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A Non-targeted Approach to Chemical Discrimination Between Green Tea Dietary Supplements and Green Tea Leaves by HPLC/MS

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Abstract

Green tea-based dietary supplements (GTDSs) have gained popularity in the U.S. market in recent years. This study evaluated the phytochemical composition difference of GTDS in comparison with green tea leaves using an HPLC/MS fingerprinting technique coupled with chemometric analysis. Five components that are most responsible for class separation among samples were identified as (−) epicatechin gallate, strictinin, trigalloylglucose, quercetin-3-*O*-glucosylrhamnosylglucoside, and kaempferol-3-*O*-galactosyl-rhamnosylglucoside, according to the accurate mass measurements and MS/MS data. The similarity coefficients between the GTDSs in solid form with green tea were 0.55 to 0.91, while for the GTDSs in liquid form they were 0.12 to 0.89, which suggested that chemical composition variance across the GTDSs was significant. Flavonol aglycone concentrations were higher in GTDSs than in tea leaves, indicating the degradation of flavonol glycosides or the oxidation of catechin during the manufacturing and storage processes. In some GTDS samples, compounds were identified that were on the label. The results demonstrate the urgency of QC for GTDS products.

Tea (*Camellia sinensis*) has been consumed as the most globally popular beverage, aside from water (1). It is usually consumed in unfermented (green tea; GT), semifermented (oolong teas), and fermented (black, red, and cooked pu-erh) forms, with GT the most popular. Compared to the fermented teas, green tea contains more antioxidant polyphenolic catechins. The major GT catechins are epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (EGCG; 2). Tea catechins exhibit a large range of biological activities such as angiogenesis, alteration of cell signaling (3–7), and weight loss (8, 9). Because of its diverse health effects, green tea extract has gained popularity as the fourth most commonly used dietary supplement in the U.S. market in recent years (10). Commercial GT-based dietary supplements (GTDSs) are available in solid (capsules and tablets) and liquid forms. Commercial GTDSs claim to be “standardized” for levels of polyphenols and catechins. However, the extraction and manufacturing procedures of GTDSs are not standardized, and the recommended daily intake amounts (equivalent to the respective labeled standardized GTE amount) of different manufacturers range from 100 to 6000 mg.

As GTDSs become more and more popular in the United States, questions remain as to the quality and efficacy of these products. There are very few studies comparing GTDSs and GT. Currently, there are several reports showing that the actual content of catechin or polyphenols was not consistent with the label claims (11–13). These studies were all carried out with the traditional approach of targeted compound quantitation. The traditional

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approach requires multiple standards and is expensive, laborious, and time-consuming. Moreover, this approach focuses only on selected targets (often catechins) and ignores other known or unknown constituents. A compositional comparison of GTDSs and tea leaves was not performed in those studies. This approach is not suitable for detection of adulteration or loss of compounds in GTDSs.

A non-targeted chromatographic fingerprinting technique with principal component analysis (PCA) can be effectively used to profile phytochemical differences among samples from different origins or varieties. Previously in our laboratory, this approach has been used successfully in the quality evaluation of *Ginkgo biloba* and Pycnogenol-based dietary supplements (14, 15). HPLC/MS is one of the most powerful analytical tools. Moreover, when accurate mass measurement is used, HPLC/MS allows for identification of the components if needed. HPLC/MS has been successfully used in the characterization of flavan-3-ols, flavonoids, gallic acid, quinic esters of caffeine, thearubigins, and alkaloids in different kinds of tea (16–22).

The aim of this study was to compare the phytochemical composition of GTDSs with GT leaves using an HPLC/MS fingerprinting approach and chemometric analysis. Twenty commercially available GTDS samples and eight GT samples (leaves) were evaluated. Constituents in GTDS and GT samples were identified using accurate mass measurement and MS/MS. A PCA model was established to distinguish the chemical differences between GT and GTDS samples.

Experimental

Reagents, Standards, and Samples

(a) *Water.*—Optima grade (Fisher Scientific, Pittsburgh, PA).

(b) *Acetonitrile.*—Optima grade (Fisher Scientific).

(c) *Methanol.*—Optima grade (Fisher Scientific).

(d) *Formic acid.*—MS grade (Sigma-Aldrich, St. Louis, MO).

(e) *Reference standards.*—(+)-Catechin, (−)-epicatechin, (−)-gallocatechin, (−)-epigallocation, (−)-epicatechin3-gallate, (−)-catechin 3-gallate, (−)-epigallocatechin 3-gallate, and (−)-gallocatechin 3-gallate were obtained from Sigma-Aldrich. Theaflavin and theaflavin 3,3′-digallate were obtained from Chromadex, Inc. (Irvine, CA).

