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Chromatographic Profiles and Identification of New Phenolic Components of *Ginkgo biloba* Leaves and Selected Products

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Abstract

Ginkgo biloba leaves and their extracts are one of the most widely used herbal products and/or dietary supplements in the world. A systematic study of the phenolic compounds is necessary to establish quality parameters. A modified LC-DAD-ESI/MS method was used to obtain chromatographic profiles for the flavonoids and terpene lactones of *Ginkgo biloba* leaves. The method was used to identify 45 glycosylated flavonols and flavones, 3 flavonol aglycones, catechin, 10 biflavones, a dihydroxybenzoic acid, and 4 terpene lactones in an aqueous methanol extract of the leaves. The extracted *G. biloba* leaf products contained the same flavonoids as the raw leaves except for the lack of biflavones. The detected glycosylated flavonol contents were equal to or more than 0.0008% of the dry plant material. This is the first report of the presence of more than 20 of these flavonoids in *G. biloba*.

Keywords

Ginkgo biloba; flavonoids; terpene lactones; LC-DAD-ESI/MS identification; chromatographic flavonoid profile

Introduction

The dried leaves of *Ginkgo biloba* L. (Ginkgoaceae) have been used as herbal remedies for centuries in China, and now their extracts are one of the most widely used herbal products and/or dietary supplements in the world (1–4). The flavonoids and terpene lactones (Figure 1) are considered to be the main beneficial components (5, 6). The voluntary industry standard is 24% flavonoid and 6% terpene lactones (by weight) in the powdered extracts (1–4). Over 30 flavonoids, including flavonol glycosides, flavonols, flavones, biflavones, and catechins, have been reported in the leaves (1–11). The quality of ginkgo leaves and their products and/or quantitative determination of their active components, mainly the flavonoid components and terpene lactones, have been previously reported (1–3, 12–30). However, none have developed methods for a systematic identification of all the main phenolic components.

The flavonoid components far outnumber the diterpene lactones found in *G. biloba* leaves (11, 12, 27–29). Thus, good separation and detailed identification of the indigenous flavonoid components is crucial to qualitative analysis and authentication of *G. biloba* leaves and their products. Chromatographic profiles are an important means of characterizing the leaf materials. For this purpose, a method was developed based on liquid chromatography

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with tandem diode array and mass spectrometric detection (LC-DAD-ESI/MS) to profile the flavonoids of *G. biloba* leaves and its products.

Materials and Methods

Plant Materials

Six *G. biloba* leaf samples and three *G. biloba* processed products (powdered extracts) were used in these studies. Leaf sample 1 was a standard reference material (SRM) 3246, *G. biloba* leaf, from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Sample 2 was an American Herbal Pharmacopoeia (AHP) verified Botanical Reference Standard BRS-GB. Leaf samples 3 and 4 were picked from mature *G. biloba* trees in Columbia, MD and Kunmin, Yunnan Province, China, respectively. Samples 5 and 6 were picked from female (fruit bearing) and male (nonfruit bearing) *G. biloba* trees growing in Wanzhou, Chongqing City, China, respectively. The dried leaves were finely powdered and passed through 20 mesh sieves prior to the extraction experiments.[2]

Processed product sample 1 is SRM #3247, powdered *G. biloba* extract (NIST). The other two product samples, products 2 and 3, were purchased from local stores in the greater Washington, DC area.

Reagents, Solvent, and Standards

HPLC grade methanol, acetonitrile, formic acid, acetic acid, and NaOH were purchased from VWR International, Inc. (Clarksburg, MD, USA). HPLC water was prepared from distilled water using a Milli-Q system (Millipore Laboratory, Bedford, MA, USA).

Quercetin dihydrate (≥98%), rutin trihydrate (≥95%), kaempferol (≥90%), luteolin, apigenin (95%), catechin (95%), 3,4-dihydroxybenzoic acid, bilobalide, and ginkgolide A, B, and C were obtained from Sigma Chemical Co. (Saint Louis, MO, USA). Quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, myricetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, isorhamnetin 3-O-rutinoside, isorhamnetin, myricetin, and syringetin were purchased from Extrasynthese (Genay, Cedex, France).

Patuletin 3-*O*-rutinoside was isolated from *Echinacea angustifolia* leaves and identified by NMR analysis (31). Patuletin was obtained from the acid hydrolysis of patuletin 3-*O*-rutinoside in this laboratory. Gingkolide J was a gift from Professor Zhu, Shanghai Institute of Mataria Medica, Chinese Academy of Sciences, Shanghai, China.

Extraction Method

Dried ground leaves (125 mg) were extracted with 5.0 mL of methanol–water (60:40, v/v) using sonication with a FS30 Ultrasonic sonicator (40 KHz, 100 W) (Fisher Scientific, Pittsburgh, PA, USA) for 60 min at room temperature (<35 °C at the end). Powdered leaf products (5 mg) were extracted with 5.0 mL of methanol–water (60:40, v/v). The extracts were filtered through a 0.45 μm Nylon Acrodisk 13 filter (Gelman, Ann Arbor, MI, USA). A 50 μL aliquot of the extracts was injected onto the analytical column for analysis. The analysis was completed less than 24 h after the extracts were prepared.

Acid Hydrolysis of Extracts

Filtered extracts (0.50 mL) were mixed with concentrated HCl (37%, 0.1 mL) and heated in a capped tube at 85 °C for 2 h. Then, 0.40 mL of methanol was added to the mixture, and the solution was sonicated for 10 min. The solution was refiltered prior to HPLC injection (32).

