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Analysis of Hydrolyzable Tannins and Other Phenolic Compounds in Emblic Leafflower (*Phyllanthus emblica* L.) Fruits by High Performance Liquid Chromatography–Electrospray Ionization Mass Spectrometry

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Supporting Information

ABSTRACT: Phenolic compounds were extracted from dried emblic leafflower (*Phyllanthus emblica* L.) fruits with methanol and separated by Sephadex LH-20 column chromatography. The raw extracts and fractions were analyzed with HPLC coupled with diode array UV spectroscopy, electrospray ionization mass spectrometry, and tandem mass spectrometry. Mucic acid gallate, mucic acid lactone gallate, monogalloylglucose, gallic acid, digalloylglucose, putranjivain A, galloyl-HHDP-glucose, elaeocarpusin, and chebulagic acid were suggested to be the most abundant compounds in the crude methanol extracts of the fruits. In addition, 144 peaks were detected, of which 67 were tentatively identified mostly as ellagitannins, flavonoids, and simple gallic acid derivatives in the fractions. The results indicated the presence of neochebulagic acid, isomers of neochebuloyl galloylglucose, chebuloyl neochebuloyl galloylglucose, ellagic acid glycosides, quercetin glycosides, and eriodictyol coumaroyl glycosides in the fruits. The study provides a systematic report of the retention data and characteristics of UV, MS, and MS/MS spectra of the phenolic compounds in the fruits of emblic leafflower. The fruits of two varieties (Ping Dan No 1 and Fruity) from Guangxi Province differed from those of wild Tian Chuan emblic leafflower from Fujian Province in the content and profile of phenolic compounds.

KEYWORDS: *emblic leafflower fruits, phenolic compounds, Phyllanthus emblica, tannins*

INTRODUCTION

Phyllanthus emblica L. (syn: *Emblica officinalis* Gaertn.), commonly known as emblic leafflower fruit, Indian gooseberry, or Amla, belongs to the genus *Phyllanthus* in the family *Euphorbiaceae*. The species is naturally distributed in the tropical and subtropical area of Asia such as Southern China and India. The fruits have been consumed as food and used as traditional medicinal materials for a long time in China, India, and the Southeast Asian countries.[1]

In vitro and *in vivo* studies showed that the extracts of the fruits had strong antioxidative and radical scavenging activities against DPPH, $O_2^{\bullet-}$, $OH^{\bullet}$, and NO radicals.[2−4] Moreover, preclinical and clinical studies carried out in past decades have shown that fruits of emblic leafflower possess antibacterial, antidiabetic, hypolipidemic, anticancer, anti-inflammatory, immunomodulatory, antiatherogenic, antihypercholesterolemic, gastroprotective, hepatoprotective, cardiovascular protective, and neuroprotective properties.[1,2,5−7] Most of the reports suggest that these health effects could be attributed to the antioxidative activities of the fruits.

Phenolic compounds, especially hydrolyzable tannins and flavonoids in combination with vitamin C, are considered to be the major antioxidants and bioactive components in the extracts of emblic leafflower fruits.[8−11] More than 20 hydrolyzable tannins have been reported in emblic leafflower fruits in the literature.[9,10,12,13] However, there are contradictory reports among different studies on the compositional profiles of hydrolyzable tannins in emblic leafflower fruit. For instance, Ghosal et al. reported that the fruits contained hydrolyzable tannins, emblicanin A (2,3-di-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-2-keto-gluconolactone), and emblicanin B (2,3,4,6-bis-(*S*)-hexahydroxydiphenoyl-2-ketogluconolactone), along with pedunculagin and punigluconin.[12] However, according to Majeed et al., the fruits of emblic leafflower do not contain emblicanin A and B. The compounds identified as emblicanin A and B by Ghosal were in fact 1-*O*-galloyl-*β*-D-glucose and mucic acid 1,4-lactone 5-*O*-gallate, respectively.[13]

In addition, previous studies on the phenolic compounds of emblic leafflower fruits are commonly carried out by the isolation and purification of individual compounds.[9,10,12,13] There is a lack of information on the overall composition of the phenolic compounds of the fruits and the chromatographic behaviors of these compounds in a modern chromatographic system. A systematical investigation of the phenolic compounds in the fruits with high performance liquid chromatography combined with mass spectrometry is essential to obtain an overall profile of the phenolic compounds and for the quantitative analysis of these compounds.

In the current study, phenolic compounds were extracted from the fruits of emblic leafflower with methanol and analyzed

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by high performance liquid chromatography (HPLC) combined with diode array detection (HPLC-DAD) and electrospray ionization mass spectrometry in negative ion mode (HPLC-ESI(−)-MS) with two different mass analyzers (Quadrupole and Time-of-Flight). In order to improve the separation between compounds, the crude extract was further fractionated by Sephadex LH-20 column chromatography, followed by the analysis of each of the fractions by HPLC-DAD and HPLC-ESI-MS. In addition, HPLC-ESI-MS/MS analyses were performed to further support compound identifications.

Emblic leafflower is naturally distributed and cultivated in subtropical areas with significant genetic variations. The size, shape and taste, and basic nutrient composition of the fruits differ among different natural populations and varieties. So far, no studies have been reported on the difference in the phenolic profiles among the fruits of different natural populations and varieties of emblic leafflower. In the current study, we investigated the phenolic compounds in fruits of two varieties (Ping Dan No. 1) and (Fruity) from Guangxi Province and two natural populations of wild emblic leafflower "Tian Chuan" from Fujian Province in order to compare the phenolic composition of these major sources of emblic leafflower fruits in China.

MATERIALS AND METHODS

Material. Four emblic leafflower fruit (*P. emblica*) samples were collected from Guangxi and Fujian provinces in China in 2006. The fruits of the varieties Ping Dan No. 1 (EMB1) and Fruity (EMB2) were provided by Guangxi Dayu Emblic Fruit Ltd. (Pingnan County, Guangxi Province, China), and the fruits were collected from a cultivation site in Danzhu town, Pingnan County, Guangxi Province, China. The samples were identified by Huang Xiongfang, the developer of the varieties. Fruit samples of wild Tian Chuan emblic leafflower were provided by Huian Old Father's Food Company (Huian County, Fujian Province, China), and the fruits were collected from two different certified natural growth sites of Tian Chuan Emblic Leafflower in Lantian County (EMB3) and Weishan County (EMB4), Fujian Province, China (EMB3 and EMB4). After harvesting, the fruits were sliced and air-dried for one day, followed by hot air-drying at 60 °C for 8 h.

Gallic acid was purchased from Sigma-Aldrich (St. Louis, MO) and formic acid (eluent additive for HPLC-MS) from J. T. Baker (Deventer, The Netherlands). Methanol (HPLC grade), acetone (HPLC grade), and acetonitrile (HPLC grade) were purchased from VWR International Oy (Espoo, Finland) and Sigma-Aldrich (St. Louis, MO). Sephadex LH-20 was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden).

For the HPLC-DAD-ESI(−)-QTOF-MS/MS system, acetonitrile (LC-MS CHROMASOL grade) and formic acid were purchased from Sigma-Aldrich (Steinheim, Germany). Water was filtered through an Elgastat UHQ-PS purification system (Elga, Kaarst, Germany).

Sample Preparation. Dried, seedless emblic leafflower fruits were milled into a fine powder using a Retsch electric mill (Haan, Germany) with a sieve sized 0.5 mm and stored in a desiccator until extraction.

Preparation of Crude Methanol Extract. A sample of 0.5 g of dry emblic leafflower fruit powder was extracted with 10 mL of methanol in an ultrasonicator bath at room temperature for 5 min. The obtained crude extract was filtered through a 0.45 μm filter and immediately analyzed with HPLC-DAD and HPLC-ESI(−)-MS.

Preparation of Sephadex LH-20 Column Chromatography Fractions. A sample of 20 g of emblic leafflower fruit powder was extracted with 100 mL of methanol in an ultrasonicator bath for 20 min, followed by centrifugation (4420*g*) for 15 min, and collection of the supernatant after centrifugation. The extraction was repeated, and the two supernatants were combined. The extract was evaporated into thick slurry using a vacuum rotary evaporator at 35 °C. The slurry was washed out and dissolved in Milli-Q water (3−5 mL). An aliquot of extract equivalent to 5 g of dried fruit powder was applied to a 22 mm i.d. × 470 mm L. glass column (Wright Scientific Ltd., England) packed with 20 g Sephadex LH-20 medium (dry particle size 18−111 μm). The Sephadex LH-20 dry powder was swollen in Milli-Q water overnight. The column was packed and equilibrated with Milli-Q water as recommended by the manufacturer. After that, the column was sequentially eluted with solvents according to Table 1 to yield 10 fractions of 150 mL each. The elution speed was maintained at 1.4 mL/min by an Alitea-XV peristaltic pump (Bioengineering, Sweden). Between samples, the column was regenerated by washing it with 10 column volumes of Milli-Q water.

