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Ultra High-Performance Liquid Chromatography with High-Resolution Mass Spectrometry Analysis of African Mango (*Irvingia gabonensis*) Seeds, Extract, and Related Dietary Supplements

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Supporting Information

ABSTRACT: Dietary supplements based on an extract from *Irvingia gabonensis* (African mango, AM) seeds are one of the popular herbal weight loss dietary supplements in the U.S. market. The extract is believed to be a natural and healthy way to lose weight and improve overall health. However, the chemical composition of AM-based dietary supplements (AMDSs) has never been reported. In this study, the chemical constituents of AM seeds, AM seeds extract (AMSE), and different kinds of commercially available AMDSs have been investigated using an ultra high-performance liquid chromatography with high resolution mass spectrometry method. Ellagic acid, mono-, di-, and tri-*O*-methyl-ellagic acids, and their glycosides were found as major components in AM seeds. These compounds may be used for quality control of AM extract and related dietary supplements.

KEYWORDS: *Irvingia gabonensis, African mango, UHPLC-HRMS, ellagic acid, O-methyl-ellagic acids*

INTRODUCTION

Irvingia gabonensis (African mango or AM) belongs to the *Irvingiaceae* family. It is also known as wild mango or bush mango,[1] which is different from regular mango (*Mangifera indica* L., which belongs to *Anacardiacea*). The mangolike fruits of AM are especially valued for their dietary fiber and fat- and protein-rich seeds. The consumption of dried *I. gabonensis* seeds is common in West African countries and, for centuries, have been part of the local diets.[2]

In recent years, AM-based dietary supplements (AMDS) have appeared as a popular herbal weight loss dietary supplement in the United States. The labels of AMDS products sold in the United States all claim the use of African mango seed extract (AMSE) as the major ingredient. AMSE is believed to be a natural and healthy way to lose weight and improve overall health. A randomized, double-blind, placebo-controlled study demonstrated significant differences between the treatment and the placebo groups in weight and fat loss, as well as reductions in hip and waist circumference.[3] AMSE combined with Veldt Grape (*Cissus quadrangularis*) resulted in significant reductions of six anthropomorphic and serological measurements (body weight, body fat, and waist sizes; total plasma cholesterol, LDL cholesterol, and fasting blood glucose level).[4] In another study, AMSE showed positive effects on body weight and a variety of parameters characteristic of metabolic syndrome in a double-blind, randomized, placebo-controlled clinical trial investigating the antiobesity and lipid profile-modulating effects.[5] It is believed that the high soluble fiber content may cause the lowering of plasma cholesterol, triglycerides, and glucose concentrations.[6] However, detailed chemical analyses of the seeds of AM, AMSE, and AMDS have not been reported.

To advance our understanding of the health benefits of AMSE and/or AMDS, it is important to investigate their chemical composition and to find marker compounds for the authentication of AMDS products. To date, the only report on the analysis of "AM" was performed on the peel and flesh of the fruit, not on the seeds. The study was conducted using gas chromatography−mass spectrometry (GC-MS), and 33 volatile compounds were reported including eight monoterpene alcohols, five aldehydes, four acids, seven esters, and five C_{13} norisoprenoids.[7] Unfortunately, the authors confused the name of "AM" with mango growing in Africa and incorrectly used the name "AM" in the paper, as the research was actually done on mangos growing in Africa (*M. indica* L.), not AM (*I. gabonensis*). Other studies on mango (*M. indica* L.) revealed that flavonol, carotenoids, tocopherols, and xanthone glycosides were the major compounds from mango peel and flesh,[8−11] while the major compounds from the seeds were shown to be benzophenone and gallotanins by LC-MS^n methods.[12−14]

Currently, AMDS sold in the U.S. market can be divided into three groups according to their labels: (1) AMSE only, (2) AMSE with green tea extracts, and (3) AMSE with other botanical extracts, such as berries, kelp, and caffeine, as well as green tea extracts. Some manufacturers claim on their labels that *I. gabonensis* seed fiber and flavonoids are major components in their products. Others claim mangiferin, a known constituent from mango, but not from AM, for their products. Clearly, there is a lot of confusion on the manufacturer and distributor sides.

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Figure 1. AM seeds (left) and Mexican mango seeds (right).

In this study, the chemical constituents of AM (*I. gabonensis*) seeds, mango (*M. indica* L) seeds, AMSE, and different kinds of AMDS have been investigated using an ultra high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) method. Ellagic acid, mono-, di-, and tri-*O*-methyl-ellagic acid, and their related glycosides were found to be the major constituents in AM seeds, which may be used for future quality control and assurance for AMSE or AMDS as marker compounds. A wide variation in the constituents was found among AMSE products from China and AMDS products sold in the United States.