Alkali Hydrolysis of Extracts

The filtered extract (1.00 mL) was dried, and the residue was mixed with 0.30 mL of 4 M NaOH and kept at room temperature under a N_2 atmosphere for 18 h. Then, 0.15 mL of HCl (37%) was added to the reaction mixture to bring the pH to approximately 1.0, and 0.55 mL of MeOH was added. The solution was refiltered prior to HPLC injection (32).

LC-DAD and ESI-MSD Conditions

The LC-DAD-ESI/MS system used has been previously described. It consisted of a quaternary pump with a vacuum degasser, a thermostatted column compartment, an autosampler, a diode array detector, and a quadrupole mass spectrometer from Agilent Technologies (Palo Alto, CA, USA). A 250 × 4.6 mm i.d., 5 μm Symmetry C18 column with a 20 × 3.9 mm i.d., 5 μm sentry guard column (Waters Corp., Milford, MA, USA) was used at a flow rate of 1.0 mL/min. The column oven temperature was set at 25 °C. The mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient increased linearly from 10% to 26% B (v/v) at 40 min, to 65% B at 70 min, and to 100% B at 100 min and was held at 100% B to 105 min. The last step, increasing from 65% B at 70 min to 100% B at 100 min, was a modification necessary for elution of the biflavones. The DAD was set at 270, 330, and 350 nm for real time monitoring of the peak intensities. UV spectra were recorded from 190–650 nm and were available after completion of the chromatographic run for plant component identification. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization (PI and NI) modes at low and high fragmentation voltages (100 and 250 V) over the range of *m/z* 100–2000. A drying nitrogen gas flow of 13 L/min and temperature of 350 °C, a nebulizer pressure of 50 psi, and capillary voltages of 4000 V for PI and 3500 V for NI were used. The LC system was directly coupled to the MSD without stream splitting (32).

In order to detect less intense peaks, in the presence of more intense peaks, and to provide more sensitivity, positive and negative selective ion monitoring (PI/NI SIM) was used. For detection of all the flavones, ions at *m/z* 271/269 (to detect all the trihydroxyflavones), 285/283 (dihydroxy-methoxyflavone), 285/287 (tetrahydroxyflavone), 301/299 (trihydroxy-methoxyflavone), 303/301 (pentahydroxyflavone), 317/315 (tetrahydroxy-methoxyflavone), 319/317 (hexahydroxyflavone), 333/331 (pentahydroxy-methoxyflavone), and 347/345 (tetrahydroxydimethoxyflavone) were monitored. For the terpene lactones, ions at *m/z* 327/325 (bilobalide), 409/407 (ginkgolide A), 425/423 (ginkgolides B and J), and 441/439 (ginkgolide C) (11, 12, 27–29) were monitored.

Results and Discussion

Identification of Ginkgo Leaf Flavonoids and Phenolic Compounds

UV chromatograms for the extract (270 and 350 nm) and alkali hydrolyzed extract (310 nm) of sample 1 are shown in Figure 2A–C. The NI SIM chromatograms for the extract (masses for flavones and terpene lactones) and the acid hydrolyzed extract (masses for flavones) for sample 1 are shown in Figure 2D–F. The structures of some ginkgo phenolic components and their terpene lactones are shown in Figure 1. The retention times (t_R), wavelengths of maximum absorbance (λ_{max}), protonated/deprotonated molecules ($[M + H]^+/[M - H]^-$), major PI fragment ions, and PI/NI aglycone ions ($[A + H]^+/[A - H]^-$) are listed in Table 1. Peak identification was based on the data in Table 1, standards, and published literature.

The identification process can be illustrated by the identification of peak 7 in Figure 3. The $[M + H]^+/[M - H]^-$ ions at *m/z* 757/755, $[A + H]^+/[A - H]^-$ ions at *m/z* 303/301, and PI fragment ions at 611 (loss of the third glycosyl, a rhamnosyl, from the glycoside) and 465

(loss of the secondary glycosyl, a rhamnosyl, from the produced fragment) (Table 1) suggested the peak was quercetin-dirhamnosylhexoside. Its UV data (λ_{max} 256, 266 sh, and 354 nm) and retention time (t_R 19.27 min) strongly suggested that the glycoside was located at the 3-position of the aglycone (30, 31). This flavonoid was finally identified as quercetin 3-*O*-2″,6″-dirhamnosylglucoside since this compound has been previously reported in *G. biloba* leaves (1–3) and is the only detected flavonoid that matches the listed retention time and UV and mass spectrometric data of peak 7. In the same manner, peak 12 (λ_{max} 256, 266 sh, and 354 nm, and PI ions at *m/z* 771, 625, 479, and 317) was identified as isorhamnetin 3-*O*-2″,6″-dirhamnosylglucoside and peak 11 (λ_{max} 266 and 348 nm, PI ions at *m/z* 741, 595, 449, 287) was identified as kaempferol 3-*O*-2″,6″-dirhamnosylglucoside. Both flavonoids have been previously reported in *G. biloba* leaves (1–3, 5).

Peak 3 had protonated molecular and aglycone ions at *m/z* 773 and 303 suggesting it was a triglycoside like peak 7. Its retention time (t_R 10.46 min), however, was much shorter than that of peak 7 (t_R 19.27 min), suggesting that the compound might have a sugar at the 3-position and a second at one of the remaining positions (7, 5, 4′, or 3′). The UV spectra suggested that the sugars were located at the 3 and 7 positions of quercetin (32, 33). This quercetin *O*-triglycoside lost its first glycosyl, a rhamnosyl of the second sugar at the 3- or 7-position, to form the fragment *m/z* 627 (i.e., 773 – 627 = 146). Then, the diglycoside lost its first hexosyl at the 3- position to form the fragment at *m/z* 465 (i.e., 627 – 465 = 162). Finally, the molecule lost its third glycosyl, the hexosyl at the 7-position, to form its aglycone, *m/z* 303 (i.e., 465 – 303 = 162) (32, 34). This compound was tentatively identified as quercetin 3-*O*-rhamnosylglycoside-7-*O*-glucoside or its isomer quercetin 3-*O*-glucoside-7-*O*-rhamnosylglycoside since without a direct standard comparison they cannot be distinguished by LC-MS. Similarly, on the basis of retention time, UV, and mass spectrometric data listed in Table 1, peaks 5 and 6 were identified as kaempferol 3-*O*-rhamnosylglycoside-7-*O*-glucoside and isorhamnetin 3-*O*-rhamnosylglycoside-7-*O*-glucoside, or their isomers, respectively. These three glycosides (peaks 3, 5, and 6) were formed from the same saccharides connected to the same positions of the three different flavonols and have not been previously reported in *G. biloba* leaves.