(f) *HPLC mobile phase.*—Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile.

(g) *Samples.*—Twenty GTDS samples representing most of the big dietary supplement manufacturers were purchased commercially. Table 1 shows the label claims of the 20 GTDS samples. All of the samples were labeled as GTE, and no details were provided about extraction procedures. Ten of the samples were encapsulated extracts, two were tablets, and the remaining eight were liquids. All of the extracts had label claims as total catechin, total polyphenol, or total standardized GTE contents. Product J claimed to be decaffeinated, and Products N and Q specified that Luo Han Guo (the fruit of *Siraitia grosvenorii*) and stevia leaf extracts were added. Eight GT leaf samples from China, Japan, and Taiwan were used as listed in Table 2. Six of them were common grade tea and two of them (T5 and T8) were special grade (much more expensive), categorized commercially. The eight GT leaves were selected based on their chemical composition according to a previous study (21). The goal was to select

a group of GT samples with as much chemical difference as possible to be representative of all types.

Apparatus

(a) *HPLC system*.—Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) consisting of a quaternary pump with a vacuum degasser, a thermostatted column compartment, an autosampler, and a diode array detector (DAD).

(b) *Mass spectrometer*.—LCQ Classic ion-trap mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) and Agilent 6530 accurate-mass quadrupole-time of flight (Q-TOF) mass spectrometer equipped with an electrospray ionization (ESI) probe were used for accurate mass measurement.

(c) *Centrifuge*.—IEC Clinical Centrifuge (Danon/IEC Division, Needham Heights, MA).

(d) *HPLC column*.—Phenomenex (Sigma-Aldrich) Hydro RP C_{18} column (4 μm particle size, 250 × 2.0 mm id) with Column-Saver™ precolumn filter (MAC-MOD Analytical, Inc., Chadds Ford, PA).

(e) *HPLC conditions*.—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 25% B (v/v) at 30 min and to 65% B at 60 min with a flow rate of 0.25 mL/min. The post-run time for re-equilibrating the system with the beginning mobile phase was 15 min. The DAD was set at 350 and 270 nm, and UV spectra were continuously recorded from 190 to 450 nm.

(f) *MS conditions*.—ESI was performed in the negative ion and positive ion modes over the range of *m/z* 100–1500. For the LCQ, the following conditions were used: sheath gas flow rate, 80 arbitrary units; auxiliary gas flow rate, 10 arbitrary units; spray voltage, 4.50 kV; heated capillary temperature, 220°C; capillary voltage, 34 V for positive and −7 V for negative mode; and tube lens offset, 25 V.

For accurate mass measurement, the Q-TOF was operated in both the positive and negative modes with a capillary voltage of 3500 V; nebulizer 30 psig; drying gas, 12.0 L/min; sheath gas flow, 16.3 L/min, gas temperature, 300°C; fragmentor, 165 V; skimmer, 60 V; OCT DC1, 46 V; and OCT RFV, 750 V. The samples were run in the data-dependent MS/MS mode. Mass spectra were recorded over a mass range of 100 to 1600 Da. The multichannel plate detector voltage was 650 V and the photomultiplier tube voltage was 659 V. Mass calibration was performed with an Agilent tune mix from 100 to 1600 Da.

Sample Preparation

(a) *Solid form GTDS samples*.—Twenty-five tablets of each sample were weighed to 0.1 mg accuracy and ground into a fine powder; 25 capsules of each capsule sample were opened and the contents weighed to 0.1 mg accuracy. A 100 mg amount of powder from each sample was mixed with 10.00 mL methanol–water (6 + 4, v/v) in a 15 mL centrifuge tube and sonicated for 1 h at room temperature. The slurry mixture was centrifuged at 5000 × *g* for 10 min. Then the supernatant was filtered through a 0.20 μm PVDF syringe filter (VWR Scientific, Seattle, WA). Finally, a 2 μL aliquot was injected for HPLC/MS analysis.

(b) *Liquid form GTDS samples*.—Each bottle was vortexed 30 s prior to extraction. Then 200 μL of the sample was diluted by 10 with methanol–water (60 + 40, v/

v) to 2.00 mL, and votexed again for 30 s. The mixture was filtered through a 0.20 mm PVDF syringe filter, and 2 μL was injected for HPLC/MS analysis.