MATERIALS AND METHODS

Chemicals and Materials. HPLC grade methanol, acetonitrile, and formic acid were purchased from VWR International, Inc. (Clarksburg, MD). HPLC water was purchased from Sigma-Aldrich (St. Louis, MO). Authentic AM seeds and AMSE were kindly provided by Strategic Sourcing, Inc. (Reading, PA), imported directly from Africa. The authentication vouchers of the samples were deposited in the Food Composition and Methods Development Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture. As shown in Figure 1, the AM seeds are quite different from regular mango seeds. Two AMSE commercial products were obtained from Changsha, Hunan Province, China, courtesy of the Hunan Food Test and Analysis Center (Changsha, Hunan, China). The label claims them to be 20:1 and 10:1 AM ethanol extract, respectively. Five AMDS commercial products were purchased from Internet distributors in the United States. The Mexican mango fruit (*M. indica* L.) was purchased from a local supermarket.

Ellagic acid, 3-*O*-methyl-ellagic acid, quercetin 3-*O*-rhamnoside (quercitrin), and diosmetin were purchased from Chromadex Inc. (Irvine, CA). Kaempferol 3-*O*-glucoside and caffeine were purchased from Sigma-Aldrich.

Sample Preparation. The AM seeds were ground into powder and then passed through a 60 mesh sieve. Five hundred milligrams of seeds powder/seeds extract or dietary supplement sample equal to one serving size was extracted with 5.00 mL of methanol−water (60:40, v/v) with sonication for 20 min at room temperature. The slurry mixture was centrifuged at 5000*g* for 15 min. The supernatant was filtered through a 17 mm (0.20 μm) PVDF syringe filter (VWR Scientific, Seattle, WA) and stored at 4 °C before analysis. All analyses were done within 24 h of extraction. The injection volume for all samples was 1 μL.

UHPLC-HRMS Conditions. The UHPLC-HRMS system consisted of a LTQ Orbitrap XL mass spectrometer with an Accela 1250 binary Pump, a PAL HTC Accela TMO autosampler, an Accela PDA detector (Thermo Fisher Scientific, San Jose, CA), and a G1316A column compartment (Agilent, Santa Clara, CA). The separation was carried out on a Hypersil Gold C_{18} column (200 μm × 2.1 mm, 1.9 μm) (Thermo Fisher Scientific) with a flow rate of 0.3 mL/min. The mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The linear gradient was from 4 to 20% B (v/v) at 40 min, to 35% B at 60 min, to 100% B at 61 min, and was then held at 100% B to 65 min. The column temperature was set at 50 °C, and UV/vis spectra were recorded from 200 to 700 nm. The high accurate mass measurements were carried out under both positive and negative mode. The MS conditions were set as follows: sheath gas at 70 (arbitrary units), auxiliary and sweep gas at 10 (arbitrary units), spray voltage at 4.5 kV for positive mode and 4 kV for negative mode, capillary temperature at 250 °C, capillary voltage at 40 V for positive mode and −50 V for negative mode, and tube lens at 150 V. For FTMS, the mass range was from 200 to 2000 m/z with a resolution of 15000, AGC target value of 200000 and 100000 in full scan and FTMS/MS AGC target at 1e5, isolation width of 1 amu, and max ion injection time of 750 ms; the ion trap settings used were as follows: AGC target value of 30000 and 10000 in full scan and MS^n mode, respectively, and max ion injection time of 200 ms. The most intense ion was selected for the data-dependent scan with normalization collision energy at 30%. The formulas of compounds of interest were calculated based on their measured mass using the Xcalibur software (Thermo Fisher Scientific). The compounds of interest were identified or tentatively identified based on their respective calculated formulas, retention times, UV spectra, MS fragmentation patterns, the literature, and reference standards (when available).