There are two pairs of flavonol 3-*O*-diglycosides consisting of one rhamnoside and one glucoside, i.e., two quercetin 3-*O*-diglycosides (peaks 14 and 19) and two kaempferol 3-*O*-diglycosides (peaks 20 and 26). They all have the same UV spectra and the same molecular and aglycone ions, but the fragments for loss of the second sugar are different. Peak 14 has a fragment *m/z* 465 for the loss of rhamnosyl, indicating that it is quercetin 3-*O*-rhamnosylglucoside, while peak 26 has a fragment *m/z* 433 for the loss of glucosyl, indicating it is kaempferol 3-*O*-glucosylrhamnoside (not shown). By direct comparison with standards and references to the literature on *G. biloba* flavonoids (1–3, 6–11), these four peaks were further identified as quercetin 3-*O*-6″-rhamnosylglucoside (rutin) (peak 14), quercetin 3-*O*-2″-glucosylrhamnoside (peak 19), kaempferol 3-*O*-6″-rhamnosylglucoside (peak 20), and kaempferol 3-*O*-2″-glucosylrhamnoside (peak 26).

It was found that using the LC conditions of this study, rutin (peak 14) eluted nearly 2 min earlier than quercetin 3-*O*-glucoside (peak 17) and 4 min earlier than quercetin 3-*O*-2″-glucosylrhamnoside (peak 19). The pair of kaempferol diglycosides (peaks 20 and 26) also showed the same elution order (30.06 and 34.71 min). Using this elution order, a pair of isorhamnetin diglycosides (peaks 21A, 31.03 min and 28A, 36.90 min) were identified, and an additional isorhamnetin 3-*O*-rhamnosylglucoside (peak 21B, 31.35 min) was detected, which is most likely isorhamnetin 3-*O*-2″-rhamnosylglucoside. This identification was based on the fact that the flavonol 3-*O*-2″-rhanomosylglucoside (i.e., 3-*O*-hesperidoside) elutes slightly later than its isomer 3-*O*-6″-rhamnosylglucoside (i.e., 3-*O*-rutinoside) under these LC conditions (32).

The remaining nonacylated glycosylated flavones were identified as myricetin 3-*O*-rutinoside (peak 8), myricetin 3-*O*-glucoside (peak 9), patuletin 3-*O*-rutinoside (peak 15), patuletin 3-*O*-hesperidoside (peak 16), kaemperol 3-*O*-glucoside (peak 22), quercetin 3-*O*-rhamnoside (peak 23), isorhamnetin 3-*O*-glucoside (peak 24A), syringetin 3-*O*-2″-glucosylrhamnoside (peak 24B), apigenin 7-*O*-glucoside (peak 25), and luteolin 3′-*O*-glucoside or kaempferol *O*-hexoside (peak 29). Some of the identifications were further confirmed with standards as indicated in Table 1. Of these compounds, two patuletin 3-*O*-diglycosides, isorhamnetin 3-*O*-hesperidoside, myricetin 3-*O*-glucoside, and syringetin 3-*O*-2″-glucosylrhamnoside have not been previously reported in *G. biloba* leaves.

Twenty-one acylated flavone glycosides were detected and 20 of them (except peak 18) were identified as listed in Table 1. The main characteristics of the *p*-coumaroylglycosylated flavonols are the shift of absorption band II to 312–316 nm and PI/NI molecular ions 146 amu larger than their parent glycosides. Thus, peaks 31 and 33 were identified as quercetin 3-*O*-2″-(6″-*p*-coumaroylglucosyl)rhamnosides and kaempferol 3-*O*-2″-(6″-*p*-coumaroylglucosyl)rhamnosides. They have been previously reported (1–4, 11).

Peak 13 (24.78 min, $[M + H]^+/[M - H]^-$ at *m/z* 903/901, $[A + H]^+/[A - H]^-$ 303/301) is either kaempferol 3-*O*-2″-(6″-[4-glucosyl-*p*-coumaroy]glucosylrhamnoside) or kaempferol 3-*O*-6″-rhamnosyl-2″-(6″-*p*-coumaroylglucosyl) glucoside. The former has been reported previously (1–3), and the latter may be more prevalent since kaempferol 3-*O*-2″-glucosyl-6″-rhamnosyl-glucoside (peak AH-1 in Figure 2C, and Table 1) was detected in the alkaline hydrolyzed extract. This compound has its t_R = 16.20 min, λ_{max} 262, 342 nm, $[M + H]^+/[M - H]^-$ at *m/z* 757/755, PI fragments at *m/z* 595 (from the loss of the third glycosyl, a hexosyl), *m/z* 449 (from the loss of the second glycosyl, a rhamnosyl), and $[A + H]^+$ at *m/z* 287 (from the loss of the first glycosyl, a hexosyl). The assignment of the glucosyl at the 2″ position and the rhamnosyl at the 6″-position was based on the fact that the glycosyl at 2″ position is removed first at this LC-MS condition when both positions are glycosylated (32).