Table 1. Eluotropic Series for Sephadex LH-20 Column Chromatography

fraction	H_2O (%)	MeOH (%)	acetone (%)
F1	100	0	0
F2	70	30	0
F3	50	50	0
F4	50	40	10
F5	50	30	20
F6	50	20	30
F7	50	10	40
F8	50	0	50
F9	30	0	70
F10	20	0	80

After the removal of organic solvents by nitrogen flow, the fractions were frozen at −20 °C and subsequently freeze-dried with a Dura-Dry freeze-dryer (FTS Systems, Inc., Stone Ridge, NY). After that, the dry material was washed out using 3 mL of Milli-Q water. The water was evaporated, and each fraction was redissolved in 1 mL of methanol, filtered through a 0.45 μm filter, and analyzed with HPLC-ESI(−)-MS. At the same time, the peaks were monitored with a diode-array detector at wavelengths of 280 and 360 nm.

HPLC-DAD Analysis. The HPLC-DAD system consisted of a GT-154 vacuum degasser, two LC-10AT pumps, a SIL-10A automatic injector, a CTO-10A column oven, a SPD-M10A VP photodiode array detector (DAD), and a SCL-10A VP system controller (Shimadzu, Kyoto, Japan). The system was operated using Class-VP 6.1 Workstation software. A Phenomenex Prodigy RP-18 ODS (3) column (5 μm, 250 × 4.60 mm, Torrance, CA) combined with a Phenomenex Prodigy guard column (5 μm, 30 × 4.60 mm, Torrance, CA) was used. A binary solvent system was employed consisting of 0.1% formic acid in Milli-Q water as solvent A and acetonitrile/methanol (4:1, v/v) as solvent B. The gradient program was 0−5 min with 0% solvent B, 5−15 min with 0−5% B, 15−20 min with 5−10% B, 20−25 min with 10−15% B, 25−30 min with 15−20% B, 30−35 min with 20−25% B, 35−40 min with 25% B, 40−55 min with 25−60% B, 55−60 min with 60−0% B, and 60−65 min with 0% B. The flow rate of the mobile phase was 1 mL/min, and the injection volume was 10 μL.

HPLC-ESI(−)-MS Analysis. HPLC-ESI(−)-MS analysis was performed using a Waters Acquity Ultra Performance LC system in combination with a Waters Quattro Premier mass spectrometer (Waters Corp., Milford, MA) equipped with an ion-spray interface. The column and chromatographic conditions were the same as in the HPLC-DAD analyses.

The capillary voltage was set to 3.0 kV, the cone voltage to 25 V, and the extractor voltage to 8 V. The source temperature was 120 °C and the desolvation temperature 300 °C. Mass spectra were obtained by scanning ions in the range of m/z 100 and 1300. The HPLC-ESI(−)-MS system was operated using MassLynx 4.1 software (Waters Corp., Milford, MA).

HPLC-DAD-ESI(−)-QTOF-MS/MS Analysis. The crude extract and the 10 fractions of sample EMB1 were also analyzed with the HPLC-DAD-ESI(−)-QTOF-MS/MS system, which consisted of an Agilent HPLC 1200 Series equipped with a diode array detector

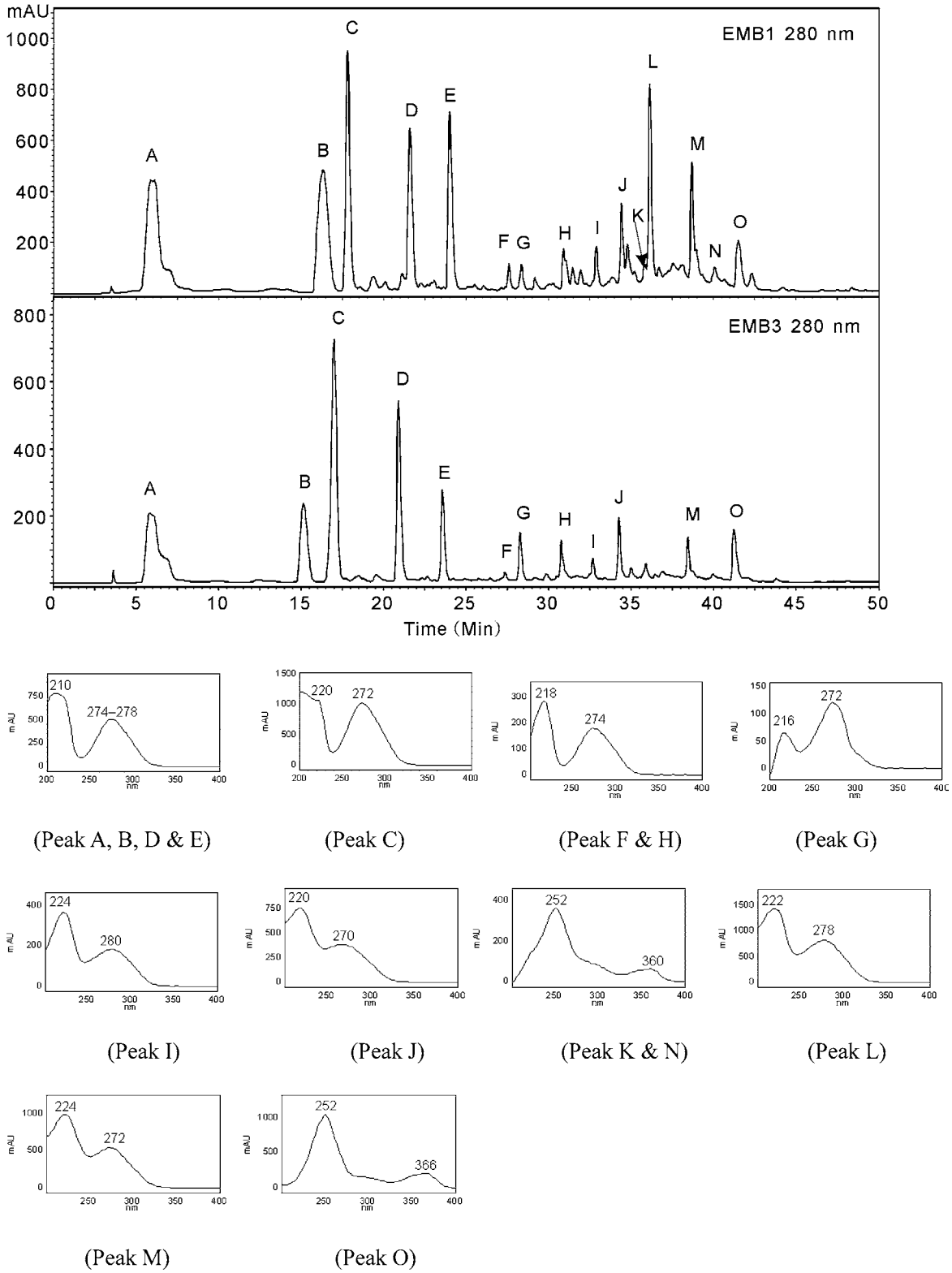

Figure 1. HPLC-DAD chromatograms of the crude extracts of EMB1 and EMB3 and the UV spectra of major compounds at 280 nm. The slight difference in the retention times between corresponding peaks of EMB1 and EMB3 was due to some inter-run shift of retention times.

