developed to directly identify major phenolic compounds (lignans) in methanolic crude extracts from burdock seeds within a few minutes. Also, a comparison of antioxidant activity between different burdock genotypes and several common vegetables and fruits was conducted. This study would contribute to a better understanding of all antioxidant phenolic compounds of burdock as a root vegetable as well as a medicinal plant. The analytical techniques described in the present study may be used for the authentication and quality evaluation of burdock roots and seeds during their production, storage, and process.

MATERIALS AND METHODS

Materials and Chemicals/Reagents. Six burdock (*Arctium lappa* L.) genotypes (HEB, HUB, JL, JS, SD, and ZJ) collected from different provinces (Hebei, Hubei, Jilin, Jiangsu, Shandong, and Zhejiang) in China were grown in an experimental farm of Guankou Town (Xishui, Hubei, China) at the end of April, 2010. Soil type of the experimental field was sandy loam, which was rich and loose, and drained well. Plot design was random in arrangement, and each plot was 16 m^2 (3.2 × 5.0 m) with four rows (row distance was 80 cm, and distance each plant was around 10 cm). Normal field managements were practiced according to the cultivation requirements of burdock. Seeds and roots of six different burdock genotypes were harvested between September and October. The harvested seed samples were solar-dried and stored in the sealed plastic bags, and the harvested fresh root samples were packaged using preservative film and stored in a cold room (∼4 °C) until analysis. Additionally, eight common vegetables and fruits (Chinese cabbage, carrot, cucumber, eggplant, kiwi fruit, orange, tomato, and Washington red apple) collected in a Hong Kong local supermarket were used as controls for comparison of antioxidant activity assays.

Sodium iodide (NaI), trifluoroacetic acid (TFA), 2,5-dihydroxybenzoic acid, and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane 2-carboxylic acid) were obtained from Fluka Chemie (Buchs, Switzerland); formic acid, Folin-Ciocalteu reagent, and HPLC grade organic reagents were from BDH (Dorset, England). Authentic standards (5-*O*-caffeoylquinic acid, gallic acid, arctigenin, and matairesinol) and most of the chemicals for assaying antioxidant activity were purchased from Sigma/Aldrich (St. Louis, MO). The 1,3- and 1,5-di-*O*-caffeoylquinic acids were from Roth (Karlsruhe, Germany) and ChromaDex, Inc. (Santa Ana, CA), respectively.

The reference compounds of the lignans and caffeoylquinic acids without commercial standards (e.g., arctiin, lappaol A, B, C, and H, arctignan B, C, D, and E; 1,5-di-*O*-caffeoyl-3-*O*-maloylquinic acid, 1,5-di-*O*-caffeoyl-3-*O*-succinoylquinic acid) were prepared and purified with a preparative HPLC (Hewlett-Packard HP 1100 series) on a 250 × 9.4 mm i.d., 5 μm, Zorbax SB-C18 column (before purification, various fractions were collected using a 100 cm × 2.5 cm i.d. Sephadex LH-20-100 column) or a silica gel column, according to our previous methods[18] and also with reference to other previous methods.[2,19] The separated and purified standards were verified and identified according to the retention time, UV spectroscopic, and mass spectrometric data by LC−ESI-MS by comparison with literature data. They were freeze-dried and stored at −18 °C in a refrigerator until use.

Sample Preparation. Burdock seeds were air-dried in a ventilated oven at 40 °C for 24 h. Fresh roots were freeze-dried by a Heto FD3 freeze-dryer (Heto-Holten A/S, Allerod, Denmark). Dried seeds and roots were ground into fine powder. Sample preparation for LC−MS analysis was as follows: the powdered sample (20 mg) was added in a 1.5 mL vial and extracted with 1 mL of 80% methanol at room temperature (∼23 °C) for 8 h. The extract was then filtered using a Millipore filter (nylon membrane, 0.2 μm i.d.), and the filtrate was stored at 23 °C until use. Sample preparation for MALDI-QIT-TOF MS analysis was as follows: the powdered sample (20 mg) was added in a 1.5 mL vial and extracted with 1 mL of 80% methanol at 23 °C for 8 h, and then filtered directly for MALDI-QIT-TOF MS analysis. 2,5-Dihydroxybenzoic acid (100 mM) was used as a matrix and dissolved in 0.1% TFA methanol with 3 mM NaI. The filtered crude extract (0.5 μL) and 2,5-dihydroxybenzoic acid solution (0.5 μL) were spotted on a sample plate, and then allowed to air-dry at room temperature before loading into the Amixa-QIT instrument. Sample preparation for antioxidant activity assays and total phenolic content determination followed our previous method.[20]

LC−MS. LC−MS analysis was performed with an LC−MS-2010EV system consisting of a LC-20AD binary pump, SIL-20AC autosampler, photodiode-array detector (PDA), central controller, and single quadrupole MS detector with ESI (electrospray ionization) interface (Shimadzu, Tokyo, Japan). The system was equipped with a 250 mm × 2.0 mm i.d., 5 μm, VP-ODS C_{18} column (Nomura Chemical Co., Seto, Japan). The mobile phase consisted of 0.1% formic acid (solvent A) and MeOH with 0.1% formic acid (solvent B). To completely separate and simultaneously identify all lignans and caffeoylquinic acids in the crude extract samples, a long gradient procedure was used in this study. The gradient program was as follows: 0−5 min, 5% B; 5−15 min, 5−30% B; 15−40 min, 30−40% B; 40−60 min, 40−50% B; 60−65 min, 50−55% B; 65−90 min, 55−100% B; 90−100 min, 100% B. Flow rate was 0.2 mL/min, and injection volume was 5 μL. The LC eluate was introduced directly into the ESI interface without flow splitting. The ESI voltage was 4.5 kV in positive-ion mode and 3.5 kV in negative-ion mode. A nebulizing gas of 1.5 L/min and a drying gas of 10 L/min were applied for ionization using nitrogen in both cases. Mass spectra were recorded in the range m/z 160−800.