Peak 10 (21.64 min, $[M + H]^+/[M - H]^-$ at *m/z* 919/917, $[A + H]^+/[A - H]^-$ 303/301) and its molecular and aglycone ions were 16 amu more than those of peak 13, suggesting a quercetin glycoside with the same saccharide. Considering the biosynthetic passways for peaks 13 and 10, peak 10 is most likely quercetin 3-*O*-6″-(*p*-4-glucosylcoumaroyl)-2″-glucosylrhamnoside rather than its isomer quercetin 3-*O*-6″-rhamnosyl-2″-(6″-*p*-coumaroylglucosyl)glucoside. If so, neither compounds has been previously reported in ginkgo leaves (1–3, 5, 11).

Nine of the remaining 16 peaks were identified as kaempferol 3-*O*-*p*-coumaroyldigylcosides and seven as quercetin 3-*O*-*p*-coumaroyldigylcosides. The diglycosides consisted of one glucoside and one rhamnoside. The sugar sequence and the position for *p*-coumaroyl or other acyls could not be determined by MS or MS^n. Some of these peaks did not show the UV data described above, but they all disappeared following alkaline hydrolysis with the accompanying appearance of *p*-coumaric acid (AH-2 in Figure 2C) and a significant enhancement of peak 26 (kaempferol 3-*O*-2″-glucosyl-6″-rhanoside).

So far, only the 3-*O*-2″-(6″-*p*-coumaroyl)glucosylrhamnosides of kaempferol, quercetin, and isorhamnetin, and 7-*O*-rhamoside-3-*O*-2″-(6″-feruloyl)glucosylrhamnosides, 7-*O*-glucoside-3-*O*-2″-(6″-*p*-coumaroyl)glucosylrhamnosides, and 3-*O*-2″-(6″-[4-glucosyl-p-coumaroy]glucosyl)rhamnoside of kaempferol and quercetin, have been previously isolated and detected from ginkgo leaves (1–3, 5–11). Therefore, more than half of the acylated flavonol glycosides have not been previously reported in *G. biloba* leaves.

Nine flavonol and flavone aglycones, i.e., quercetin, kaempferol, myricetin, apigenin, 4′-*O*-methylapigenin (acacetin), luteolin, 3′-*O*-methylquercetin (isorhamnetin), 4′-*O*-methylquercetin (tamarixetin), and 3′,5′-*O*-dimethylmyricetin (syringetin), have been reported in *G. biloba* leaves (1–3, 5, 11), but only three of them, myricetin (peak 28B), quercetin (peak 43A), and kaempferol (peak 48), were observable in this study using UV or MS (total ion counts) detection. With SIM detection, 14 naturally occurring aglycones were observed in the leaf extracts (NI SIM in Figure 2E; PI SIM not shown) and the acid hydrolyzed extract (Figure 2D). Among them, peaks A-5 (quercetin) and A-8 (kaempferol) were considerably larger than others. The identification of myricetin (A-1), patuletin (A-3), luteolin (A-4), apigenin (A-6), syringetin (A-7), and isorhamnetin (A-9) was confirmed with standards, but the remaining peaks (A-10 through A-14) could only be identified as polyhydroxyflavones or as the mono- or dimethyl derivatives. Their specific structures could not be determined. The amplitude of peaks A-1, A-3, and A-5 through A-10 were relatively higher in the acid hydrolyzed extract (Figure 2D), indicating that their glycosides were present in the leaf extract (Figure 2E) and contributed to the aglycone concentration following acid hydrolysis.

Peaks 2 and 4 (clearly shown in Figure 2B) were identified as 3,4-dihydroxybenzoic acid (protocatechuic acid) and catechin, respectively. Peak 1 and several other small peaks (not numbered) in the 5–20 min and 80–100 min region (Figure 2A and B and 9) could not be identified at this stage since their UV and/or mass data were not sufficiently intense to provide positive identification.

The detection (UV and MS TIC) limits of this method (32), using the extract of 125 mg dried leaves in 5.00 mL of the solvent and 50 μL of injection volume, all of the detected glycosylated flavonols will have their content equal to or over 0.0008% dry plant material by weight.

Identification of Ginkgo Biflavones

Biflavones are compounds distinctive to *G. biloba* leaves and, in some cases, constitute a major fraction of the flavonoid components. So far, six biflavones and some of their glycosides have been reported (1–3, 5, 6, 9, 11). In this study, 10 biflavones were detected in the unprocessed leaf samples. Peaks 49, 51, 54, 55, 56, and 57, the relatively major biflavone peaks, were identified as amentoflavone, bilobetin, sequoiaflavone, ginkgetin, isoginkgetin, and sciadopitysin, on the basis of the fact that all of them were previously reported as major Ginkgo biflavones, and their UV and mass data (Table 1) matched previously published values, including the elution order on reverse phase columns (1–3, 6). Similarly, on the basis of the similarity of the retention times, UV spectra, and masses, the remaining 4 minor compounds (peaks 47, 50, 52, and 53) were tentatively identified as the isomers of some of Gingko major biflavones. Unfortunately, their exact structures could not be determined from the MS data, and none of them have been previously reported in Ginkgo leaves.

No significant amounts of biflavones were detected in the *G. biloba* processed products. The commercial extraction process appears to leave the biflavones behind. The relatively nonpolar biflavones are not efficiently extracted by the polar solvents used by most commercial companies. The biflavones are reported to be COX-2 inhibitors with the potential to serve as unique anti-inflammatory agents (35).