(Agilent Technologies, Waldbronn, Germany) and micrOTOF$_Q$ ESI-mass spectrometer (Bruker Daltonics, Bremen, Germany). Chromatographic separations were performed using an XBridge column (2.1 × 100 mm, Phenyl, 3.5 μm, Waters, Dublin, Ireland). The binary mobile phase consisted of acetonitrile (A) and water and formic acid (99.9:0.1, v/v) (B). The elution profile was as follows: 0−1 min 0% A in B, 1−30 min 0−30% A in B, 30−35 min 30−40% A in B, and 35−37 min 40−80% A in B, 37−47 min 80% A in B, 47−49 min 80−0% A in B, and 49−65 min 100% B. The flow rate was 0.3 mL/min, and the injection volume was 5 μL. Chromatograms were recorded at 190−950 nm. The HPLC system was controlled by Hystar software (version 3.2.; Bruker BioSpin, Rheinstetten, Germany). The mass spectrometer was controlled by Bruker Compass micrOTOF control software (Bruker Daltonics, Bremen, Germany) and operated in

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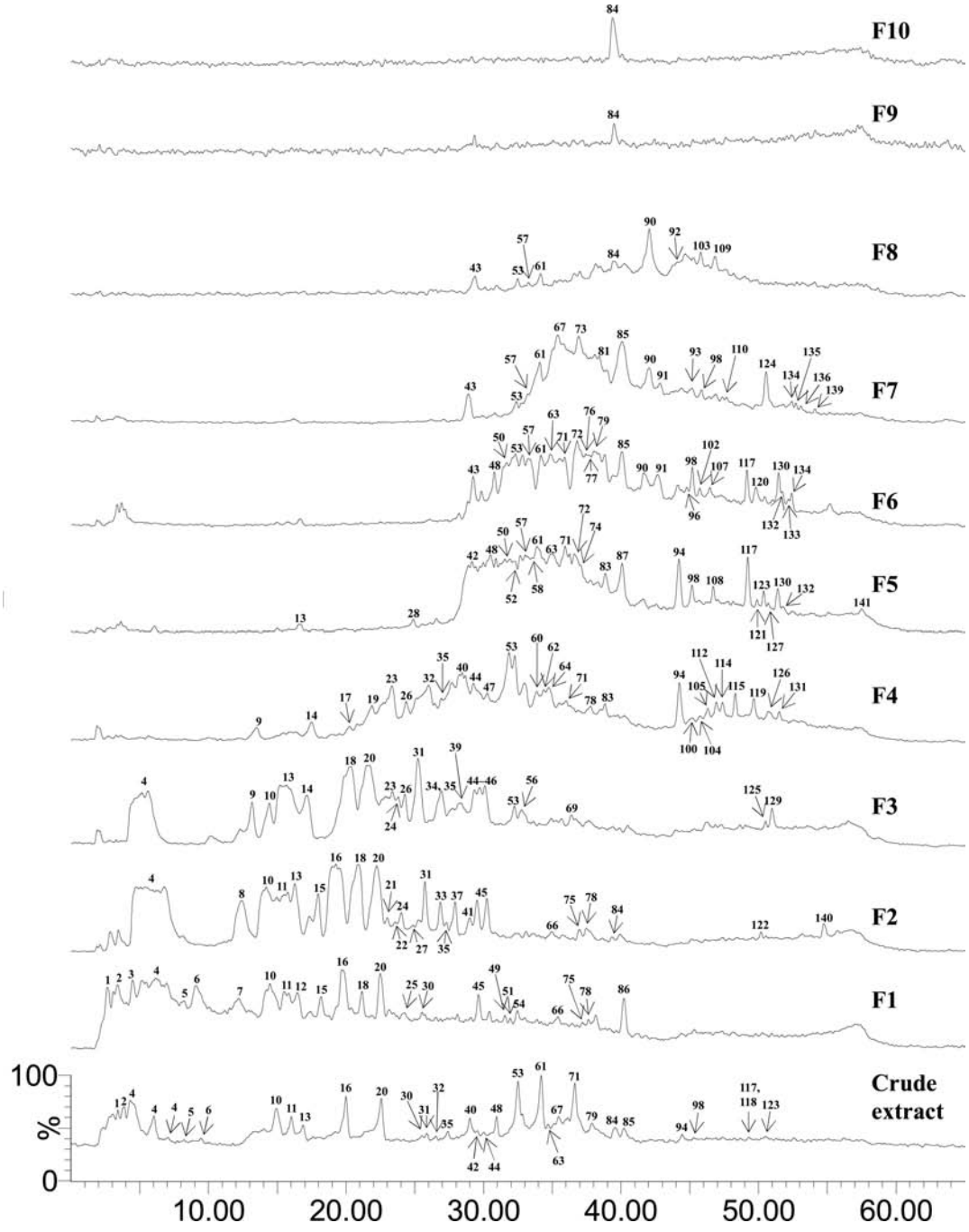

Figure 2. HPLC-ESI(−)-MS total ion chromatograms of the crude extract and fractions F1−10 from the sample EMB1.

negative ion mode. The capillary voltage was maintained at +4000 V with the end plate offset at −500 V. The pressure for nebulizer gas (N_2) was set at 1.6 bar, and the drying gas (N_2) flow was 12.0 L/min and the drying gas temperature 200 °C. The full scan mass ranged from m/z 50 up to m/z 3000. For HPLC-MS/MS experiments, a collision sweeping mode was used for the collision induced dissociation in the collision cell. The collision gas was argon. The calibration with 5 mM sodium formate injected via the six-port valve was used at the end of HPLC-MS/MS experiment in order to provide high-accuracy mass measurements. The data were handled by Bruker Compass DataAnalysis (version 4.0; Bruker Daltonics, Bremen, Germany). The internal mass spectrum calibration was performed with HPC mode, and the minimal number of calibration points was seven and standard deviation below 1.5 ppm.

RESULTS AND DISCUSSION

Analysis of Crude Methanol Extracts of *P. emblica* Fruit. The HPLC-UV chromatograms at 280 nm of EMB1 and EMB3 and the UV spectra of the major peaks are displayed in Figure 1. The total ion chromatogram of the crude extract of EMB1 is presented in Figure 2. The mass spectra of peaks A−O are shown in the Supporting Information, Figure 1. Fifteen peaks were tentatively identified on the basis of the UV and mass spectra.

Peaks A, B, D, and E had similar UV spectra with maxima at 210 and 274−278 nm (Figure 1), which are the characteristics of the UV spectra of gallate esters.[13] Analyses of the mass spectra of these compounds also supported the identification. Peak A was tentatively identified as a mucic acid gallate. The

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mass spectrum had the $[M - H]^-$ and $[2M - H]^-$ ions at m/z 361 and 723, respectively. The fragment ion at m/z 209 represented the ion $[M - H - galloyl]^-$, the deprotonated ion of the mucic acid moiety. The mass spectra of peaks B, D, and E showed $[M - H]^-$, $[2M - H]^-$, and $[M - H - galloyl]^-$ ions at m/z 343, 687, and 191, respectively. These three compounds were tentatively identified as mucic acid lactone gallates.

Peaks F and H had UV spectra typical for simple gallate esters of glucose.[14] There were two clear absorption maxima at 218 and 274 nm, respectively, separated by a deep valley at 250 nm (Figure 1). Peak F exhibited overlapping peaks of digalloylglucose and malic acid gallate, which was verified by the mass spectra. Peak H was also identified as a digalloylglucose. On the basis of the mass spectra, peak C consisted of galloylglucose (C1) and gallic acid (C2), which were well separated in the HPLC-ESI(−)-MS chromatogram (Figure 2, peaks 11 and 13, respectively). Peak G had an UV profile, which was very different from the rest (Figure 1); this was possibly caused by two or more compounds eluting at the same time.

Peak I, J, L, and M had the typical UV absorption profiles of ellagitannins.[14,15] These compounds all had two maximal UV absorptions at around 220 nm and 270−280 nm, respectively (Figure 1). The differences in UV spectra among individual ellagitannins are caused by different numbers of C−C linkages between galloyl groups in the molecules. The ratio of galloyl to HHDP (hexahydroxydiphenoyl) groups esterified to the polyol determines the depth of the valley between the absorption maxima in the UV spectra of ellagitannins.[14,15] For example, the valleys are deeper in the UV spectra of galloylglucose (ratio 1:0) than those in the UV spectra of galloyl-HHDP-glucose (ratio 1:1). On the basis of the UV spectra of I, J, L, and M, the ratio was likely between 1:1 and 3:1. The valley was the lowest in the spectrum of peak I, suggesting that it had the highest galloyl/HHDP ratio. Peak I was tentatively identified as putranjivain A. The mass spectrum presented an $[M - H]^-$ ion at m/z 1083 and $[M - 2H]^{2-}$ and $[M - 2H - galloyl]^{2-}$ ions at m/z 541 and 467, respectively. The data were identical with those of previous reports.[10,16] Peaks J and M were tentatively identified as galloyl-HHDP-glucose and chebulagic acid, respectively, according to their UV and MS spectra and the literature.[10,20] Peak L represented a compound with a molecular weight equal to that of elaeocarpusin, a compound previously reported in emblic leafflower fruits.[10]

Some minor components with absorption maxima around 360 nm were found in *P. emblica* crude extracts. Peak O was identified as ellagic acid (λ_{max} at 252 and 366 nm, Figure 1). Peaks K and N had UV spectra similar to that of peak O, but the λ_{max} was at 252 and 360 nm (Figure 1). Peaks K and N were identified as ellagic acid glycosides.