MALDI-QIT-TOF MS. MALDI-QIT-TOF MS analysis was performed according to our previous method.[21] The MALDI-TOF mass spectra (MS and MS^n) were acquired on an Axima MALDI-QIT-TOF MS instrument (Shimadzu Biotech, Kratos, U.K.). Acquisition and data processing were controlled by Launchpad software (Shimadzu Biotech, Kratos, U.K.). The instrument was operated in the positive ion mass mode (100−700 Da). Mass spectra from a sum of 200−1000 laser shots were recorded using a laser power of 70 arbitrary units (range of laser power 0−180). External mass calibration was performed daily using fullerite deposited on the sample plate.

Quantitative Analysis of Individual and Total Phenolic Compounds. Quantitative analysis of individual phenolic compounds (caffeoylquinic acids and lignans) in the crude extracts of the tested burdock samples was performed using LC-PDA system at the same chromatographic conditions described above and by comparison with two external standards, that is, 5-*O*-caffeoylquinic acid (chlorogenic acid) and arctigenin, respectively, and their contents were expressed as mg/g of dry weight (d.w.). The standard curves were constructed with their corresponding maximum absorbance wavelength at 325 nm for 5-*O*-caffeoylquinic acid to quantitate the categories of caffeoylquinic acids and at 280 nm for arctigenin to quantitate the categories of lignans. Total content of caffeoylquinic acids in the crude extracts of the burdock samples was the sum of contents of individual caffeoylquinic acids, and total content of lignans was the sum of contents of individual lignans.

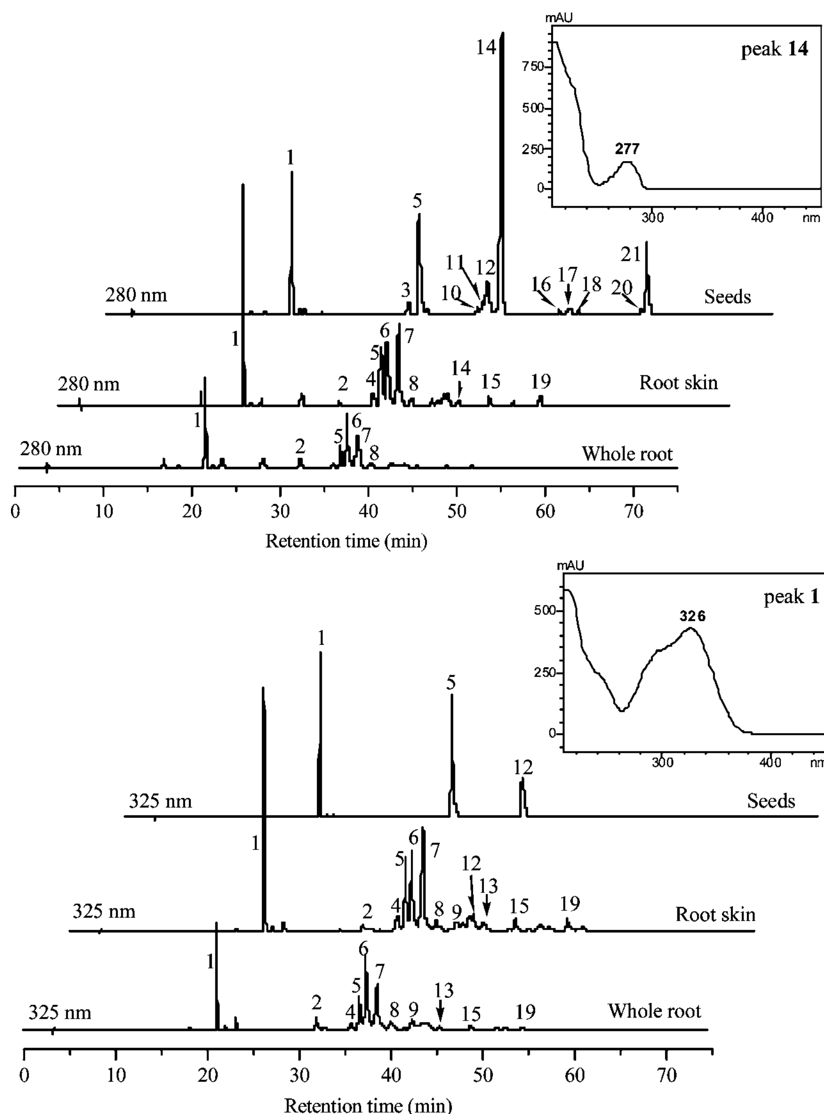

Figure 1. Typical LC chromatograms and UV spectra of lignans (280 nm) and caffeoylquinic acids and their derivatives (325 nm) separated in methanolic crude extracts of seeds and roots (whole root and root skin) from a representative burdock genotype (HUB). For peak assignments, see Table 1. The peak numbers in this figure correspond to the peak numbers in Table 1 and the numbers of compound molecular structures in Figure 2.

Total phenolic content in the crude extracts of the burdock samples was estimated using the Folin−Ciocalteu colorimetric method described previously.[20] Total phenolic content was expressed as milligrams of gallic acid equivalent per 100 g of dry weight (mg GAE/100 g d.w.). All determinations were performed in triplicate.

Antioxidant Activity Assays. Three common methods were used for the estimation of antioxidant activity in the present study. Total antioxidant capacity of the crude extracts was assayed using a Spectronic Genesys 5 spectrophotometer (Milton Roy, NY) with the improved ABTS method with minor modification.[20] The results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC) (millimol Trolox equivalents per 100 g dry weight) (mM Trolox/100 g d.w.). Ferric reducing antioxidant power (FRAP) assay was performed as previously described by Benzie et al.[22] and Zhu et al.[20] FRAP was expressed as micromol Trolox equivalents per gram on dried basis (μM Trolox/g d.w.). The hydroxyl radical-scavenging activity was determined using the deoxyribose method described previously[20] and expressed as percentage (%). All determinations were performed in triplicate.