Detection of Ginkgo Terpene Lactones

Five *G. biloba* terpene lacotones were identified in the extracts by direct standard comparison. Since these compounds lack the conjugated bonds of the flavonoids, they are

undetectable by UV absorption (11, 12, 27–29) and can only be detected by the mass spectrometric data from the MS TIC chromatograms, specifically the $[M + H]^+/[M - H]^-$ and $[2M - H]^-$ ions. To obtain their accurate retention times, SIM detection at *m/z* 327/325 showed the existence of bilobalide C (BB in Figures 2F and 6), which coeluted with quercetin 3-*O*-glucoside (peak 17). Similarly, monitoring $[M + H]^+/[M - H]^-$ and $[2M - H]^-$ ions at *m/z* 441/439 and 879, *m/z* 409/407 and 815, and *m/z* 425/423 and 847 allowed the identification of ginkgolides A, B, and C (GA, GB, and GC in Figures 2F and 4). SIM detection permitted identification of these compounds even though they coeluted with flavonol glycosides (ginkgolide C coeluted with peak 18, and ginkgolides A and B coeluted with peak 35).

It is worth mentioning that using the chromatographic conditions described for this study, the $[2M - H]^-$ ions for ginkgolides A, B, and C, three major ginkgo terpene lactones in the extract, could be observed while that for bilobalide, another major ginkgo terpene lactone with different structure (Figure 1), was not detected. Ginkgolide J (GJ) is barely discernible as a shoulder on the leading edge of bilobide C (BB) (GJ is not labeled in Figure 2F), and its $[M - H]^-$ was detected at *m/z* 423 (Figure 4). The retention times were t_R 27.02 min for GJ, 27.41 min for BB, 28.04 min for GC, 44.32 min for GA, and 44.84 min for GB, respectively, and perfectly matched those of the standards (not shown).

Comparison of Leaves and Processed Products Using the Phenolic Component Profile

The samples consisted of six *G. biloba* leaf samples and three processed products from local stores. The four leaf samples that were personally collected (samples 3–6) came from different locations with significantly different altitudes, weather, and soil conditions. Two of the Chinese samples (samples 5 and 6) were from male and female trees at the same location. The female tree produces fruit yearly, and the male tree does not. Both of them grow widely in China. Over 70% of ginkgo leaves and their products come from China, and many studies on the quality of the leaves and products, including profiling methods, have been carried out there (2, 29, 36–38). Despite the difference in growing conditions and sex, all 6 leaf samples had similar chromatographic profiles; only the relative intensities of the peaks were different (Figure 5). Thus, the data reported in this study suggest that the *G. biloba* chromatographic profiles might be similar for ginkgo trees growing all over the world. This is not surprising since *G. biloba* does not have diverse phenotypes.

The three processed products contained most of the glycosylated flavonoids found in the raw materials (Figure 6), even acylated flavonoid glycosides (37–50 min). The only substantial difference between the raw leaf and processed materials was the lack of significant amounts of the biflavones in the processed materials.

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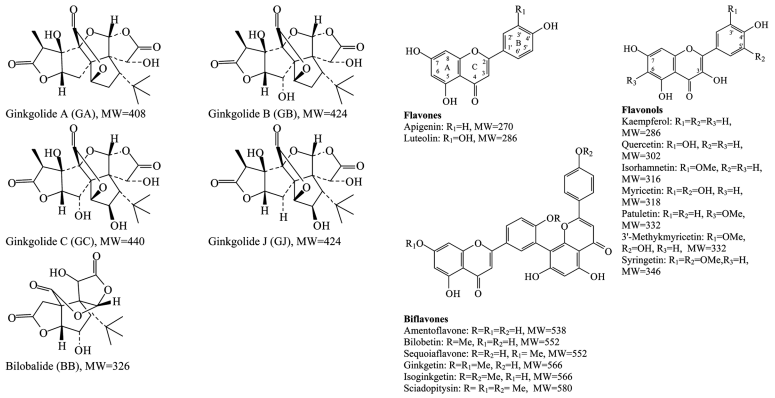

Figure 1.
Structures of the phenolic components and terpene lactones of *Ginkgo biloba* leaves.

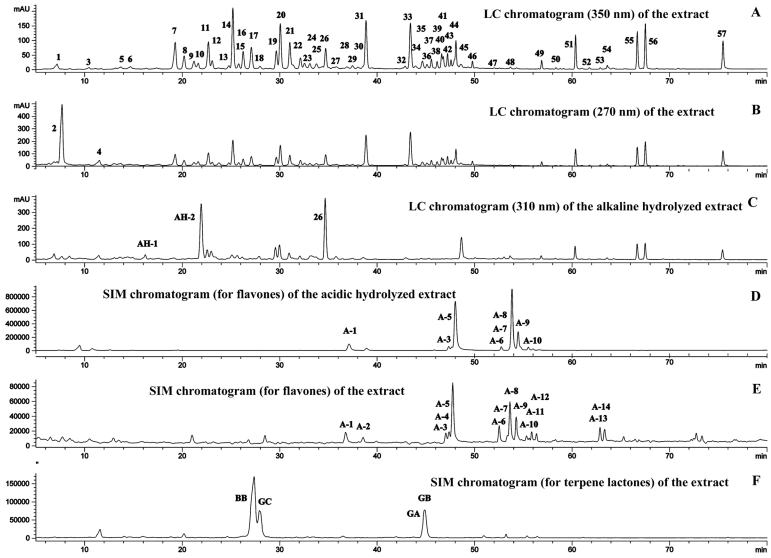

Figure 2.

LC Chromatograms of ginkgo extracts of leaf sample 1: (**A**) extract, 350 nm; (**B**) extract, 270 nm; (**C**) alkali hydrolyzed extract, 310 nm; (**D**) acid hydrolyzed extract, SIM; (**E**) untreated extract, SIM; and (**F**) untreated extract, SIM. See text for SIM masses and Table 1 for peak identification.