In addition to the above peaks in the HPLC-UV chromatogram of the crude extracts at 280 nm, gallic acid was detected and identified based on the UV and MS spectra and with the aid of a reference compound in the total ion chromatograms of *P. emblica* fruit extracts.

On the basis of the above HPLC-UV and HPLC-MS analyses of the total extracts, mucic acid gallate, mucic acid lactone gallate isomers, monogalloylglucose, gallic acid, digalloylglucose, putranjivain A, galloyl-HHDP-glucose, elaeocarpusin, and chebulagic acid were the most abundant compounds in the crude methanol extracts of emblic leafflower fruits. These results were in congruence with earlier reports by Zhang et al.[17]

The UV and total ion chromatograms showed the complex profiles of phenolic compounds of the crude methanol extracts of *P. emblica* fruits. In addition to the major compounds identified or tentatively identified, there were a large number of minor compounds. Identification of these compounds was difficult because of their low concentrations and insufficient separation in the chromatograms of the crude extracts.

Identification of the Components in Sephadex LH-20 Column Chromatographic Fractions by HPLC-MS and HPLC-MS/MS. In order to achieve a more detailed characterization of the methanol extract, Sephadex LH-20 column chromatography was employed to fractionate the crude extract of *P. emblica* fruits into 10 fractions. These fractions were analyzed by HPLC-ESI(−)-MS. The total ion chromatograms of HPLC-ESI(−)-MS of the crude extract of EMB1 and its fractions are shown in Figure 2.

The HPLC-MS analyses were carried out using both an HPLC-ESI(−)-MS with a quadrupole mass spectrometer and an HPLC-ESI(−)-MS with a Time-of-Flight (TOF) mass spectrometer. The TOF-MS gave more accurate ion masses than the quadrupole mass spectrometer, which helped to fully differentiate between ions with approximately the same mass (for example, ellagic acid and quercetin, with calculated monoisotopic ion mass of 300.9990 and 301.0354, respectively). It was also easier to identify the multiply charged ions with the TOF-MS. Selected fractions were subjected to additional MS/MS analyses with the HPLC-ESI(−)-TOF-MS/MS system to get more detailed structural information.

Table 2 presents a summary of the phenolic compounds identified or tentatively identified in the methanol extract of fruits of emblic leafflower and in its fractions obtained by Sephadex LH-20 column chromatography, together with characteristic information on the UV, MS, and MS/MS spectra of these compounds. The proposed structures of the major groups of compounds identified in this study are presented in Figure 3. In the following sections, the characteristics of the mass spectra of some major compounds are reviewed. The numbers presented in parentheses following the names of compounds correspond to the peak numbers presented in Figure 2 and Table 2.

Acids. Mucic acid (**1**),[18] mucic acid lactone (**2**), and malic acid (**3**) were detected from the water fraction (F1) with $[M - H]^-$ ions at m/z 209, 191, and 133, respectively. Chebulic acid (**8**) was identified based on its fragmentation pattern with ions at m/z 355 $[M - H]^-$, m/z 337 $[M - H - H_2O]^-$, and m/z 711 $[2M - H]^-$.[19,20] Gallic acid (**13**) was identified based on its UV and MS spectra and by comparison to a reference compound. Gallic acid had an $[M - H]^-$ ion at m/z 169 and a $[M - COOH]^-$ ion at m/z 125 and UV maxima at 217 and 271 nm.[9,20,21]

Ellagic acid (**84**) was identified based on the $[M - H]^-$ and $[2M - H]^-$ ions at m/z 301 and 603, respectively, and the characteristic UV spectrum with λ_{max} at 253 and 367 nm.[14,20,21] Cinnamic acid (**129**) eluted to the third fraction (F3) and exhibited $[M - H]^-$ and $[M - COOH]^-$ ions at m/z 147 and m/z 103, respectively.[21]

Simple Gallic Acid Esters. Several isomers of mucic acid gallate and mucic acid lactone gallates, -digallates, and their mono- and dimethyl esters were identified, as reported earlier by Zhang et al. and Yokosawa et al.[9,11] UV absorbance maxima at 214−216 and 274−277 nm and the ions resulting from the loss of the galloyl moieties (152 Da) in the mass spectra were characteristics of these compounds. In HPLC-ESI(−)-MS

Table 2. Compounds Determined by HPLC-ESI(−)-MS and HPLC-DAD-ESI(−)-QTOF-MS/MS in the Sephadex LH-20 Column Chromatographic Fractions of Four *P. emblica* Fruit Samples EMB1−4

		m/z data[c]							exact mass		
peak[a]	R_t[b] (min)	$[M-H]^-$	$[2M-H]^-$	fragments	λ_{max} (nm)[d]	sample containing the compd	tentative identification[e]	molecular formula	measd[f]	calcd	error (ppm)
1	2.71	209	419	191 $[M-H-H_2O]^-$	nd	EMB1−4	mucic acid	$C_6H_{10}O_8$	210.0383	210.0376	3.33
2	3.46	191	383		nd	EMB1−4	mucic acid lactone	$C_6H_8O_7$	192.0279	192.0270	4.69
3	4.54	133		115 $[M-H-H_2O]^-$	nd	EMB1−4	malic acid	$C_4H_6O_5$	nd	134.0215	
4	5.85	361	723	209 $[M-H-galloyl]^-$, 191 $[M-H-galloyl-H_2O]^-$	215, 275	EMB1−4	mucic acid gallate	$C_{13}H_{14}O_{12}$	362.0510	362.0485	6.91
8	12.22	355	711	337 $[M-H-H_2O]^-$	217, 282	EMB1−4	chebulic acid	$C_{14}H_{12}O_{11}$	356.0406	356.0380	7.30
9	13.45	513	1027	361 $[M-H-galloyl]^-$, 209 $[M-H-galloyl-galloyl]^-$	214, 277	EMB1−4	mucic acid digallate	$C_{20}H_{18}O_{16}$	514.0595	514.0595	0.00
10	14.31	343	687	191 $[M-H-galloyl]^-$	215, 276	EMB1−4	mucic acid lactone gallate	$C_{13}H_{12}O_{11}$	344.0411	344.0380	9.01
11	15.57	331	663		215, 272	EMB1−4	galloylglucose	$C_{13}H_{16}O_{10}$	332.0775	332.0743	9.64
12	16.14	375	751	223 $[M-H-galloyl]^-$, 191	217, 274	EMB1−4	mucic acid methyl ester gallate	$C_{14}H_{16}O_{12}$	376.0694	376.0642	13.84
13	16.47	169		125 $[M-COOH]^-$	217, 271	EMB1−4	gallic acid	$C_7H_6O_5$	170.0232	170.0215	10.00
14	17.57	513	1027	361 $[M-H-galloyl]^-$, 209 $[M-H-galloyl-galloyl]^-$, 191 $[M-H-galloyl-galloyl-H_2O]^-$	215, 274	EMB1−4	mucic acid digallate	$C_{20}H_{18}O_{16}$	514.0618	514.0595	4.47
15	18.01	375	751	223 $[M-H-galloyl]^-$, 191	215, 276	EMB1−4	mucic acid methyl ester gallate	$C_{14}H_{16}O_{12}$	376.0772	376.0642	21.27
16	19.46	343	687	191 $[M-H-galloyl]^-$	215, 276	EMB1−4	mucic acid lactone gallate	$C_{13}H_{12}O_{11}$	344.0414	344.0380	9.88
17	20.40	513	1027	361 $[M-H-galloyl]^-$, 209 $[M-H-galloyl-galloyl]^-$, 191 $[M-H-galloyl-galloyl-H_2O]^-$	215, 274	EMB1−3	mucic acid digallate	$C_{20}H_{18}O_{16}$	514.0618	514.0595	4.47
18	20.76	375	751	223 $[M-H-galloyl]^-$, 191	216, 276	EMB1−4	mucic acid methyl ester gallate	$C_{14}H_{16}O_{12}$	376.0692	376.0642	13.30
19	22.11	513	1027	361 $[M-H-galloyl]^-$, 209 $[M-H-galloyl-galloyl]^-$, 191 $[M-H-galloyl-galloyl-H_2O]^-$	215, 274	EMB1−3	mucic acid digallate	$C_{20}H_{18}O_{16}$	514.0565	514.0595	−5.84
20	22.18	343	687	191 $[M-H-galloyl]^-$	215, 277	EMB1−4	mucic acid lactone gallate	$C_{13}H_{12}O_{11}$	344.0414	344.0380	4.94
22	23.21	389	779	241, 169	216, 277	EMB1−4	mucic acid dimethyl ester gallate	$C_{15}H_{18}O_{12}$	390.0817	390.0798	4.87
23	23.51	513	1027	361 $[M-H-galloyl]^-$, 209 $[M-H-galloyl-galloyl]^-$, 191 $[M-H-galloyl-galloyl-H_2O]^-$	215, 274	EMB1−4	mucic acid digallate	$C_{20}H_{18}O_{16}$	514.0655	514.0595	−1.95
26	24.48	527	1055	375 $[M-H-galloyl]^-$, 223 $[M-H-galloyl-galloyl]^-$	216, 274	EMB1−4	**mucic acid methyl ester digallate**	$C_{21}H_{20}O_{16}$	528.0746	528.0751	−0.95
28	24.97	633		463 $[M-H-galloyl-H_2O]^-$, 301 $[M-H-galloyl-H_2O-Hex]^-$	219, 277	EMB1, 2, 4	galloyl-HHDP-glucose	$C_{27}H_{22}O_{18}$	634.0790	634.0806	−2.52
29	25.14	483	967	331 $[M-H-galloyl]^-$, 271, 211, 169	215, 273	EMB3, 4	digalloylglucose	$C_{20}H_{20}O_{14}$	484.0849	484.0853	−0.83
31	25.60	285	571	169, 133 $[M-H-galloyl]^-$	216, 275	EMB1−4	malic acid gallate	$C_{11}H_{10}O_9$	286.0335	286.0325	3.50
32	26.30	483	967	331 $[M-H-galloyl]^-$, 271, 211, 169	216, 275	EMB1−4	digalloylglucose	$C_{20}H_{20}O_{14}$	484.0852	484.0853	−0.21
34	26.81	669		337 $[M-H-Hex-H_2O-galloyl]^-$/[chebulic acid $-H-H_2O]^-$, 249, 205	215, 283	EMB1, 2, 4	**neochebuloyl galloylglucose**	$C_{27}H_{26}O_{20}$	670.0992	670.1017	−3.73
37	27.84	357	715	169	216, 277	EMB1−4	mucic acid lactone methyl ester gallate	$C_{14}H_{14}O_{11}$	358.0569	358.0536	9.22
38	27.91	527	1055	375 $[M-H-galloyl]^-$, 223 $[M-H-galloyl-galloyl]^-$, 191	216, 274	EMB1−4	**mucic acid methyl ester digallate**	$C_{21}H_{20}O_{16}$	528.0748	528.0751	−0.57