Table 1. UHPLC-HRMS Data of Constituents from AM Seeds

peak no.	RT (min)	formula	$[M - H]^-$	error (mmu)	UV (λ_{max}, nm)	main MS2−MS4 product ions	tentative identification
1	1.81	$C_{12}H_{22}O_{11}$	341.1081	−0.88	233	179 (100), 161 (20), 143 (21), 131 (7), 119 (15), 113 (17)	hexosyl-hexose
2	1.87	$C_6H_8O_7$	191.0191	−0.60	N.D.[b]	173 (21), 111 (100)	citric acid or its isomer
3	2.15	$C_{20}H_{18}O_{14}$	481.0613	−1.07	233	301 (100), 275 (12)	hexahydroxydiphenoyl (HHDP)-hexose
4	3.28	$C_{34}H_{24}O_{22}$	783.0669	−2.70	N.D.	765 (3), 481 (35), 301 (100), 275 (16)	di-HHDP-hexose
5	4.9	$C_{34}H_{24}O_{22}$	783.0658	−2.85	N.D.	765 (3), 481 (35), 301 (100), 275 (16)	di-HHDP-hexose
6	5.64	$C_{34}H_{24}O_{22}$	783.0658	−2.85	N.D.	481 (35), 301 (100), 275 (16)	di-HHDP-hexose
7	7.02	$C_{34}H_{24}O_{22}$	783.065	−3.65	271	481 (35), 301 (100), 275 (16)	di-HHDP-hexose
8	8.16	$C_{34}H_{24}O_{22}$	783.0667	−1.94	271	481 (35), 301 (100), 275 (16)	di-HHDP-hexose
9	9.16	$C_{21}H_{32}O_{10}$	443.1907	−1.57	223, 275, 349	425 (33), 375 (56), 237 (100), 219 (67), 189 (67), 161 (56)	unknown
10	10.66	$C_{18}H_{18}O_8$	361.0951	2.20	223, 271, 343	317 (12), 293 (3), 281 (100), 237 (27)	unknown
11	11.33	$C_{33}H_{24}O_{22}$	771.0655	−3.16	222, 281, 398	753 (17), 481 (100), 469 (29), 379 (6), 301 (17), 289 (27)	unknown ellagitannin
12	11.89	$C_{33}H_{24}O_{22}$	771.0651	−3.53	295	481 (100), 469 (75), 307 (12), 301 (29), 289 (71), 263 (25)	unknown ellagitannin
13	15.35	$C_{18}H_{28}O_9$	387.1647	−1.39	271, 349	370 (12), 360 (4), 343 (6), 341 (4), 207 (100), 163 (40)	unknown
14	18.82	$C_{33}H_{24}O_{21}$	755.0698	−3.91	255, 345	727 (27), 453 (89), 393 (23), 301 (51), 291 (99), 247 (100)	unknown ellagitannin
15[a]	21.51	$C_{14}H_6O_8$	300.9978	−1.16	223, 253, 367	301 (100), 300 (14), 284 (24), 257 (59), 229 (50), 185 (22)	ellagic acid
16	26.29	$C_{22}H_{20}O_{13}$	491.0817	−1.444	222, 250, 358	476 (19), 328 (100), 313 (9), MS3: 313 (100), MS4: 298 (100), 285 (49)	di-*O*-methyl-ellagic acid hexoside
17[a]	26.83	$C_{15}H_8O_8$	315.0135	−1.09	222, 254, 354	300 (100), MS3: 300 (100), 283 (16), 272 (64), 271 (31), 244 (98), 243 (31), 228 (36), 216 (20), 200 (20)	methyl-ellagic acid
18	27.61	$C_{15}H_8O_8$	315.0136	−1.00	220, 250, 360	300 (100), 272 (13), 244 (21), 200 (10)	methyl-ellagic acid
19	28.26	$C_{34}H_{28}O_{20}$	755.1072	−2.88	220, 251, 364	711 (15), 603 (4), 453 (7), 301 (100), 284 (3), 275 (6)	galloyl-HHDP-ellagic acid
20	28.94	$C_{34}H_{28}O_{20}$	755.1063	−3.80	N.D.	711 (9), 453 (6), 301 (100), 291 (5), 275 (10), 247 (5)	galloyl-HHDP-ellagic acid
21	29.38	$C_{25}H_{16}O_{15}$	555.0732	1.51	220, 250, 358	475 (100), MS3: 460 (23), 328 (100), 313 (7), MS4: 313 (100)	di-*O*-methyl-ellagic acid deoxyhexide with an 80 amu group
22[a]	29.91	$C_{21}H_{20}O_{11}$	447.0919	−1.43	N.D.	357 (10), 327 (19), 301 (8), 285 (75), 284 (100), 255 (15)	kaemferol 3-*O*-glucoside
23[a]	30.34	$C_{21}H_{20}O_{11}$	447.0917	−1.55	N.D.	301 (100), 300 (40)	quercetin 3-*O*-rhamnoside
24	30.73	$C_{21}H_{18}O_{12}$	461.0708	−1.78	220, 253, 349	315 (100), MS3: 300 (100), MS4: 300 (100), 284 (13), 272 (17), 271 (33), 244 (33), 243 (10), 228 (20), 216 (27), 200 (17)	mono-*O*-methyl ellagic acid deoxyhexoside
25	32.47	$C_{16}H_{10}O_8$	329.0290	−1.30	220, 247, 363	314 (100), MS3: 299 (100), MS4: 271 (100)	di-*O*-methyl ellagic acid
26	33.08	$C_{21}H_{18}O_{12}$	461.0712	−1.30	220, 248, 364	446 (17), 328 (100), MS3: 313 (100), MS4: 298 (100), 285 (43)	di-*O*-methyl ellagic acid-*O*-pentoside
27	33.7	$C_{34}H_{42}O_{20}$	769.2166	−3.06	220, 250, 349	607 (100), 477 (23), 315 (14)	rhamnetin or isorhamnetin with one hexose group and one rhamnosyl-rhamnose
28	35.91	$C_{41}H_{30}O_{24}$	905.1021	−3.32	N.D.	603 (55), 301 (100)	di-HHDP-ellagic acid
29	36.45	$C_{22}H_{20}O_{12}$	475.0867	−1.47	N.D.	460 (100), 329 (8), 328 (16), 327 (9), 313 (15), 299 (7)	di-*O*-methyl-ellagic acid deoxyhexoside
30	36.9	$C_{22}H_{20}O_{12}$	475.0869	−1.29	N.D.	460 (25), 328 (100), 313 (6), 313 (100), 298 (100), 285 (45)	di-*O*-methyl-ellagic acid deoxyhexoside
31	37.85	$C_{30}H_{26}O_{17}$	657.1069	−2.82	220, 244, 364	491 (48), 329 (100), 315 (14), 314 (31), 313 (36), 299 (13)	di-*O*-methyl-ellagic acid with one 166 amu group and one hexose goup
32	39.57	$C_{16}H_{10}O_8$	329.0289	−0.84	N.D.	314 (100)	di-*O*-methyl-ellagic acid