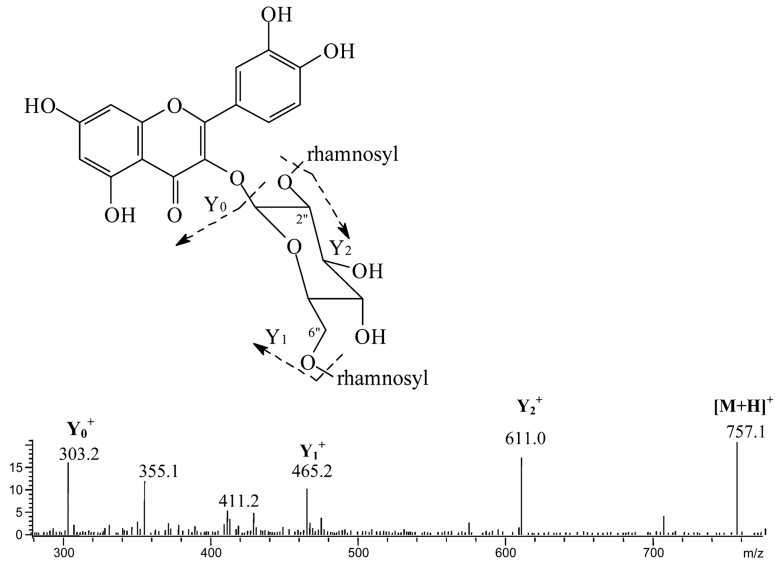

Figure 3.

Mass spectrum of quercetin 3-*O*-2″,6″-dirhamnosylglucoside (peak 7 in Figure 2A), obtained with positive ionization and 100 V fragmentation energy.

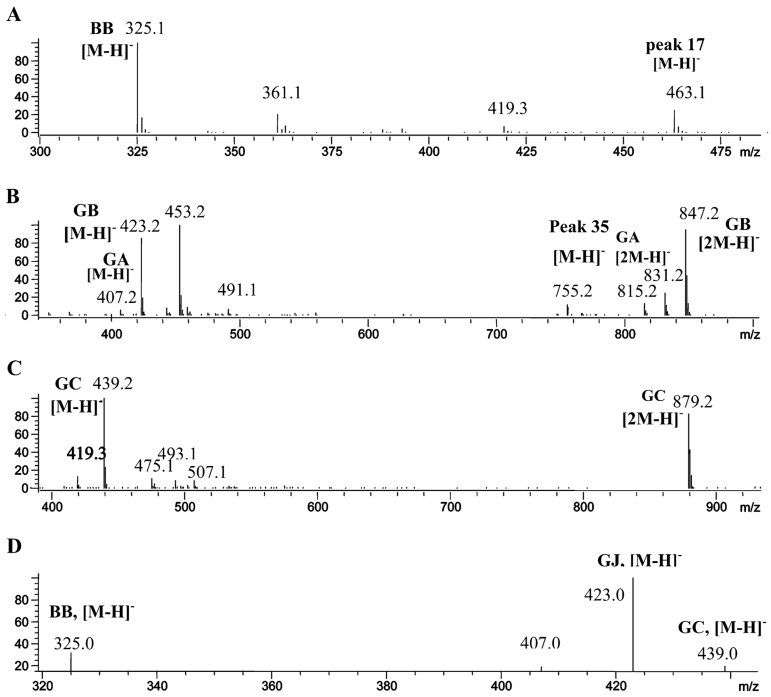

Figure 4.

Mass spectra of ginkgo terpene lactones, obtained with negative ionization and 100 V fragmentation energy: (**A**) bilobalide (BB), (**B**) ginkolides A (GA) and B (GB), (**C**) ginkolide C (GC), and (**D**) ginkolide J (GJ). Their retention times were recorded from the mass SIM chromatogram (Figure 2F), where the peaks were labeled as BB, GC, GA, and GB, with the exception of GJ, which is overlapped with BB, and not indicated.

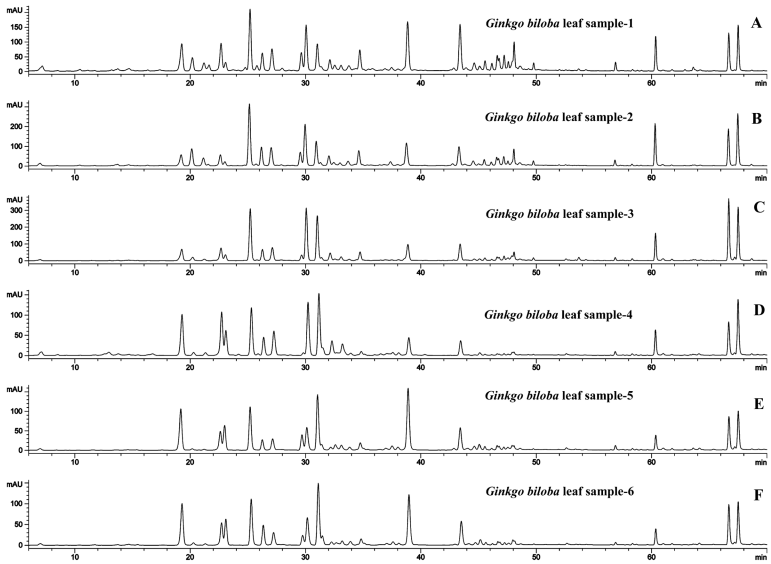

Figure 5.

LC Chromatograms of *Ginkgo biloba* leaf samples: (**A**) sample-1, NIST SRM 3246; (**B**) sample-2, AHP standard; (**C**) sample-3, the leaves from the tree in MD, USA; (**D**) sample-4, the leaves from the tree in Kunming, China; (**E**) sample-5, the leaves from the female tree in Chongqing, China; and (**F**) sample-6, the leaves from the male tree in Chongqing, China.