Statistical Analysis. All of the results were calculated as mean ± SD (standard deviation). Statistical comparisons of the mean values were performed by one-way ANOVA, followed by Duncan's multiple-range test at $p < 0.05$ confidence levels using the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Identification of Caffeoylquinic Acids and Lignans in Burdock Seeds and Roots. LC−MS was successfully employed in this study to simultaneously separate and identify a large number of lignans and caffeoylquinic acids in methanolic crude extracts of both burdock seeds and roots. Most peaks (Figure 1) of the crude extracts from burdock seeds and whole root or root skin samples were well-separated under the present chromatographic conditions, and both positive and negative ESI-MS full scan modes were sensitive for the determination of the peaks separated by LC. The retention times (R_t), UV spectra (λ_{max}), MS adduct ions observed in both positive and negative modes, and calculated molecular masses of the caffeoylquinic acids and lignans separated in burdock seeds and roots are shown in Table 1. The results showed that the major phenolic compounds in burdock seeds were identified as nine lignans (compounds **3**, **10**, **11**, **14**, **16−18**, **20**, and **21**)

Table 1. LC−MS Analysis of Caffeoylquinic Acids and Lignans in Methanolic Crude Extract from Roots and Seeds of a Representative Burdock Genotype (HUB)

peak no.[a]	R_t (min)	UV λ_{max} (nm)	mass of observed ions (m/z)	calcd MW	tentative identification of compounds[b]	quantitative analysis (mg/g of d.w.)[c] whole root	seeds
1	21.0	214, 297sh, 327	$[M - H]^-$ (353), $[M + H]^+$ (355), $[M + Na]^+$ (377)	354	5-*O*-caffeoylquinic acid (chlorogenic acid)	5.3 ± 0.07	13.2 ± 0.12
2	31.7	215, 300sh, 328	$[M - H]^-$ (631), $[M + H]^+$ (633), $[M + Na]^+$ (655)	632	dicaffeoyl-maloylquinic acid	2.4 ± 0.02	nd
3	34.0	210, 280	$[M - H]^-$ (749), $[M + Na]^+$ (773)	750	lappaol H	nd	1.5 ± 0.03
4	35.5	215, 300sh, 329	$[M - H]^-$ (631), $[M + H]^+$ (633), $[M + Na]^+$ (655)	632	dicaffeoyl-maloylquinic acid	2.0 ± 0.01	nd
5	35.8	215, 298sh, 329	$[M - H]^-$ (515), $[M + H]^+$ (517), $[M + Na]^+$ (539)	516	1,3-di-*O*-caffeoylquinic acid	3.8 ± 0.05	20.7 ± 0.25
6	36.9	215, 300sh, 329	$[M - H]^-$ (631), $[M + H]^+$ (633), $[M + Na]^+$ (655)	632	1,5-di-*O*-caffeoyl-3-*O*-maloylquinic acid	7.6 ± 0.07	nd
7	38.2	213, 300sh, 329	$[M - H]^-$ (615), $[M + H]^+$ (617), $[M + Na]^+$ (639)	616	1,5-di-*O*-caffeoyl-3-*O*-succinoylquinic acid	6.0 ± 0.07	nd
8	39.6	215, 300sh, 329	$[M - H]^-$ (631), $[M + Na]^+$ (655)	632	1,5-di-*O*-caffeoyl-4-*O*-maloylquinic acid	2.3 ± 0.02	nd
9	41.9	213, 300sh, 329	$[M - H]^-$ (747), $[M + H]^+$ (749), $[M + Na]^+$ (771)	748	dicaffeoyl-dimaloylquinic acid	2.1 ± 0.01	nd
10	41.8	210, 280	$[M - H]^-$ (553), $[M + H]^+$ (555), $[M + Na]^+$ (577)	554	lappaol C	nd	1.2 ± 0.02
11	42.4	210, 280	$[M - H]^-$ (553), $[M + H]^+$ (555), $[M + Na]^+$ (577)	554	isolappaol C	nd	2.3 ± 0.04
12	42.9	215, 298sh, 328	$[M - H]^-$ (515), $[M + H]^+$ (517), $[M + Na]^+$ (539)	516	1,5-di-*O*-caffeoylquinic acid	2.0 ± 0.02	8.6 ± 0.11
13	44.8	213, 300sh, 329	$[M - H]^-$ (731), $[M + Na]^+$ (755)	732	1,5-di-*O*-caffeoyl-3-*O*-succinoyl-4-*O*-maloyquinic acid	1.8 ± 0.02	nd
14	44.5	228sh, 277	$[M - H]^-$ (533), $[M + H]^+$ (535), $[M + Na]^+$ (557)	534	arctiin	0.3 ± 0.00	45.8 ± 0.62
15	48.3	213, 300sh, 330	$[M - H]^-$ (715), $[M + Na]^+$ (739)	716	1,5-di-*O*-caffeoyl-3,4-di-*O*-succinoylquinic acid	1.5 ± 0.01	nd
16	50.9	210, 278	$[M - H]^-$ (357), $[M + H]^+$ (359), $[M + Na]^+$ (381)	358	matairesinol	nd	0.3 ± 0.00
17	52.1	210, 281	$[M - H]^-$ (731), $[M + Na]^+$ (755)	732	arctignan D	nd	0.4 ± 0.01
18	53.1	210, 281	$[M - H]^-$ (731), $[M + Na]^+$ (755)	732	arctignan E	nd	0.3 ± 0.00
19	53.9	213, 298sh, 328	$[M - H]^-$ (777), $[M + Na]^+$ (801)	778	1,3,5-tri-*O*-caffeoyl-4-*O*-succinoylquinic acid	1.4 ± 0.01	nd
20	60.0	210, 280	$[M - H]^-$ (535), $[M + Na]^+$ (559)	536	lappaol A	nd	0.4 ± 0.01
21	60.7	229sh, 279	$[M - H]^-$ (371), $[M + H]^+$ (373), $[M + Na]^+$ (395)	372	arctigenin	nd	11.8 ± 0.17

[a]Peak numbers correspond to the peak numbers in Figure 1 and the numbers of compound molecular structures in Figure 2. [b]Identified with standards, reference compounds, or previously reported data. [c]Contents of individual caffeoylquinic acids and lignans were expressed as mg 5-*O*-caffeoylquinic acid/g d.w. and mg arctigenin/g d.w., respectively. nd, not detected.

and three caffeoylquinic acids (**1**, **5**, and **12**). The main phenolic compounds in burdock roots were identified as 12 caffeoylquinic acids (compounds **1**, **2**, **4**−**9**, **12**, **13**, **15**, and **19**) and one lignan (**14**). The chemical structures of the lignans (mono-, sesqui-, and dilignans) and caffeoylquinic acids (mono- and dicaffeoylquinic acids as well as caffeoylquinic acids incorporating one or more residues of aliphatic acids) identified in burdock seeds and roots are illustrated in Figure 2.