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Table 1. continued

peak no.	RT (min)	formula	$[M - H]^-$	error (mmu)	UV (λ_{max} nm)	main MS2–MS4 product ions	tentative identification
33	41.52	$C_{16}H_{10}O_8$	329.0292	−0.57	223, 246, 375	314 (100), MS3: 299 (100), MS4: 271 (100)	di-*O*-methyl-ellagic acid
34	43.01	$C_{34}H_{42}O_{20}$	769.217	−2.14	239	697 (3), 315 (100), 300 (25), 271 (25), 255 (5)	galloyl-HHDP-methyl-ellagic acid
35	44.96	$C_{17}H_{12}O_8$	343.0449	−0.50	222, 246, 362	328 (100), 313 (100), 298 (100, MS4), 285 (38, MS4)	tri-*O*-methyl-ellagic acid
36	45.78	$C_{22}H_{22}O_{11}$	461.1074	−0.97	220, 236, 341	315 (100), 300 (5)	mono-*O*-methyl-ellagic acid rhamnoside
37	46.94	$C_{35}H_{28}O_{10}$	607.1649	−3.95	220, 236, 347	315 (100), 300 (17), MS3: 300 (100), MS4: 271 (100), 255 (55)	mono-*O*-methyl-ellagic acid rhamnosyl-rhamnoside
38	47.76	$C_{30}H_{26}O_{17}$	657.1071	−2.66	ND	343 (100), 329 (11), 328 (28), 313 (48), 285 (3)	galloyl-tri-*O*-methyl-ellagic acid hexoside
39[a]	49.75	$C_{16}H_{12}O_6$	299.0549	−1.18	ND	284 (100), 271 (16), 227 (5)	diosmetin
40	53.3	$C_{17}H_{12}O_8$	343.0449	−1.05	220, 236, 375	328 (100), 313 (100), 298 (100), 285 (40)	tri-*O*-methyl-ellagic acid

[a]In comparison with reference standards. [b]ND, not detected or very weak absorbance.

RESULTS AND DISCUSSION

Tentative Identification of Constituents from AM Seeds. The UHPLC-PDA analysis revealed that most of the constituents in AM seeds have three UV maximum absorbance bands at 220–230, 240–260, and 350–370 nm. Forty-one phenolic compounds were found in AM seeds including ellagic acid, methyl-ellagic acid, ellagitannins, and flavonol glycosides (Table 1). As shown in Figure 2, the chemical profile of AM seeds is quite different from that of mango seeds. The constituents identified from mango seeds are mostly flavonol, xanthone glycosides, benzophenone, and gallotanins, which is consistent with the literature[8−11] (Figure S1 and Table S1 in the Supporting Information).