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Table 2. continued

peak[a]	R_t[b] (min)	m/z data[c] [M − H]⁻	m/z data[c] [2M − H]⁻	m/z data[c] fragments	λ_{max} (nm)[d]	sample containing the compd	tentative identification[e]	molecular formula	exact mass measd[f]	exact mass calcd	exact mass error (ppm)
39	28.42	669		337 [M − H − Hex − H_2O − galloyl]⁻/ [chebulic acid − H − H_2O]⁻	226, 279	EMB1, 4	**neochebuloyl galloylglucose**	$C_{27}H_{26}O_{20}$	670.0999	670.1017	−2.69
40	28.74	483	967	331 [M − H − galloyl]⁻, 271, 211	217, 275	EMB1−4	digalloylglucose	$C_{20}H_{20}O_{14}$	484.0878	484.0853	5.16
42	29.23	807		463, 301	219, 280	EMB1−4	mallonin	$C_{33}H_{28}O_{24}$	808.0942	808.0970	−3.46
43	29.24	183			215, 270	EMB1−4	methyl gallate	$C_8H_8O_5$	184.0385	184.0372	7.06
44	29.53	495	991	343 [M − H − galloyl]⁻, 209, 191	215, 273	EMB1, 3, 4	mucic acid lactone digallate	$C_{20}H_{16}O_{15}$	496.0489	496.0489	0.00
45	30.02	357	715	169	217, 277	EMB1−4	mucic acid lactone methyl ester gallate	$C_{14}H_{14}O_{11}$	358.0577	358.0536	31.84
47	30.31	527	1055	375 [M − H − galloyl]⁻, 223 [M − H − galloyl − galloyl]⁻, 191	217, 277	EMB1, 3, 4	**mucic acid methyl ester digallate**	$C_{21}H_{20}O_{16}$	528.0794	528.0751	8.14
48	30.66	1083		541 [M − 2H]²⁻, 467 [M − 2H − galloyl]²⁻, 301	220, 279	EMB1−4	putranjivain A	$C_{46}H_{36}O_{31}$	1084.1249	1084.1240	0.83
50	31.57	971		953 [M − H − H_2O]⁻, 935 [M − H − H_2O − H_2O]⁻, 467 [M − 2H − H_2O − H_2O]²⁻, 301	219, 279	EMB1−4	**neochebulagic acid**	$C_{41}H_{32}O_{28}$	972.1068	972.1080	−1.23
52	32.19	651		633 [M − H − H_2O]⁻, 405, 300, 275	220, 277	EMB1−4	chebulanin	$C_{27}H_{24}O_{19}$	652.0934	652.0912	3.37
53	32.32	633	1267	301 [M − H − Hex − H_2O − galloyl]⁻	219, 272	EMB1−4	galloyl-HHDP-glucose	$C_{27}H_{22}O_{18}$	634.0813	634.0806	1.10
55	32.82	669		337 [M − H − Hex − H_2O − galloyl]⁻/ [chebulic acid − H − H_2O]⁻, 301, 275, 249, 205	219, 276	EMB4	**neochebuloyl galloylglucose**	$C_{27}H_{26}O_{20}$	670.0992	670.1017	−3.73
57	33.16	951		301	220, 278	EMB1−4	geraniin	$C_{41}H_{28}O_{27}$	952.0808	952.0818	−1.05
58	33.51	953		454 [M − H − COOH]²⁻, 301	220, 275	EMB1, 2, 4	chebulagic acid	$C_{41}H_{30}O_{27}$	954.0962	954.0974	−1.26
59	33.70	463	927	301 [M − H − Hex]⁻	252, 360	EMB1−4	**ellagic acid hexose**	$C_{20}H_{16}O_{13}$	464.0611	464.0591	4.31
61	34.08	1109		554 [M − 2H]²⁻, 463, 301	220, 278	EMB1−4	elaeocarpusin	$C_{47}H_{34}O_{32}$	1110.1010	1110.1033	−2.07
62a	34.37	635		331 [M − H − galloyl − galloyl]⁻, 301, 271	217, 274	EMB1−4	**trigalloylglucose**	$C_{27}H_{24}O_{18}$	636.0955	636.0962	−1.10
63	34.62	785		633 [M − H − galloyl]⁻	218, 277	EMB1−4	digalloyl-HHDP-glucose	$C_{34}H_{26}O_{22}$	786.0895	786.0915	−2.54
64	34.68	989		425, 337 [chebulic acid − H − H_2O]⁻, 249, 205, 169	224, 253	EMB1, 2, 4	chebuloyl neochebuloyl galloylglucose	$C_{41}H_{34}O_{29}$	990.1173	990.1185	−1.51
66	35.05	433	867	300	252, 360	EMB1−4	ellagic acid pentose	$C_{19}H_{14}O_{12}$	434.0469	434.0485	−3.69
70	36.15	989		337 [chebulic acid − H − H_2O]⁻, 494 [M − 2H]²⁻	224, 253	EMB4	chebuloyl neochebuloyl galloylglucose	$C_{41}H_{34}O_{29}$	990.1173	990.1185	−1.21
71a	36.50	953		476 [M − 2H]²⁻, 301	220, 277	EMB1−4	chebulagic acid	$C_{41}H_{30}O_{27}$	954.0956	954.0974	−2.62
71b	36.50	635		465 [M − H − galloyl − H_2O]⁻, 313 [M − H − galloyl − galloyl − H_2O]⁻, 301, 271, 211, 169	220, 277	EMB1−4	**trigalloylglucose**	$C_{27}H_{24}O_{18}$	636.0990	636.0962	4.40
72	36.71	785		633 [M − H − galloyl]⁻, 301	220, 276	EMB1−4	digalloyl-HHDP-glucose	$C_{34}H_{26}O_{22}$	786.0923	786.0915	1.02
75	37.10	433	867	301 [M − H − Pent]⁻	252, 360	EMB1, 2, 4	**ellagic acid pentose**	$C_{19}H_{14}O_{12}$	434.0467	434.0485	−4.15
76	37.50	787		483 [M − H − galloyl − galloyl]⁻, 465 [M − H − galloyl − galloyl − H_2O]⁻, 331, 313, 301, 271	218, 278	EMB1−4	tetragalloylglucose	$C_{34}H_{28}O_{22}$	788.1097	788.1072	3.17
78	37.62	447	895	300	252, 360	EMB1−4	**ellagic acid deoxyhexose**	$C_{20}H_{16}O_{12}$	448.0630	448.0642	−2.68
80	38.20	989		337 [chebulic acid − H − H_2O]⁻, 287, 169	nd	EMB2, 4	**chebuloyl neochebuloyl galloylglucose**	$C_{41}H_{34}O_{29}$	990.1153	990.1185	−3.23
81	38.26	937		633 [M − H − galloyl − galloyl]⁻, 468 [M − 2H]²⁻, 301	218, 277	EMB1−4	trigalloyl-HHDP-glucose	$C_{41}H_{30}O_{26}$	938.1024	938.1025	−0.11
83	38.87	463	927	301 [M − H − Hex]⁻	223, 267, 349	EMB1, 2, 4	**quercetin hexose**	$C_{21}H_{20}O_{12}$	464.0972	464.0955	3.66
84	39.33	301	603	283 [M − H − H_2O]⁻, 270, 216, 201, 185	252, 367	EMB1−4	ellagic acid	$C_{14}H_6O_8$	302.0093	302.0063	9.93