The peaks separated in the tested burdock genotypes were identified according to available authentic standards, by comparison of retention times and UV spectroscopic and MS data, and with reference to literature data. Twelve peaks (peaks 1, 2, 4−9, 12, 13, 15, and 19) had typical UV spectroscopic characteristics of caffeoylquinic acids and their derivatives with λ_{max} at 327−330 nm, whereas nine peaks (peaks 3, 10, 11, 14, 16−18, 20, and 21) possessed typical UV spectroscopic characteristics of lignans with λ_{max} at 278−281 nm (Table 1 and Figure 1). By direct comparison with available authentic standards or reference compounds, three peaks (1, 5, and 12) separated in burdock roots and seeds were readily identified as 5-*O*-caffeoylquinic acid, 1,3-di-*O*-caffeoylquinic acid, and 1,5-di-*O*-caffeoylquinic acid, respectively, and six peaks (3, 14, 16, 17, 18, and 21) separated in burdock seeds were also easily identified as lappaol H, arctiin, matairesinol, arctignan D, arctignan E, and arctigenin, respectively. For other caffeoylquinic acids and lignans without reference standards, the identification of the corresponding peaks (2, 4, 6−9, 10, 11, 13, 15, 19, and 20) was based on their R_t and λ_{max} values as well as MS data including the $[M + H]^+$ and/or $[M + Na]^+$ ions in positive mode and the prominent $[M - H]^-$ ions in negative mode (Table 1) and by comparison with literature data.[1,9−11,16,17,19,23] These peaks were tentatively identified as seven known caffeoylquinic acids (**6**−**9**, **13**, **15**, and **19**) and four known lignans (**10**, **11**, and **20**), and the names of these known compounds are shown in Table 1 and Figure 2. Interestingly, four regio-isomers (M_r = 632 and similar UV spectra) (peaks 2, 4, 6, and 8) were detected in burdock roots. Two of them (peaks 6 and 8) were tentatively identified as 1,5-

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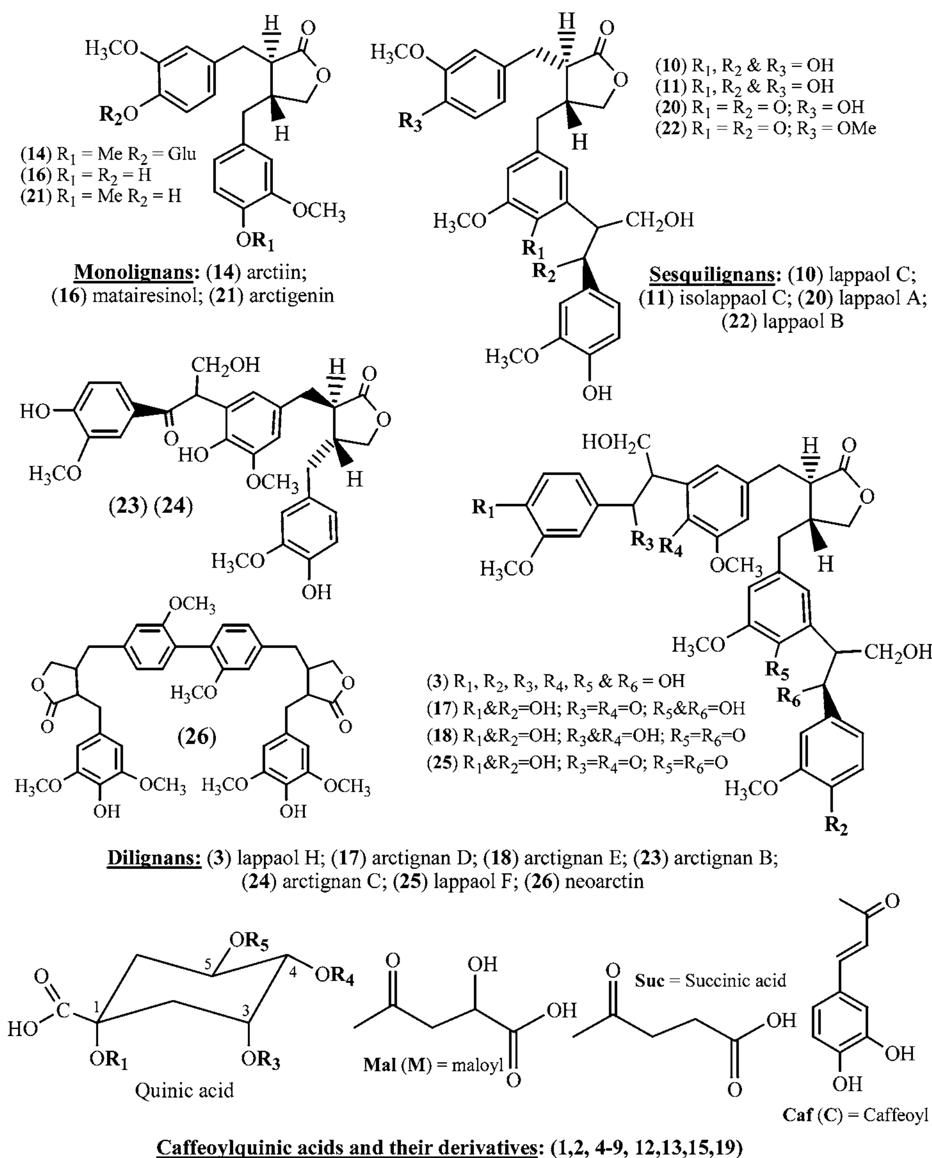