Ellagic Acid, Mono-, Di-, and Tri-Methyl-Ellagic Acids, and Their Glycosides. Peaks 15, 25, 33, 35, 37, 39, and 40 were found to be the major constituents in the PDA and total ion chromatograms (Figure 2). Peak 15 was identified as ellagic acid. Its molecular ion was 300.9978, indicating the formula of $C_{14}H_6O_8$ (−1.16 mmu). Its major product ions were *m/z* 257, 229, 284, 185, and 201, consistent with previous literature for ellagic acid. It was further confirmed by analysis of a reference standard. Peaks 25, 33, 35, 37, 39, and 40 displayed similar UV spectra to peak 15, suggesting that they were ellagic acid derivatives. Peaks 25, 33, and 32 (minor contituent) all had a formula of $C_{16}H_{10}O_8$, and the deprotonated ions of 25, 33, and 32 underwent the successive losses of CH_3 radicals. Thus, two methyl groups existed in the structures. These three compounds were tentatively identified as di-*O*-methyl ellagic acids. The molecular ion of peaks 35 and 40 was ($[M - H]^-$, $C_{17}H_{12}O_8$), and the major product ions were *m/z* 328, *m/z* 313, and *m/z* 298 via the successive losses of CH_3 radicals. Thus, three methyl groups exist in the structures of peaks 35 and 40. Hence, these two compounds were tentatively identified as tri-*O*-methyl ellagic acids. Peaks 17 and 18 had the formula of $C_{15}H_8O_8$, and the ion at *m/z* 300 was the predominate ion in the MS^2 spectra. Further fragmentation of this ion showed an almost identical behavior of that of ellagic acid; thus, these two compounds were tentatively identified as monomethyl-ellagic acids.

Peak 16 had the $[M - H]^-$ ion at *m/z* 491 ($C_{22}H_{20}O_{13}$). Its product ion was *m/z* 328 via the loss of 163 amu, corresponding to a cleavage of a glycosidic bond. The MS^3 and MS^4 spectra of the ion *m/z* 328 were almost identical with that of di-*O*-methyl ellagic acid. Thus, peak 16 was tentatively identified as di-*O*-methyl-ellagic acid hexoside. It is worth noting that the HRMS data can easily differentiate between a hexosyl ($C_6H_{10}O_5$) and a caffeoyl ($C_9H_6O_3$) group or between a deoxy-hexosyl and a coumaroyl group. Similarly, peak 24 was tentatively identified as methyl ellagic acid-*O*-deoxyhexose. Peaks 26 and 36 were tentatively identified as di-*O*-methyl ellagic acid-*O*-pentoside. Peaks 29 and 30 were tentatively identified as di-*O*-methyl-ellagic acid deoxyhexoside. Peak 41 had the deprotonated ion at *m/z* 607.1649 ($C_{35}H_{28}$ O_{10}), and the loss of 292 Da unit was observed to produce the product ion at *m/z* 315. The successive fragmentation of *m/z* 315 was very similar to peak 17 and 18 (methyl ellagic acid). Hence, this compound was tentatively identified as methyl-ellagic acid-*O*-rhamnosyl-rhamnoside.

Ellagitannins. Ellagitannins are hydrolyzable tannins containing one or more hexahydroxydiphenoyl (HHDP) groups esterified to a sugar. The $[M - H]^-$ ion of peak 3 was 481.0613, indicating the formula of $C_{20}H_{18}O_{14}$. The ion at