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Table 2. continued

peak[a]	R_t[b] (min)	m/z data[c] [M − H]⁻	[2M − H]⁻	fragments	λ_{max} (nm)[d]	sample containing the compd	tentative identification[e]	molecular formula	exact mass measd[f]	calcd	error (ppm)
87	40.14	785		633 [M − H − galloyl]⁻, 316 [M − 2H − galloyl]$^{2-}$, 301	220, 276	EMB1−4	digalloyl-HHDP-glucose	$C_{34}H_{26}O_{22}$	786.0902	786.0915	−1.65
90	41.89	917		458 [M − 2H]$^{2-}$	225, 276	EMB1−4	mallotusinin	$C_{41}H_{26}O_{25}$	918.0752	918.0763	−1.20
93	44.22	937		392 [M − 2H − galloyl]$^{2-}$	217, 277	EMB1−4	trigalloyl-HHDP-glucose	$C_{41}H_{30}O_{26}$	938.1023	938.1025	−0.21
94	44.25	447	895	300, 271, 255	204, 256, 347	EMB1−4	**quercetin deoxyhexose**	$C_{21}H_{20}O_{11}$	4448.1016	448.1005	2.45
98	45.14	937		633, 468 [M − 2H]$^{2-}$, 301	219, 278	EMB1−4	trigalloyl-HHDP-glucose	$C_{41}H_{30}O_{26}$	938.1023	938.1025	−0.21
123	50.35	595		287 [M − H − coumaroylhexose]⁻	234, 284	EMB1, 2	**eriodictyol coumaroylhexose**	$C_{30}H_{28}O_{13}$	596.1512	596.1530	−3.02
124	50.48	301		243	231, 255, 369	EMB1−4	quercetin	$C_{15}H_{10}O_7$	302.0447	302.0426	6.95
127	50.74	595		287 [M − H − coumaroylhexose]⁻	280	EMB1−2	**eriodictyol coumaroylhexose**	$C_{30}H_{28}O_{13}$	596.1523	596.1530	−1.12
129	51.00	147		103 [M − COOH]⁻	272	EMB1−4	cinnamic acid	$C_9H_8O_2$	nd	148.0524	

[a]The peak numbers are according to the peak labels in Figure 2. [b]The retention times are according to the HPLC-ESI(−)-MS system. [c]*m/z* data consist of ions observed in HPLC-ESI(−)-MS and HPLC-DAD-ESI(−)-TOF-MS/MS systems and includes the fragment ions produced both in the ion source (MS) and by collision induced dissociation (MS/MS). [d]nd, not detected. [e]Compounds not reported previously are in bold letters. [f]nd, not detected in the HPLC-DAD-ESI(−)-QTOF-MS/MS system; accurate mass not available.

analyses, mucic acid gallate (**4**) appeared as a very broad peak, which supposedly consisted of several isomeric forms. Mucic acid digallates were readily identified by the fragment ion in MS and MS/MS spectra, representing a monogallate after the loss of a galloyl moiety. Previously unreported mucic acid methyl ester digallates (**26**, **38**, and **47**) had an $[M - H]^-$ ion at *m/z* 527, a $[2M - H]^-$ ion at *m/z* 1055, and an $[M - H - galloyl]^-$ ion at *m/z* 375, which corresponded to the $[M - H]^-$ ions of mucic acid methyl ester gallates (**12**, **15**, and **18**). Some methyl esters are possibly artifacts produced during the methanol extraction process,[20] although Zhang et al. have claimed that small amounts of methyl esters of mucic acid occur naturally in *P. emblica* fruit juice.[9]

Galloyl esters of glucose had UV absorbance maxima at 215−217 and 272−275 nm. Identification of galloylglucose (**11**) was based on the $[M - H]^-$ ion at *m/z* 331 and $[2M - H]^-$ ion at *m/z* 663 and the literature.[9,13−16,20] Monogalloylglucose or *β*-glucogallin (1-*O*-galloyl-*β*-D-glucose, Figure 3) was one of the most abundant phenolic compounds in *P. emblica* fruit samples.[16] Isomers of digalloylglucose (**29**, **32**, and **40**, Figure 3) and trigalloylglucose (**62a** and **71b**), as well as a tetragalloylglucose (**76**), were also found in the extracts.[10,14−16,20]

A malic acid gallate (**31**) was identified with an $[M - H]^-$ ion at *m/z* 285, a $[2M - H]^-$ ion at *m/z* 571, and an $[M - H - galloyl]^-$ ion at *m/z* 133 in the mass spectrum.[9] Minor peaks of methyl gallate (**43**) were observed in several fractions; they might have been produced by methanolysis of some compounds containing galloyl groups during the sample preparation.[20]

Ellagitannins. The structures of major ellagitannins in emblic leafflower fruit extract are presented in Figure 3. Typically ellagitannins give a fragment of 301 Da due to the loss of an HHDP-group. Galloyl-HHDP-glucose had two isomers (**28** and **53**). The two galloyl-HHDP-glucose forms reported in *P. emblica* fruit were corilagin (1-*O*-galloyl-3,6-(*R*)-HHDP-*β*-D-glucose) and its *α*-isomer isocorilagin.[17,22,23] In reversed phase HPLC, the *β*-isomer elutes before the *α*-isomer; therefore, in this case peak **28** was tentatively identified as corilagin and peak **53** as isocorilagin. Tercatain (1,4-di-*O*-galloyl-3,6-(*R*)-HHDP-*β*-D-glucose) and punicafolin (1,2,4-tri-*O*-galloyl-3,6-(*R*)-HHDP-*β*-D-glucose) are known compounds in *P. emblica* fruit.[10] Both digalloyl-HHDP-glucoses (**63**, **72**, and **87**) and trigalloyl-HHDP-glucoses (**81**, **93**, and **98**) were also found. However, it was not possible to differentiate between the isomers of digalloyl-HHDP-glucose and trigalloyl-HHDP-glucose based on solely MS, MS/MS, and UV spectra.