no.	caffeoylquinic acids and their derivatives	abbreviation	R_1	R_3	R_4	R_5
1	5-*O*-caffeoylquinic acid (chlorogenic acid)	5-CQA	H	H	H	Caf
2	dicaffeoyl-maloylquinic acid	diC-MQA	Caf	C/M	H/C	C/M
4	dicaffeoyl-maloylquinic acid	diC-MQA	Caf	C/M	C/M	H/C
5	1,3-di-*O*-caffeoylquinic acid	1,3-diCQA	Caf	Caf	H	H
6	1,5-di-*O*-caffeoyl-3-*O*-maloylquinic acid	diC-MQA	Caf	Mal	H	Caf
7	1,5-di-*O*-caffeoyl-3-*O*-succinoylquinic acid	1,5-diC-3-SQA	Caf	Suc	H	Caf
8	1,5-di-*O*-caffeoyl-4-*O*-maloylquinic acid	diC-MQA	Caf	H	Mal	Caf
9	dicaffeoyl-dimaloylquinic acid	diC-diMQA	C/M	C/M	C/M	C/M
12	1,5-di-*O*-caffeoylquinic acid	1,5-diCQA	Caf	H	H	Caf
13	1,5-di-*O*-caffeoyl-3-*O*-succinoyl-4-*O*-maloyquinic acid	1,5-diC-3-S-4-MQA	Caf	Suc	Mal	Caf
15	1,5-di-*O*-caffeoyl-3,4-di-*O*-succinoylquinic acid	1,5-diC-3,4-diSQA	Caf	Suc	Suc	Caf
19	1,3,5-tri-*O*-caffeoyl-4-*O*-succinoylquinic acid	1,3,5-riC-4-SQA	Caf	Caf	Suc	Caf

Figure 2. Structures of lignans (mono-, sesqui-, and dilignans) and caffeoylquinic acids and their derivatives identified in burdock seeds and roots. The compound numbers (**1**−**26**) in this figure correspond to the peak numbers of Table 1 and Figures 1 and 3.

dicaffeoyl-3-maloylquinic acid (**6**) and 1,5-dicaffeoyl-4-maloylquinic acid (**8**) with reference to the recent report.[16] The other two regio-isomers (M_r = 632) (peaks 2 and 4) might be new ones. Because of the absence of NMR and reference data, these new regio-isomers (M_r = 632) (peaks 2 and 4) were tentatively identified as dicaffeoylmaloylquinic acids (**2** and **4**) (Table 1). Their structures need further assignment by NMR.

As far as we know, the caffeoylquinic acids identified in burdock seeds and the lignan identified in burdock roots were scarcely reported before. Many previous studies[2,8,9,16,23]

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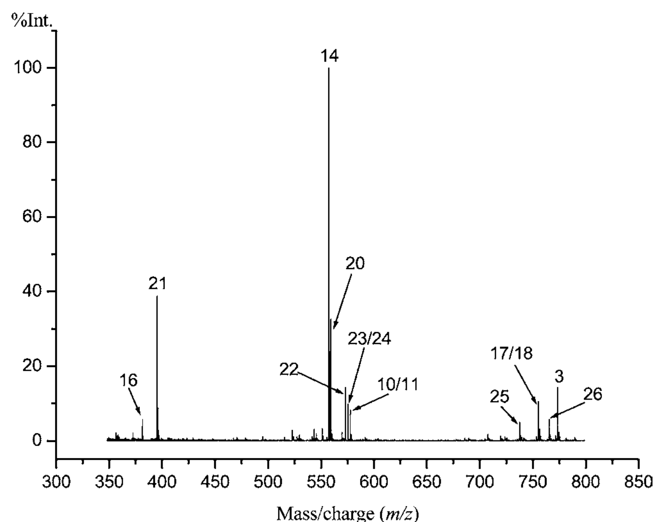
focused on identification of the caffeoylquinic acids in burdock roots and the lignans in burdock seeds and leaves. In this study, it was found that burdock seeds contained three major caffeoylquinic acids (5-*O*-caffeoylquinic acid, 1,3-di-*O*-caffeoylquinic acid, and 1,5-di-*O*-caffeoylquinic acid), and burdock roots had one minor lignan component (arctiin). Although a recent study[17] first reported the occurrence of two caffeoylquinic acids (5-*O*-caffeoylquinic acid and 1,3-di-*O*-caffeoylquinic acid) in burdock seeds, we detected three major caffeoylquinic acids (the third one was 1,5-di-*O*-caffeoylquinic acid) in burdock seeds in the present study (Table 1 and Figure 1). Moreover, the numbers of the lignans identified in burdock seed samples by LC–MS in this study were much more than those identified in the seeds of burdock reported previously,[17] but the numbers of the caffeoylquinic acids identified in burdock root samples were less than those identified in the roots of burdock from previous studies.[16,23] It was likely due to various burdock genotypes and different analytical methods. In the present study, LC–MS simultaneously determined two different categories of phenolic compounds (lignans and caffeoylquinic acids) in the crude extracts of burdock seeds and roots at the same chromatographic conditions.

MALDI-QIT-TOF MS is a new rapid technique for identification of low molecular weight compounds. It was applied in the present study to identify the lignans and caffeoylquinic acids in the crude extracts of burdock seeds and roots. The results showed that the relevant adduct ions of many lignans in burdock samples could be easily observed. Because 2,5-dihydroxybenzoic acid was used as a matrix in 0.1% TFA methanol with 3 mM NaI for desorption and ionization of the lignans, all of the adduct ions observed in the crude extracts of burdock samples were the $[M + Na]^+$ ion. However, unexpectedly, the corresponding adduct ions of the caffeoylquinic acids in burdock samples could not be found. It was likely because the caffeoylquinic acids were not sensitive to MALDI-QIT-TOF MS determination under the present experimental conditions (2,5-dihydroxybenzoic acid was used as a matrix). Successful MALDI-QIT-TOF MS analysis of the caffeoylquinic acids may require using a redox-silent matrix for sample preparation.

Typical MALDI-QIT-TOF MS positive ion spectra, MS data, and the names of the lignans identified in the methanolic crude extracts of burdock seeds are shown in Figure 3. A total of 14 lignans (including nine lignans identified by LC–MS) in the crude extracts of burdock seeds were identified by MALDI-QIT-TOF MS, based on the comparison of their mass spectrometric behavior and MS data with those of the relevant authentic standards or reference compounds and with reference to literature data.[1,9–11,19,24] This suggested that MALDI-QIT-TOF MS could identify more lignans in the crude extracts of burdock seeds than LC–MS. The additional lignans identified by MALDI-QIT-TOF MS were, respectively, lappaol B (**22**), arctignan B (**23**) and C (**24**), lappaol F (**25**), and neoarctin (**26**) (Figure 3), and their structures are shown in Figure 2.