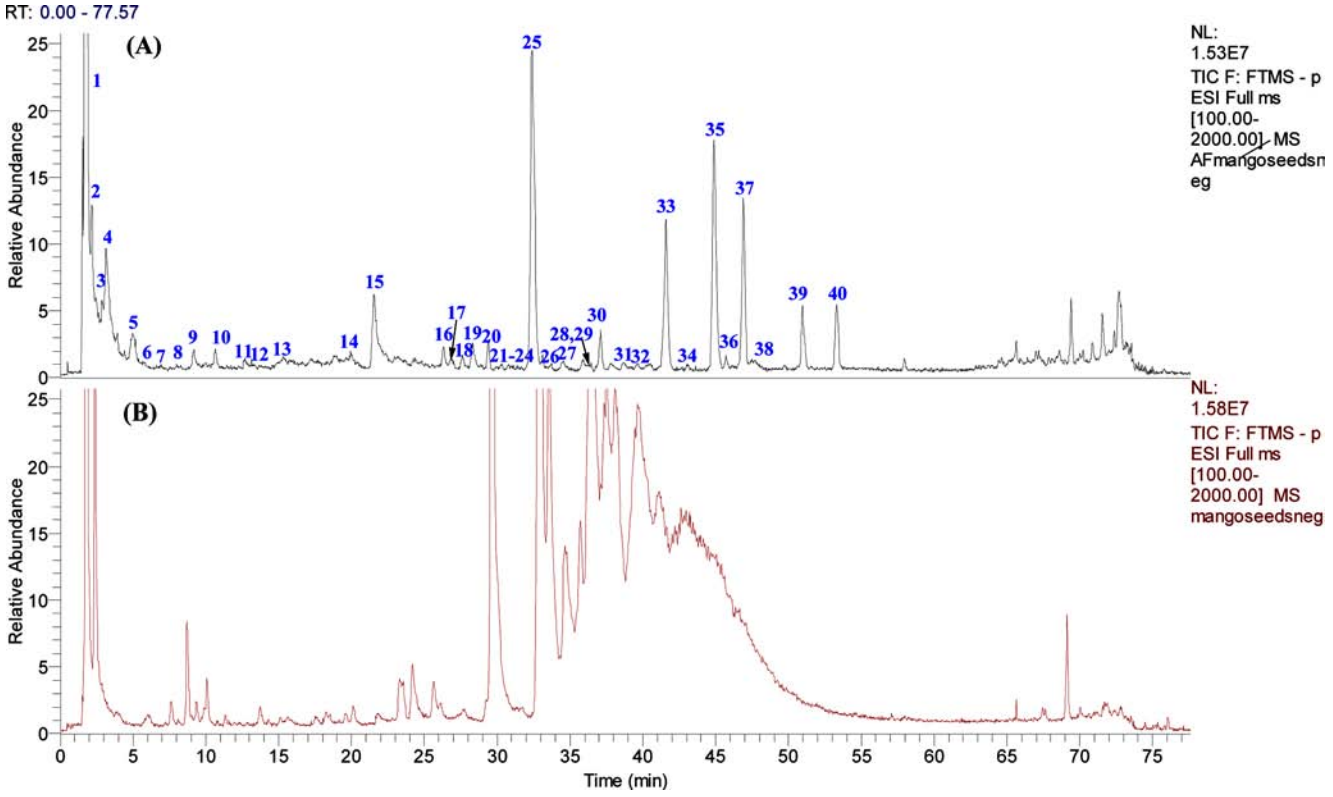

Figure 2. Total ion chromatograms of AM (A) and Mexican mango (B) seeds.

m/*z* 301 was observed as the major product ion via the loss of 180 amu in the MS/MS spectrum. Hence, this compound was considered to contain one HHDP unit esterified to hexose. The loss of 180 amu corresponded to a hexosyl unit (162 amu) and H_2O (18 amu) simultaneously.

Peaks 4−8 showed the $[M - H]^-$ ion at 783 with the formula of $C_{34}H_{24}O_{22}$. According to MS analysis, all of these five constituents likely contain HHDP-hexose in their makeup due to the presence of the product ions at *m*/*z* 481 (HHDP + hexose) and 303 (HHDP). These four compounds were characterized as di-HHDP-hexose. Peaks 11, 12, and 15 were identified as unknown ellagitannins due to the characteristic neutral losses observed (loss of 302 amu unit, $C_{14}H_6O_8$). Peaks 11 and 12 had the formula of $C_{33}H_{24}O_{22}$, with a mass 14 amu less than that of peak 4. These two compounds may lack one $-CH_2$ group in the structure in comparison with peaks 4−8.

Flavonoids. Peaks 22 and 23 were kaempferol 3-*O*-glucoside and quercetin 3-*O*-rhamnoside according to their HRMS measurements and UV spectra. Their identities were confirmed by reference standards. Peak 27 had a formula of $C_{34}H_{42}O_{20}$, and the losses of one 162 and 292 group were observed in the MS^2 to MS^4 spectra, which correlated to one glucose group and one rhamnosyl rhamnose group. The aglycone part of peak 27 was $C_{16}H_{12}O_7$, a rhamnetin or isorhamnetin. Peak 39 had a formula of $C_{16}H_{12}O_6$, and the retention time and MS^2 fragmentation were consistent with the reference standard of diosmetin, an *O*-methylated flavone.