Mallonin (**42**), putranjivain A (**48**), geraniin (**57**), and elaeocarpusin (**61**) are also compounds which are characteristic ellagitannins for *P. emblica* fruit.[10,11] A major compound (**90**) detected in fraction F8 was most probably mallotusinin based on its UV and MS spectra as well as the literature.[23]

Chebulanin (galloyl chebuloylglucose) (**52**) had a $[M - H - H_2O]^-$ fragment ion at *m/z* 633. Chebulagic acid (galloyl chebuloyl − HHDP-glucose) (**58** and **71**) had two ions at *m/z* 953 and 454 corresponding to $[M - H]^-$ and $[M - COOH - H]^{2-}$ ions, respectively.[20] Neochebulagic acid (galloyl neochebuloyl − HHDP-glucose) has been reported earlier in the ethanol extract of the fresh leaves and branches of *P. emblica*.[10] In the current study, the compound (**50**) was preliminarily identified as neochebulagic acid based on the ions at *m/z* 971 $[M - H]^-$, *m/z* 953 $[M - H - H_2O]^-$, *m/z* 935 $[M - H - H_2O - H_2O]^-$, and *m/z* 467 $[M - 2H - H_2O - H_2O]^{2-}$ in

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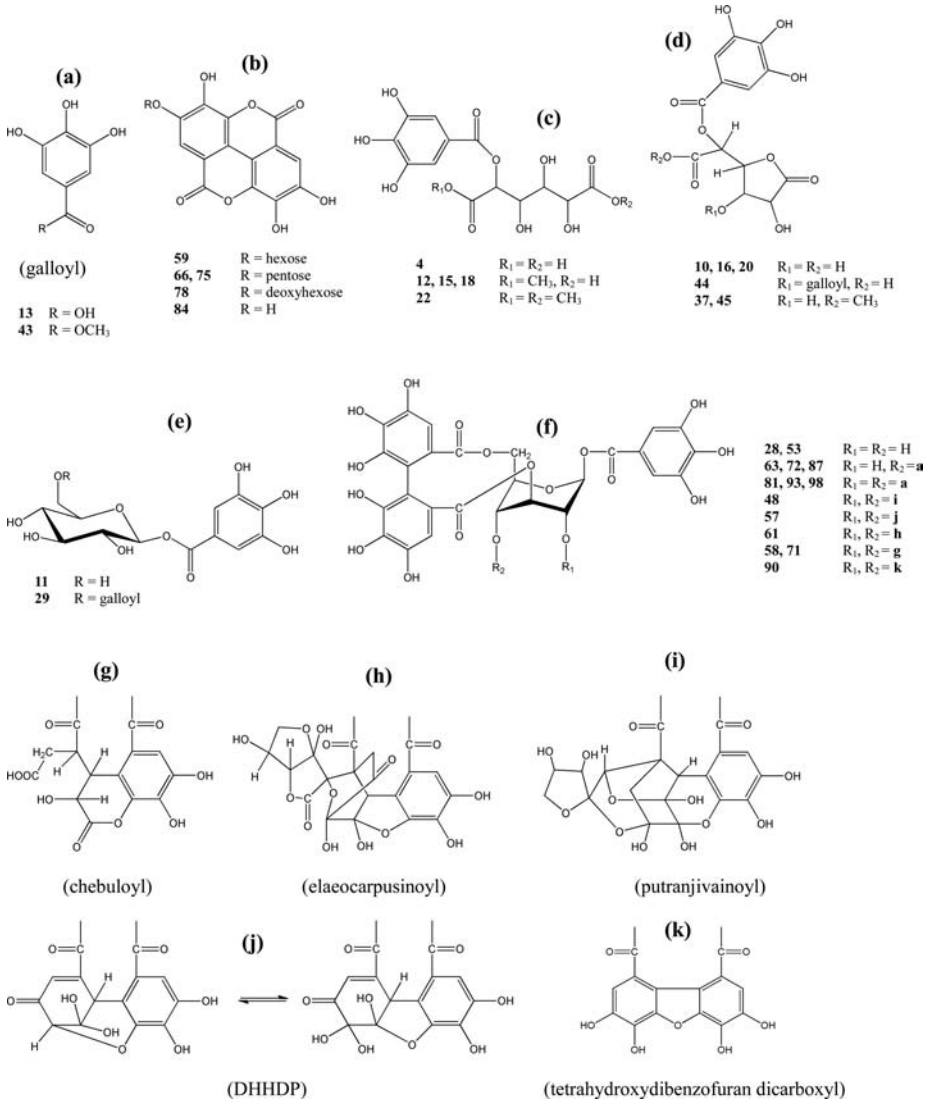

Figure 3. Structural features of the main compounds identified in *P. emblica* fruit: (a) galloyl group, (b) ellagic acid, (c) tentative structure of a mucic acid gallate isomer (2-*O*-gallate), (d) tentative structure of a mucic acid lactone gallate isomer (1,4-lactone-5-*O*-gallate), (e) galloylglucose, (f) galloyl-3,6-HHDP-glucose, and (g–k) different structural groups in ellagitannins attached to galloyl-3,6-HHDP-glucose at 2,4-positions.

the mass spectrum. The presence of this compound in the fruits of emblic leafflower has not been reported previously.

Three ellagitannins (**34**, **39**, and **55**), all having $[M - H]^-$ ions at m/z 669 and $[M - H - \text{galloyl} - \text{hexose} - H_2O]^-$ ions at m/z 337 in their mass spectra, were identified as neochebuloyl galloylglucoses from fraction F3. The 3-*O*-, 4-*O*-, and 6-*O*-neochebuloyl isomers of 1-*O*-galloyl-*β*-D-glucose isolated from *P. emblica* are also known as phyllanemblinins D, E, and F, respectively.[10] The fragment ion at m/z 337 represented the $[\text{chebulic acid} - H - H_2O]^-$ ion, resulting from the loss of the neochebuloyl group from the molecule.[20] In previous studies, these compounds were identified in the branches and leaves of emblic leafflower.[10] The results of our study suggested the presence of these compounds in the fruits. In addition, phyllanemblinin A, another isomer of neochebuloyl galloylglucose, was found in the fruit juice of emblic leafflower by Zhang et al.[10] We did not find this compound in the fruit extracts. Further, three other compounds (**64**, **70**, and **80**) in fraction F4 with ions at m/z 989 in the mass spectra and UV-maxima at 224 and 253 nm, had the same chebulic acid fragment, indicating the presence of a neochebuloyl moiety in the structures. The suggested molecular formula indicated that the compounds were isomers of carpinusnin, which, in fact, contains a neochebuloyl group (chebuloyl neochebuloyl galloylglucose).[10,24]

Ellagic Acid Derivatives. The structures of ellagic acid and major ellagic acid derivatives preliminarily identified in emblic fruit extract are presented in Figure 3b. Ellagic acid glycosides (Figure 4b) with pentose (**66** and **75**), hexose (**59**), or deoxyhexose (**78**) as a sugar moiety had similar UV spectra (λ_{max} at 252 and 360 nm).[14] An aglycone radical ion was observed at m/z 300 in the MS/MS spectra of ellagic acid glycosides. This is the first report indicating the presence of ellagic acid glycosides in the fruits of emblic leafflower.

Flavonoids. Peak **83** with an $[M - H]^-$ ion at m/z 463, a $[2M - H]^-$ ion at m/z 927, and an $[M - H - \text{hexose}]^-$ ion at m/z 301 was identified as a quercetin hexose.[14] The UV absorbance maxima at 223, 267, and 349 nm supported this identification. Peak **94** was identified as quercetin deoxyhexose based on its $[M - H]^-$ ion at m/z 447, a $[2M - H]^-$ ion at m/z 895, and the UV spectrum with λ_{max} at 204, 256, and 347 nm.