Moreover, MALDI-QIT-TOF MS could provide MS^n data for further structural elucidation of high levels of lignans (e.g., arctiin and arctigenin) in crude extracts of burdock seeds. Further evidence was the formation of product ions and characteristic fragments in the MS^2 and MS^3 experiment. Figure 4 gives an example of the typical MS^2 and MS^3 spectra of arctiin ($[M + Na]^+$ ion) and its fragmentation patterns by MALDI-QIT-TOF MS. The dotted lines in the molecular structure of



peak No.	mass of $[M + Na]^+$ ion (*m/z*)	calcd MW	tentatively identified lignan compounds
3	773.3	750	lappaol H [a]
10/11	577.2	554	lappaol C/isolappaol C [ab]
14	557.2	534	arctiin [a]
16	381.3	358	matairesinol [a]
17/18	755.3	732	arctignan D/arctignan E [a]
20	559.2	536	lappaol A [a]
21	395.3	372	arctigenin [a]
22	573.2	550	lappaol B [a]
23/24	575.2	552	arctignan B/arctignan C [ab]
25	737.3	714	lappaol F [b]
26	765.3	742	neoarctin [b]

[a] Identified with standards or reference compounds. [b] Reported previously.

Figure 3. MALDI-QIT-TOF MS positive ion ($[M + Na]^+$) spectra, MS data, and the names of the lignans identified in methanolic crude extracts of burdock seeds. 2,5-Dihydroxybenzoic acid (100 mM) as a matrix in 0.1% TFA methanol with 3 mM NaI. The peak numbers in this figure correspond to the numbers of compound molecular structures in Figure 2 and also the peak numbers (3, 10, 11, 14, 16–18, 20, and 21) in Table 1 and Figure 1.

arctiin in Figure 4 represent the postulated CID cleavage positions of fragmentation. In the MS^2 spectrum (Figure 4B), arctiin ($[534 + Na]^+$) (*m/z* 557.2) fragmented and produced a typical product ion (arctigenin), that is, $[534 + Na - 162]^+$ (*m/z* 395.2) with loss of the glucoside moiety (162.1 Da). In the MS^3 spectrum (Figure 4C), the product ion $[372 + Na]^+$ (*m/z* 395.2) further produced characteristic fragments and formed three $[M + Na]^+$ ions at *m/z* 159.8, *m/z* 174.1, and *m/z* 258.4.

LC–MS and MALDI-QIT-TOF MS analyses applied in this study had different advantages and disadvantages. The above results indicated that both LC–MS and MALDI-QIT-TOF MS could directly and simultaneously identify a large number of the phenolic compounds in crude extracts of burdock samples. MALDI-QIT-TOF MS analysis, a more rapid technique, took only several minutes (<6 min) per run, while LC–MS analysis took 100 min per run in the present study, to completely separate and simultaneously determine all lignans and caffeoylquinic acids in the samples. MALDI-QIT-TOF MS could identify more lignans (14 lignans) in crude extract samples than LC–MS (nine lignans), and also provide MS^n data for further structural elucidation of the lignans, but could not detect the caffeoylquinic acids in crude extract samples, and also could not distinguish isomers of the lignans (e.g., **10/11**, lappaol C/isolappaol C; **17/18**, arctignan D/E; **23/24**, arctignan B/C) (Figure 3). LC–MS could differentiate the