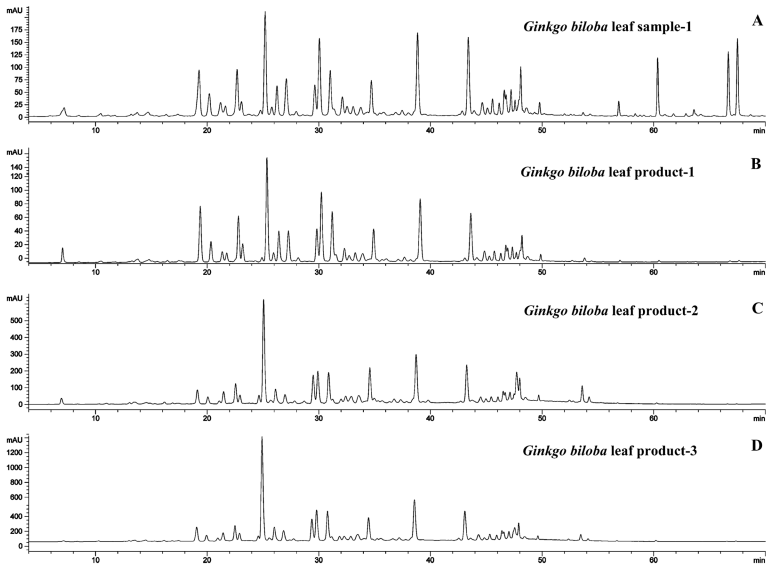

Figure 6.

LC Chromatograms of *Ginkgo biloba* raw leaf and processed product materials: (**A**) leaf sample-1, SRM 3246; (**B**) product-1 from leaf sample-1; (**C**) product-2; and (**D**) product-3.

Table 1

Peak Assignment for Aqueous Methanol Extracts of *Ginkgo biloba* Leaves

peak no.	t_R (min)	$[M + H]^+/[M - H]^-$ (*m/z*)	(PI/NI aglycone), other ion (*m/z*)	UV[a] · λ_{max} (nm)	identification
			1 phenolic acid and 1catechin		
1	7.18	nd[g]	254, 350	nd	
2	7.70	/153		260, 294	3,4-dihydroxybenzoic acid[b,d]
4	11.52	/325, 289		282	catechin[b,c]
			46 flavone *O*-glycosides		
3	10.46	773/771	627, 465, 303	256, 354	quercetin 3-*O*-rhamnosylhexoside-7-O-glucoside[d]
5	13.71	757/755	611, 449. 287	nd	kaempferol 3-*O*-rhamnosylhexoside-7-*O*-glucoside[d]
6	14.69	787/785	641, 479, 317	nd	isorhamnetin 3-*O*-rhamnosylhexoside-7-*O*-glucoside[d]
AH-1	16.20	757/755	595,449, 287,	262, 342	kaempferol 3-*O*-2″-glucosyl-6″-rhamnosyl-glucoside[d]
7	19.27	757/755	611, 465, 287	254, 266sh, 354	quercetin 3-*O*-2″,6″-dirhamnosylglucoside[c]
8	20.19	627/625	481, 319/317	254,264, 358	myricetin 3-*O*-rutinoside[c]
9	21.20	481/479	319/317	258, 356	myricetin 3-*O*-glucoside[b,c]
10	21.64	919/917	303/301	254, 266sh, 354	quercetin 3-*O*-6″-rhamnosyl-2″-(6″-*p*-coumaroylglucosyl)glucoside[d]
11	22.68	741/739	595, 449, 287	266, 348	kaempferol 3-*O*-2″, 6″-dirhamnosylglucoside[c]
12	23.07	771/769	625, 479, 317	256, 266sh, 354	isorhamnetin 3-*O*-2″,6″-dirhamnosylglucoside[b,c]
13	24.78	903/901	287/285	266, 316	kaempferol 3-*O*-6″-rhamnosyl-2″-(6″-*p*-coumaroylglucosyl)glucoside[d]
14	25.20	611/609	465, 303/301	256, 266sh, 354	quercetin 3-*O*-rutinoside[b,c]
15	25.79	641/639	495, 333/331	266, 344	patuletin 3-*O*-rutinoside[b,c]
16	26.26	641/639	495, 333/331	256, 266, 356	patuletin 3-*O*-neohesperidoside[d]
17	27.09	465/463	303/301	256, 266sh, 354	quercetin 3-*O*-glucoside[b,c]
18	27.98	881/879	nd	nd	unidentified acylated glycoside
19	29.64	611/609	449, 303/301	254, 266sh, 350	quercetin 2″-glucosylrhamnoside[c]
20	30.06	595/593	449, 287/285	266, 348	kaempferol 3-*O*-rutinoside[b,c]