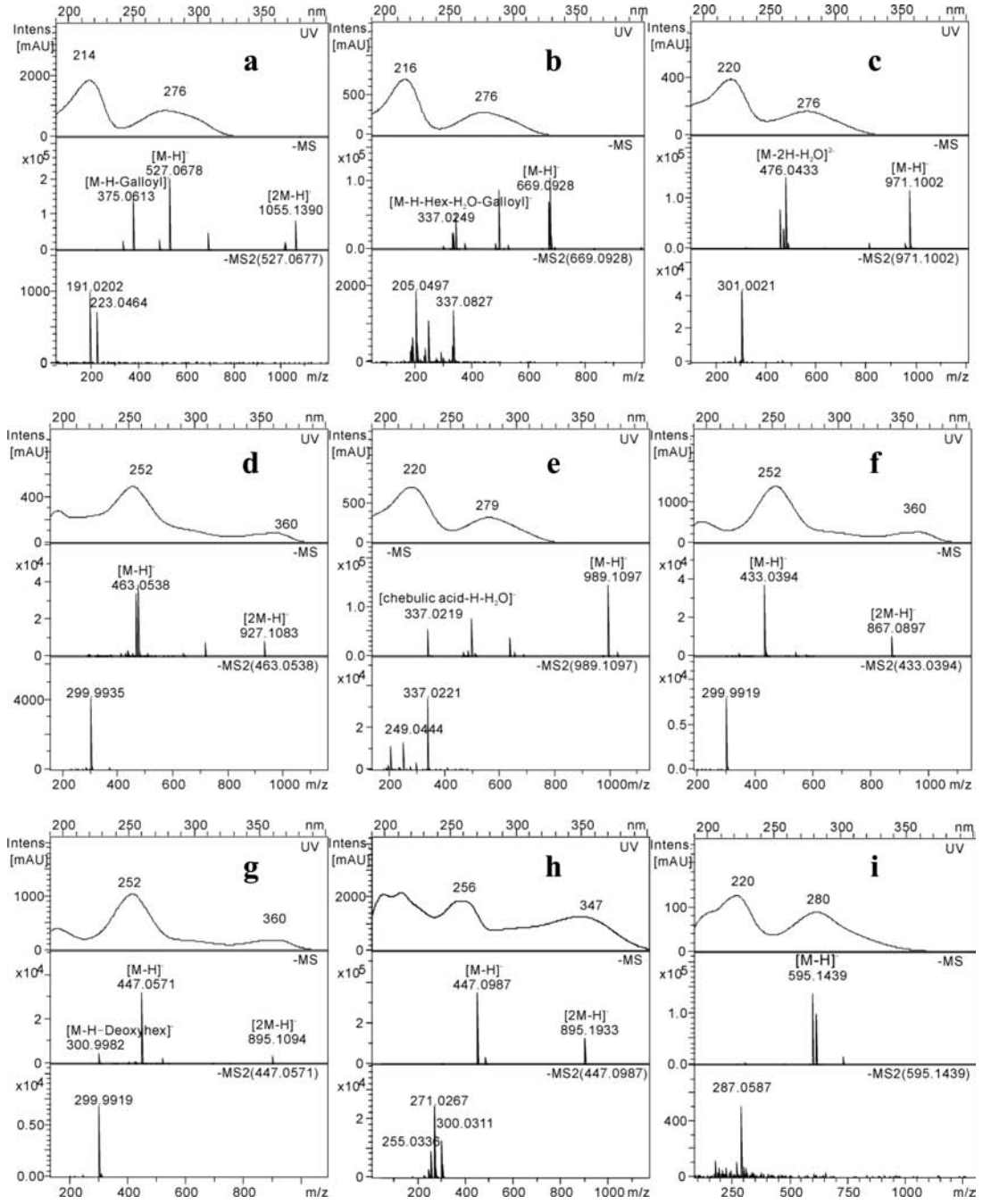

Figure 4. UV, MS, and MS/MS spectra of compounds identified or tentatively identified for the first time in the fruits of emblic leafflower. The spectra presented were obtained from HPLC-DAD-ESI(−)-QTOF-MS/MS analyses using collision sweep mode. The product ions represented in this figure were the daughters of the molecular ions. (a) Mucic acid methyl ester digallate (**26**, **38**, and **47**) (the collision energy for MS/MS was 35.82 eV), (b) neochebuloyl galloylglucose (**34**, **39**, and **55**) (40.08 eV), (c) neochebulagic acid (**50**) (49.14 eV), (d) ellagic acid hexose (**59**) (35.00 eV), (e) chebuloyl neochebuloyl galloylglucose (**64**, **70**, and **80**) (49.69 eV), (f) ellagic acid pentose (**66** and **75**) (35.00 eV), (g) ellagic acid deoxyhexose (**78**) (35.00 eV), (h) quercetin deoxyhexose (**94**) (35.00 eV), and (i) eriodictyol coumaroylhexose (**123** and **127**) (37.86 eV).

The aglycone quercetin (**124**) had an $[M - H]^-$ ion at m/z 301 and a UV spectrum with λ_{max} at 231, 255, and 369 nm.[21]

Compounds **123** and **127** were identified as eriodictyol coumaroylhexose based on their MS/MS fragmentation pattern, which showed a loss of a coumaroylhexose moiety (308 Da).[14,25] Previous reports of these compounds have been from leaves and branches of *P. emblica*.[25]

Other Compounds. Compounds that remained unidentified are presented in the Supporting Information, Table 1. Compound **6** appeared in fraction 1 (F1) of all the four samples and had ions at m/z 128 in the mass spectrum of HPLC-ESI-MS. It was not, however, detected in the HPLC-ESI-QTOF-MS analyses, probably due to its low concentration in the sample and parameters used to favor larger molecules in the TOF mass analyzer. In the same fraction, compounds **5** and **7** had ions at m/z 523, 343, and 1047. The ions at m/z 343 suggested that these compounds could be derivatives of mucic acid lactone gallate with a hexose as the sugar moiety.

Several unknown compounds, such as **67a**, **73**, and **77** with $[M - H]^-$ ions at m/z 1261 and **92**, **103**, and **109** with [M −

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$H]^-$ ions at m/z 1069, had a fragment ion at m/z 301, suggesting an ellagitannin structure. Probable molecular formulas for peaks **79**, **85**, and **91** with $[M - H]^-$ ions at m/z 1105 were $C_{48}H_{34}O_{31}$, which may belong to several different ellagitannin structures. Compound **67b** (coeluting with **67a**) exhibiting an $[M - H]^-$ ion at m/z 1103 was assigned with the molecular formula $C_{48}H_{32}O_{31}$, which may also belong to several ellagitannins. Peaks **119** and **133** were also assigned as ellagitannins.

In the present study, we confirmed the presence of some compounds reported previously for emblic leafflower. A number of compounds were found for the first time in fruits of emblic leafflower. The UV and tandem mass spectra of these compounds are presented in Figure 4. It is important to notice that UV and MS spectra from HPLC-MS and HPLC-MS/MS analyses provided only limited information on structural composition and are insufficient for the exact determination of the molecular structures of these compounds. Further studies of purified compounds with, e.g., NMR will provide important data for verifying the preliminary identification reported in this article. In previous studies, the presence of a digalloylglucose (1,6-di-*O*-), a digalloyl-HHDP-glucose [tercatain, 1,4-di-*O*-galloyl-3,6-(*R*)-HHDP-*β*-D-glucose], and a trigalloyl-HHDP-glucose [punicafolin, 1,2,4-tri-*O*-galloyl-3,6-(*R*)-HHDP-*β*-D-glucose] were reported in the emblic leafflower fruits.[9,10] The results of our study suggested that in the emblic leafflower fruits several isomers of these compounds existed with different elution characteristics in the chromatographic systems (Table 2). Some compounds identified in the fruits might be the same compounds as those reported in the leaves and branches.[10] Five isomers of mucic acid digallate (**9**, **14**, **17**, **19**, and **23**) were detected for the first time in the current study.

In addition to the compounds listed in Table 2, 77 compounds were detected. The retention data and characteristics of the UV and MS spectra of these compounds are listed in the Supporting Information, Table 1. The data provide an important basis for further studies for the identification and quantification of these compounds.

Comparison of Phenolic Profiles in the Emblic Leafflower Fruits of Different Origins. Overall, the contents of phenolic compounds were higher in the extracts of emblic leafflower fruits from Guangxi Province than in the extracts of those from Fujian Province, reflected as larger (roughly 2-fold) total peak areas in the HPLC-UV chromatograms of the crude extracts. Gallic acid and simple gallate esters represented the most abundant phenolic compounds in all of the samples analyzed (Figure 1 and Table 2). Two varieties from Dayu County, Guangxi Province also contained high levels of ellagitannins and derivatives of chebulic acid and chebuloylglucose. In contrast, these compounds were present at a much lower proportion in the two samples of Tian Chuan emblic leafflower (Figure 1). Table 2 presents a detailed list of compounds detected in the four samples analyzed. For example, elaeocarpusin and chebulagic acid, two major peaks in the crude extracts of the samples from Guangxi, appeared as minor peaks in the extracts of the Tian Chuan emblic leafflower from Fujian. Neochebulagic acid and neochebuloyl galloylglucose were not detected in the fruits from Lantian County, Fujian Province. Fruits of Tian Chuan emblic leafflower are known to taste sweeter and less astringent than the fruits of the two varieties from Guangxi Province. Phenolic compounds, especially tannins are among the major components contributing to the astringency of fruits and berries.[26] The lower content of ellagitannins in Tian Chuan emblic leafflower fruits partially explains their more pleasant taste compared with the fruits of Ping Dan No. 1 from Guangxi Province. This is also the first report of different profiles of phenolic compounds in the fruits of two major sources of emblic leafflower in China. Many potential health benefits have been associated with ellagitannins, including antioxidative, antimicrobial, and anticancer activities.[20,27] The high consumption of tannins may reduce the risk of type II diabetes and cardiovascular diseases.[28] The difference in the content and profile of phenolic compounds may result in differences in the biological activities and health benefits of emblic leafflower fruits of different origins.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Unidentified compounds determined by HPLC-ESI(−)-MS and HPLC-DAD-ESI(−)-QTOF-MS/MS in the Sephadex LH-20 column chromatographic fractions of four *P. emblica* fruit samples EMB1−4 and mass spectra of compounds A−O of the crude extract emblic leafflower fruit. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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