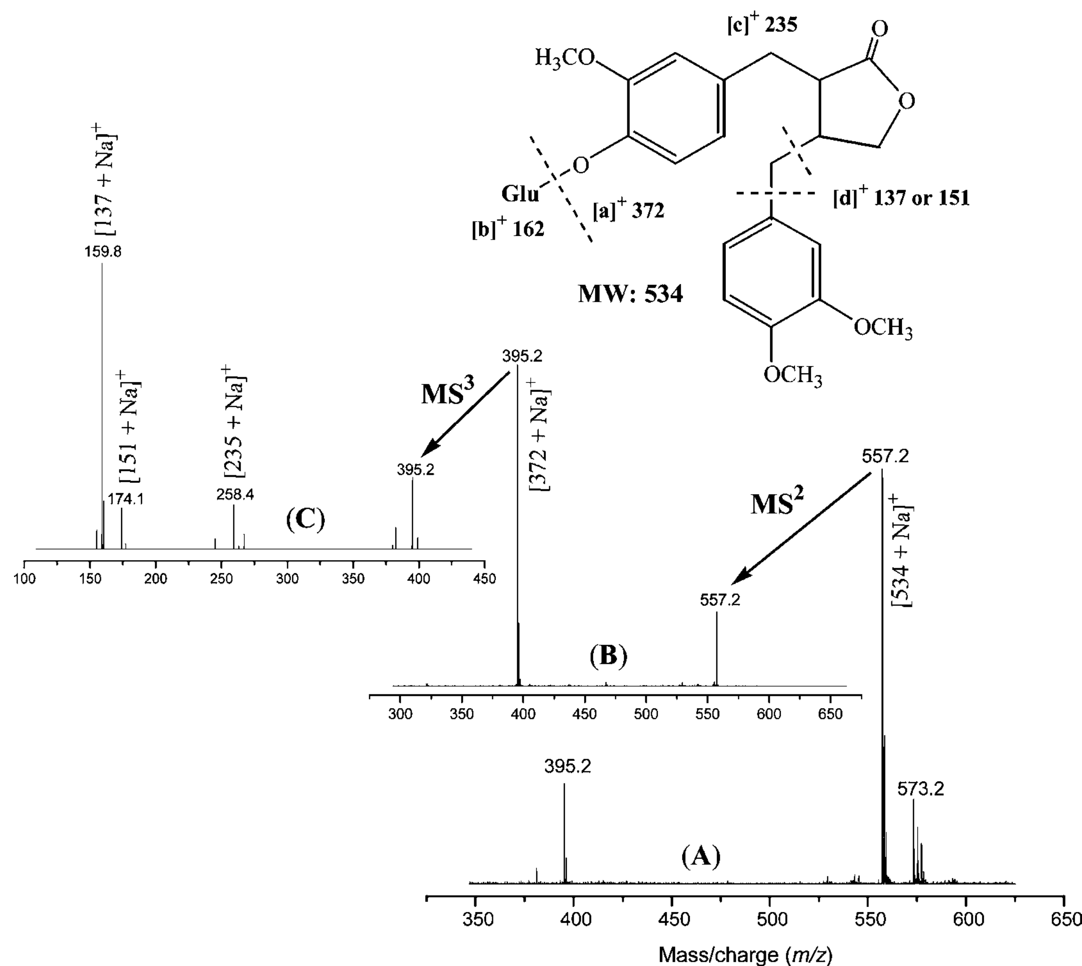


Figure 4. MS^2 (B) and MS^3 (C) spectra of MALDI-QIT-TOF MS $[M + Na]^+$ ion (A) of arctiin in methanolic crude extracts of burdock seeds (2,5-dihydroxybenzoic acid as a matrix in 0.1% TFA methanol with 3 mM NaI). The dotted lines (------) in the structure of arctiin represent the postulated CID cleavage positions of fragmentation by MALDI-QIT-TOF MS.

isomers of the known lignans, because it not only provided molecular masses but also gave retention times and UV-spectroscopic data. The isomers (peaks 10 and 11, lappaol C and isolappaol C; peaks 17 and 18, arctignan D and E) had the same molecular masses and similar UV spectra, but possessed different retention times (Table 1).

Comparison on Quantitative Analysis of Caffeoylquinic Acids and Lignans in Seeds and Roots of Different Burdock Genotypes. So far there has been little information about quantitative analysis and comparison of caffeoylquinic acids and lignans in roots and seeds among various burdock genotypes. In the present study, the quantitative analysis of individual and total phenolic compounds (caffeoylquinic acids and lignans) in seeds and roots of six burdock genotypes was systematically conducted. The data of individual caffeoylquinic acids and lignans quantitated according to their peak area (Table 1) and the typical LC profiles at 325 nm (caffeoylquinic acids) and 280 nm (lignans) (Figure 1) clearly showed that 1,3-dicaffeoylquinic acid (peak 5) was the predominant caffeoylquinic acid (20.7 mg/g d.w.) in burdock seeds, followed by 5-*O*-caffeoylquinic acid (peak 1) (13.2 mg/g), while arctiin (peak 14) was the predominant lignan (45.8 mg/g) in burdock seeds, followed by arctigenin (peak 21, 11.8 mg/g), and these two lignans accounted for 90.1% of total lignans. For burdock roots (whole root), 1,5-di-*O*-caffeoyl-3-*O*-maloylquinic acid (peak 6) was the major caffeoylquinic acid (7.6 mg/g d.w.), followed by 1,5-di-*O*-caffeoyl-3-*O*-succinoylquinic acid (peak 7) (6.0 mg/g) and 5-*O*-caffeoylquinic acid (peak 1) (5.3 mg/g), and these three compounds accounted for 49.6% of total caffeoylquinic acids. However, burdock roots lacked lignans, and only a trace amount of arctiin (0.3 mg/g) was detected in burdock roots (whole root). This was identical to the result reported by Ferracane et al.[17] who first found the occurrence of arctiin in burdock roots, but did not quantitate this component.

From Table 1, although only three caffeoylquinic acids detected in burdock seeds of a representative genotype (HUB) were much less than those (12 caffeoylquinic acids) in burdock roots, burdock seeds had quite higher levels of individual caffeoylquinic acids (mean = 14.2 mg/g) and total caffeoylquinic acids (42.5 mg/g) than burdock roots (3.2 and 38.3 mg/g, respectively). From Table 2, on average, the seeds of six burdock genotypes had considerably higher value of total caffeoylquinic acids (mean = 44.4 mg/g) than the roots (whole root) of six burdock genotypes (39.0 mg/g), and also contained significantly higher total lignans (mean = 61.1 mg/g) than the roots of six burdock genotypes (0.3 mg/g). In addition, we determined and compared the total contents of caffeoylquinic acids in three different categories of root samples (whole root, root skin, and root heart) of a representative burdock genotype (HUB) (Table 2). The root skin contained the highest levels of total caffeoylquinic acids (74.3 mg/g), which was significantly

Table 2. Total Caffeoylquinic Acids, Total Lignans, Total Phenolic Content, Total Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Power (FRAP), and Hydroxyl Radical-Scavenging Activity in Seeds and Roots of Various Burdock Genotypes[a]