Chromatographic Comparisons with AMDSs. With the chemical information acquired with authentic AM seeds and AMSE, the UHPLC-HRMS chromatograms of AMDS were compared with those of AM seeds for quality evaluation. The five AMDS and two Chinese AMSE commercial products and their label claims are listed in Table S2 in the Supporting Information. Huge variances were observed across the samples. Figure 3 shows the UV chromatograms and total ion chromatograms (TIC) in negative mode of products A−G and authentic AMSE. All commercial products displayed different TIC profiles from each other. Extracted ion chromatograms (EIC) were used to find specific peaks, if any, that might come from AMSE. Unfortunately, none of the products shared any common peaks with authentic AMSE in meaningful amounts. Product A claimed that it used AMSE only, and 15 peaks were found in its TIC (Figure S2 in the Supporting Information). Among them, six compounds were successfully identified by comparing the formula, multistage mass fragmentation, UV spectra, literature reports, and reference standards when available. The deprotonated ion $[M - H]^-$ of peak 8 is 421.0771, suggesting the chemical composition $C_{19}H_{17}O_{11}$ (−1.22 ppm). The $[M - H - 120]^-$, $[M - H - 90]^-$, and $[M - H - 18]^-$ were observed as three major product ions in the MS^2 spectra, which suggests that it is most probably a C-glycoside. Together with the UV maximum absorbance at 257, 318, and 365 nm, it was unambiguously identified as mangiferin, a xanthone glycoside, which has been extensively reported in mango (*M. indica* L)[8,11,14] but not in AMSE. Peak 9 also showed a chemical composition $C_{19}H_{17}O_{11}$ (−2.22 ppm) and was identified as isomangiferin by comparing the elution order and MS/MS data reported in the literature.[8,11,14] Peak 12 revealed a deprotonated ion $[M - H]^-$ at *m*/*z* 573.0879, suggesting a chemical composition of $C_{26}H_{21}O_{15}$. A neutral loss of 152 amu ($C_7H_4O_4$) was observed in the MS^2 spectra. The MS^3 and MS^4 spectra were almost identical with that of mangiferin. So, it can be concluded that a galloyl moiety was substituted in peak 12. This compound was then identified as galloylated mangiferin.[8] Similarly, peak 13 was identified as galloylated isomangiferin.[8] Peak 14 shows a

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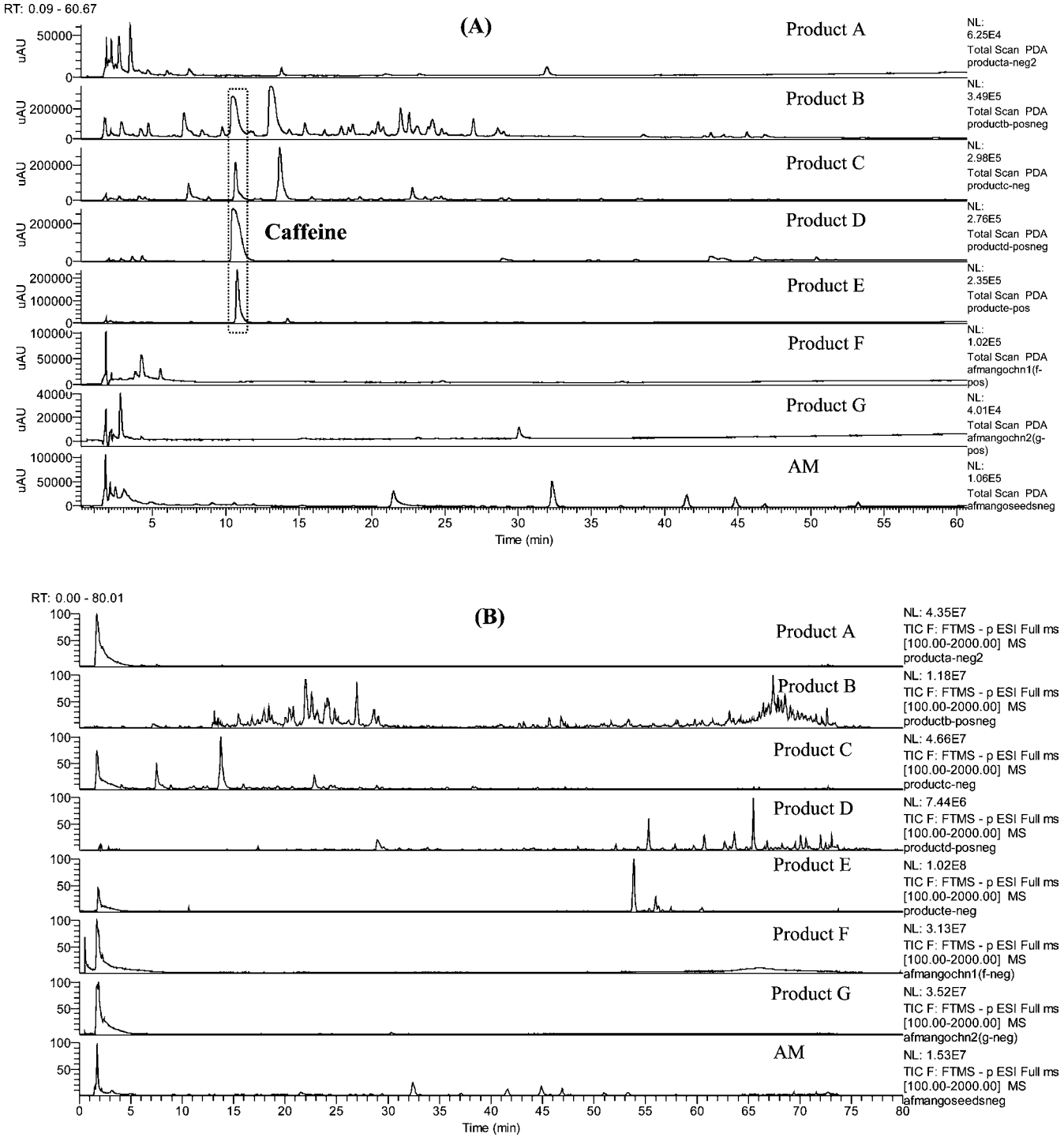