peak no.	t_R (min)	$[M + H]^+/[M - H]^-$ (*m/z*)	(PI/NI aglycone), other ion (*m/z*)	UV[a] · λ_{max} (nm)	identification
21A	31.03	625/623	479, 317/315	256, 266sh, 354	isorhamnetin 3-*O*-rutinoside[b,c]
21B	31.35	625/623	479, 317/315	256, 266sh, 354	isorhamnetin 3-*O*-neohesperidoside[d]
22	32.12	449/447	287/285	266, 348	kaempferol 3-*O*-glucoside[b,c]
23	32.54	449/447	303/301	nd	quercetin 3-*O*-rhamnoside[b,c]
24A	33.09	449/447	317	nd	isorhamnetin 3-*O*-glucoside[b,c]
24B	33.14	677/653	347/445	nd	syringetin 3-*O*-2″-glucosylrhamnoside[d]
25	33.77	433/431	271/269	270, 336	apigenin 7-*O*-glucoside[b,c]
26	34.71	595/593	433, 287/285	266, 344	kaempferol 3-*O*-2″-glucosylrhamnoside[c]
27	35.80	/739	/285	nd	kaempferol 3-*O*-*p*-coumaroylrutinosidee[d]
28A	36.90	625	463, 317/315	266, 348	isorhamnetin 3-*O*-2″-glucosylrhamnoside[c]
29	37.47	449/447	287/285	266, 342	luteolin 3′-*O*-glucoside[c] or kaempferol *O*-hexoside[d]
30	38.03	787/785	287/285	266, 328	kaempferol 3-*O*-acyldiglycoside[d,e]
31	38.86	757/755	303/301	266, 316	quercetin 3-*O*-2″-(6″-*p*-coumaroyl)glucosylrhamnoside[c]
32	42.86	757/755	303/301	262, 316	quercetin 3-*O*-*p*-coumaroyldiglycoside[d]
33	43.41	741/739	287/285	266, 316	kaempferol 3-*O*-2″-(6″-*p*-coumaroyl)glucosylrhamnoside[c]
34	43.93	741/739	287/285	nd	kaempferol 3-*O*-*p*-coumaroyldiglycoside[d]
35	44.64	/755	/300	nd	quercetin *p*-coumaroyldiglycoside[d]
36	45.10	/755	/301	nd	quercetin *p*-coumaroyldiglycoside[d]
37	45.56	/747	303/301	nd	quercetin 3-*O*-*p*-coumaroylgdiglycoside[d]
38	46.15	/747	/301	nd	quercetin 3-*O*-*p*-coumaroylgdiglycoside[d]
39	46.61	/747	/301	nd	quercetin 3-*O*-*p*-coumaroylgdiglycoside[d]
40	46.79	/747	/301	nd	quercetin 3-*O*-*p*-coumaroylgdiglycoside[d]
41	47.22	/739	287/285	nd	kaempferol *p*-coumaroyldiglycoside[d]
42	47.58	/739	287/285	nd	kaempferol *p*-coumaroyldiglycoside[d]
43B	47.92	/739	287/285	266, 316	kaempferol *p*-coumaroyldiglycoside[d]
44	48.08	/739	287/285	nd	kaempferol *p*-coumaroyldiglycoside[d]

peak no.	t_R (min)	$[M + H]^+/[M - H]^-$ (*m/z*)	(PI/NI aglycone), other ion (*m/z*)	UV[a] . λ_{max} (nm)	identification
45	48.59	/739	287/285	nd	kaempferol *p*-coumaroyldiglycoside[d]
46	49.78	/739	287/285	nd	kaempferol *p*-coumaroyldiglycoside[d]
				14 flavonoid aglycones (most by SIM) and 1phenolic acid	
A-1 (28B)	36.98	319/317		nd	myricetin[b,c]
A-2	38.95	303/301		nd	pentahydroxyflavone[d]
A-3	47.03	333/331		nd	patuletin[b]
A-4	47.36	287/285		nd	luteolin·[c]
A-5 (43A)	47.74	303/301		nd	quercetin[b,c]
A-6	52.45	271/269		nd	apigenin[b,c]
A-7	53.40	347/345		nd	syringetin[b,c]
A-8 (48)	53.68	287/285		nd	kaempferol[b,c]
A-9	54.49	317/315		nd	isorhamnetin[b,c]
A-10	55.26	347/345		nd	tetrahydroxydimethoxyflavone[d]
A-11	55.81	303/301		nd	pentahydroxyflavone[d]
A-12A	56.33	347/345		nd	tetrahydroxydimethoxyflavone[d]
A-12B	56.33	287/285		nd	tetrahydroxyflavone[d]
A-13	62.79	287/285		nd	tetrahydroxyflavone[d]
A-14	63.31	285/283		nd	dihydroxymethoxyflavone[d]
AH-2	21.94	–/163		310	*p*-coumaric acid[b]
				10 biflavones	
47	52.00	/565		nd	ginkgetin isomer[d]
49	56.87	539/537		268, 334	amentoflavone[c]
50	58.35	/537		nd	amentoflavone isomer[d]
51	60.36	553/551		222, 270, 328	bilobetin[c]
52	61.77	553/551		222, 270, 328	bilobetin isomer[f,d]
53	62.89	553/551		222, 270, 328	bilobetin isomer[f,d]

peak no.	t_R (min)	$[M + H]^+/[M - H]^-$ (*m/z*)	(PI/NI aglycone), other ion (*m/z*)	UV[a] . λ_{max} (nm)	identification
54	63.61	553/551		222, 270, 328	sequoiaflavone[c,f]
55	66.69	567/565		224, 270, 332	ginkgetin[c]
56	67.51	567/565		224, 270, 330	isoginkgetin[c]
57	75.49	581/579		224, 270, 326	sciadopitysin[c]
					4 terpene lactones
1	27.41	327/325		nd	bilobalide (BB)[b,c]
2	28.04	441/439	879 $[2M - H]^-$	nd	Gingkolide C (GC)[b,c]
3	44.32	409/407	815 $[2M - H]^-$	nd	Gingkolide A (GA)[b,c]
4	44.82	425/423	847 $[2M - H]^-$	nd	Gingkolide B (GB)[b,c]

[a] The detection limit for mono- and diglycosylated flavonols is around 0.0008% dry plant material by weight.

[b] Identified by direct comparison with standard or the completely identified compound in other plants.

[c] Previously reported in *G. biloba*.

[d] Not previously reported in *G. biloba*.

[e] Acyl might be feruloyl (+176 amu).

[f] Exchangeable.

[g] nd, not determined.