genotype codes	original provinces	sample types	total caffeoylquinic acids (mg/g d.w.)[b]	total lignans (mg/g d.w.)[c]	total phenolic content (g GAE/100 g d.w.)	TEAC (mM Trolox/100 g d.w.)	FRAP (μM Trolox/g d.w.)	hydroxyl radical-scavenging activity (%)
HEB	Hebei	seeds	37.5 ± 1.34 ef	55.3 ± 1.26 e	3.3 ± 0.12 e	21.0 ± 0.93 e	6.9 ± 0.21 ef	11.5 ± 0.41 e
HUB	Hubei	seeds	42.5 ± 1.25 c	63.9 ± 1.97 c	3.8 ± 0.16 c	23.5 ± 1.35 c	8.3 ± 0.36 c	13.4 ± 0.11 c
JL	Jilin	seeds	52.0 ± 1.41 b	66.8 ± 1.22 b	4.1 ± 0.23 b	27.4 ± 0.89 b	9.7 ± 0.67 b	15.9 ± 0.78 b
JS	Jiangsu	seeds	42.0 ± 1.56 cd	63.0 ± 1.38 cd	3.8 ± 0.10 cd	23.2 ± 1.12 cd	8.2 ± 0.43 cd	13.2 ± 0.74 bcd
SC	Sichuan	seeds	38.0 ± 1.07 e	48.3 ± 1.55 f	3.2 ± 0.15 ef	19.4 ± 0.88 f	7.0 ± 0.35 e	11.2 ± 0.39 ef
ZJ	Zhejiang	seeds	54.4 ± 1.81 a	69.5 ± 2.66 a	4.6 ± 0.19 a	29.2 ± 1.16 a	11.3 ± 0.63 a	16.8 ± 0.65 a
mean			44.4	61.1	3.8	24.0	8.7	13.7
HEB	Hebei	whole root	33.4 ± 1.18 e	0.2 ± 0.00 f	1.3 ± 0.05 f	5.9 ± 0.37 e	2.3 ± 0.09 c	8.1 ± 0.23 e
HUB	Hubei	whole root	38.3 ± 0.95 c	0.3 ± 0.00 b	1.8 ± 0.01 cd	7.0 ± 0.56 c	2.3 ± 0.02 cd	9.3 ± 0.05 b
JL	Jilin	whole root	44.8 ± 1.63 b	0.2 ± 0.00 d	2.0 ± 0.01 b	8.2 ± 0.49 ab	2.6 ± 0.11 ab	9.0 ± 0.34 cd
JS	Jiangsu	whole root	37.5 ± 0.67 cd	0.3 ± 0.00 bc	1.8 ± 0.02 c	6.7 ± 0.33 cd	2.0 ± 0.03 e	9.1 ± 0.04 bc
SC	Sichuan	whole root	32.5 ± 0.79 ef	0.2 ± 0.00 de	1.4 ± 0.03 e	5.7 ± 0.32 ef	1.9 ± 0.03 f	7.8 ± 0.17 ef
ZJ	Zhejiang	whole root	47.6 ± 1.95 a	0.4 ± 0.00 a	2.1 ± 0.02 a	8.4 ± 0.43 a	2.6 ± 0.07 a	9.7 ± 0.22 a
mean			39.0	0.3	1.7	7.0	2.3	8.8
HUB	Hubei	root skin	74.3 ± 2.46 a	1.3 ± 0.01 a	2.5 ± 0.04 a	12.8 ± 0.95 a	4.0 ± 0.08 a	11.1 ± 0.12 a
		whole root	38.3 ± 0.95 b	0.3 ± 0.00 b	1.8 ± 0.01 b	7.0 ± 0.56 b	2.3 ± 0.02 b	9.3 ± 0.05 b
		root heart	13.3 ± 0.29 c	nd	0.6 ± 0.01 c	3.9 ± 0.08 c	1.1 ± 0.01 c	3.6 ± 0.01 c
mean of eight vegetables and fruits[d]						3.8 ± 0.21	1.0 ± 0.04	3.1 ± 0.21

[a]Data are expressed as means ± SD ($n = 3$); values marked by the same letter in same column of same class are not significantly different at $p < 0.05$. [b]Total caffeoylquinic acids, the sum of the contents of individual caffeoylquinic acids identified in Table 1 that were expressed as mg 5-*O*-caffeoylquinic acid/g d.w. [c]Total lignans, the sum of the contents of individual lignans identified in Table 1 that were expressed as mg arctigenin/g d.w. [d]Eight common vegetables and fruits (Chinese cabbage, carrot, cucumber, eggplant, kiwi fruit, orange, tomato, and Washington red apple) collected in Hong Kong local supermarket were used as controls.

higher than the values of total caffeoylquinic acids of the root heart (13.3 mg/g) and the whole root (38.3 mg/g), indicating that caffeoylquinic acids were mainly distributed in the root skin of burdock. Also, trace amounts of lignans were mostly distributed in the skin root (1.3 mg/g), and no lignans were detected in the root heart.

The significant variations in contents of total caffeoylquinic acids and total lignans among six different burdock genotypes (HEB, HUB, JL, JS, SC, and ZJ) with different geographic origins were compared ($p < 0.05$) (Table 2). Total caffeoylquinic acids in seeds of six burdock genotypes varied significantly from 37.5 mg/g in HEB to 54.4 mg/g in ZJ, whereas the values of total caffeoylquinic acids in roots (whole root) of six burdock genotypes ranged considerably from 32.5 mg/g in SC to 47.6 mg/g in ZJ. Moreover, total lignans in seeds of six burdock genotypes varied significantly from 48.3 mg/g in SC to 69.5 mg/g in ZJ. Also, trace amounts of total lignans in roots of six genotypes had big variations from 0.2 mg/g in HEB to 0.4 mg/g in ZJ. The variations in the contents of individual or total caffeoylquinic acids and lignans among different burdock genotypes with different geographic origins should be attributed to the diversities in their genetic factors and environmental conditions.

In addition, total phenolic contents in crude extracts of all of the burdock genotypes were also estimated using the Folin−Ciocalteu method. The values of total phenolic contents in the seeds of six burdock genotypes varied from 3.2 to 4.6 g GAE/100 g d.w. (i.e., SC and ZJ, respectively), whereas the values of total phenolic contents in their roots (whole root) ranged from 1.3 to 2.1 g/100 g (i.e., HEB and ZJ, respectively). The seeds of all burdock genotypes had significantly higher values of total phenolic contents than their roots (whole root samples). This was identical to the results of individual caffeoylquinic acids and lignans as well as their total contents in seeds and roots of all six burdock genotypes.

Comparison of Antioxidant Activity of Seeds and Roots of Different Burdock Genotypes. The significant differences in antioxidant activities of crude extracts from seeds and roots among different genotypes were compared ($p < 0.05$) (Table 2). In comparison to the average values of the antioxidant activity assayed by TEAC, FRAP, and hydroxyl radical-scavenging activity methods, the seeds of six burdock genotypes exhibited much stronger antioxidant activity than their roots (whole root samples). Moreover, these values of antioxidant activity of both seeds and roots of all the tested burdock genotypes were significantly higher (∼2−8-fold) than the mean values of controls consisting of eight common vegetables and fruits. This suggested that both seeds and roots of different burdock genotypes could be good sources of potent natural antioxidants.

In summary, qualitative and quantitative analyses of caffeoylquinic acids and lignans in the methanolic crude

extracts of both roots and seeds of six various burdock genotypes originated from different provinces in China were systematically performed and compared in the present study for the first time. The developed method of LC−MS combined with MALDI-QIT-TOF MS may have potential for simultaneous identification and large-scale screening of a great number of lignans and caffeoylquinic acids in dietary plants and medicinal herbs. The qualitative and quantitative data may provide useful information for screening and breeding of the burdock genotypes with high levels of the bioactive phenolic components and also for their authentication and quality control during production, storage, and process. Moreover, this study could contribute to a better understanding of all antioxidant phenolic compounds of burdock as a root vegetable as well as a medicinal plant.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: (852) 25890481. Fax: (852) 28725476. E-mail: ybzhang@hku.hk.

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Notes

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