Figure 3. HPLC chromatogram (200−700 nm, A) and total ion chromatogram in negative mode (B) of products A−G and African mango seeds extract.

$[M - H]^-$ ion at 300.9984 ($C_{14}H_5O_8$, −1.34 ppm) and was identified as ellagic acid by comparing the MS/MS data and with reference standards.[9] Peak 15 with the retention time at 31.97 min shows a $[M - H]^-$ ion at 201.0295 ($C_{11}H_7O_5$, −1.81 ppm). The neutral losses of CO, H_2O, and C_2H_2O were observed in the MS^2 spectrum and are consistent with the fragmentation behavior of coumarin.[15] Obviously, product A used mango seed extract instead of AMSE.

The labels of both products B and C claim the use of AMSE (4:1) with 200 mg of green tea extract. However, no compounds related to either AMSE or xanthone glycosides (mangiferin related constituents) were detected.

Product D claims that maqui berry (*Aristotelia chilensisa*), acai fruit (*Euterpe oleracea*), green tea extract, resveratrol, caffeine, apple cider vinegar, kelp (seaweeds), and grapefruit were added in addition to AMSE. The UV chromatogram of product D (Figure 3A) shows a major peak with the retention time of 10.4 min, which constitutes more than 90% of the total peak area. The protonated ion $[M + H]^+$ at m/z 195.0883 revealed the formula of $C_8H_{10}N_4O_2$. It was identified as caffeine and

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confirmed with a reference standard. No compounds from AMSE were detected in product D.

The label of product E claimed that it contained green tea extract, cranberry, raspberry, fibersol, citric acid, and stevia along with AMSE. First, no compound from AMSE was detected. In addition, the major groups of constituents did not have strong UV absorbance and were eluted from 45 to 66 min. That means that phenolic compounds (major components of green tea extract, cranberry, and raspberry) are not a significant part of the product E.

Products F and G claimed to be pure AMSE. They were obtained directly from suppliers through Hunan Food Test and Analysis Center (Changsha, Hunan, China). The UHPLC-HRMS analysis did not show any detectable amount of components related to either AM or regular mango. If the AMDS samples B–E used AMSE as claimed in their labels, they might have used extracts similar to the products F and G, and it might be the reason that no AM related components were detected in these AMDS samples.

The chemical constituents of AM (*I. gabonensis*) seeds and related dietary supplements were studied with the developed UHPLC-PDA-HRMS method. It was found that ellagic acid, methyl-ellagic acids, and their glycosides were the main compounds in the authentic AM seeds and AMSE. The chromatographic profiles of AM seeds are distinctly different from that of regular mango (*M. indica*). Among the five representative AMDS purchased from the Internet, only one sample contained trace constituents of regular mango seeds. The other four AMDS samples and the two AMSE samples from Chinese suppliers do not contain detectable amount of authentic AMSE. This study continues to demonstrate the urgency for the enforcement of FDA's GMP requirements for the dietary supplement industry. For AMDS, the labels do not provide accurate information for the consumer. Proper standardization and quality control of raw materials and the herbal preparations should be carried out. Finally, it is worth noting that although the conclusion was based on the authentic samples from one source only, the differences between the authentic samples and the commercial products were significant that the probable cause of the difference was not due to variability of growing conditions, harvesting times, and storage conditions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures of total ion chromatogram of Mexican mango seeds and the major compounds found in product A. Tables of UHPLC-HRMS data of constituents from Mexican mango seeds and label claims of five AMDSs. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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