(c) *GT leaves*.—Each of the tea samples was finely powdered and passed through a 20-mesh sieve prior to extraction. Each powdered tea sample (100 mg) was extracted with 10.00 mL of methanol–water (60 + 40, v/v) in a 15 mL centrifuge tube and sonicated for 1 h at room temperature. The slurry mixture was centrifuged at 5000 × *g* for 10 min. Then the supernatant was filtered through a 0.20 μm PVDF syringe filter, and 2 μL was injected for HPLC/MS analysis.

Multivariate Statistical Analysis

The HPLC/MS raw files were converted to NetCDF (Network Common Data Form) format with Xcalibur (Thermo Fisher Scientific Inc.) and subsequently processed by the XCMS package (Version 1.14.1, http://metlin.scripps.edu/download/) under R (Version 2.10.1, the R project for statistical computing, www.r-project.org) using default settings of the XCMS. The resulting table from XCMS was then exported to Microsoft Excel (Chevy Chase, MD). Normalization was performed against the EGCG peak intensity prior to multivariate analyses. The resulting matrix involving peak index (*m*/*z*, retention time pair), sample names, and normalized peak area percent were introduced into a SIMCA-P 11.5 software package (Umetrics AB, Umea°, Sweden) for the PCA analysis.

Similarity Analysis

First, all peaks were aligned using the software SpectraAlign (Version 2.4, Cartwright Group, PTCL, University of Oxford, Oxford, UK), and then a reference chromatogram was calculated using the average of eight GT samples (T1–T8). The similarity of each chromatogram against this reference chromatogram was then calculated based on the average Pearson's correlation coefficient of each chromatogram with the equation:

$$r_{xy}=\frac{\sum_{i=1}^{n}\left[(x_i-\overline{x})\right](y_i-\overline{y})}{(n-1)\,S_x S_y}$$

where x and y are the sample means of X and Y, and s_x and s_y are the sample SD values of X and Y. Cluster analysis was performed based on Ward's method using R (Version 2.10.1, the R project for statistical computing, www.r-project.org).

Results and Discussion

Peak Identification

The HPLC method used in this study was an improved version of the method reported previously by our group (21) using a newer HPLC column (narrower diameter and smaller particle size) for better separation and reduced solvent usage (Figures 1–3). Accurate mass measurement was used in this study to assist in the identification of the constituents. A total of 66 constituents from various GTDSs have been identified according to their retention times, the accurate mass measurements, their MS/MS spectra, and literature reports (Table 3). The chemical differences between GTDS and GT samples were mainly in flavonoid contents and theaflavins. Many components reported in fermented tea (oolong tea, pu-erh tea, and black tea) were identified in GTDS samples, especially in the liquid form.

Flavonoids Contents

Figure 1 shows the chromatograms detected at 350 nm of GT (T1–T8) and GTDS (A–K, solid form; L–T, liquid form) samples. There were some obvious differences in peak intensities and/or additional peaks in the GTDS samples compared with the GT samples. The most obvious is Sample T, for which peaks corresponding to tea constituents were detected either by UV or MS (Figures 1 and 2, respectively). The chromatogram for Sample T was almost identical to the blank run. The label of Sample T did not provide any information regarding to the amount of GTE or other polyphenols. The product was light brown colored with sediments by visual inspection.

Among the other samples, peaks 51, 60, and 63 in Table 3, with retention times at 33.8, 43.1, and 47.7 min, respectively, were obviously higher in GTDSs than those in GT leaves. Furthermore, these three peaks were higher in liquid form GTDSs than those in solid form GTDSs. The molecular composition of Peak 51 (*m*/*z* 317.0285, M-H) is $C_{15}H_{12}O_8$ [Δm = (measured mass − theoretical mass)/theoretical mass] = 5.63 ppm. The collision induced dissociation (CID)-MS/MS generated product ions at *m*/*z* 289 ($^{0,2}A^-$), 271 ($^{0,2}A^- –H_2O$), 179 ($^{1,3}A^-$), and 151 ($^{1,3}A^- –CO$) with the loss of one CO, CO plus H_2O, $C_7H_6O_3$, and $C_8H_6O_4$, respectively. The ion at *m*/*z* 179 was generated through retro-Diels-Alder (RDA) fission. All of the above information was consistent with myricetin, and a flavonol glycone previously characterized in tea. Similarly, peaks at 60 and 63 min were identified as quercetin and kaempferol by their high resolution MS and MS/MS data, respectively. These three flavonols were considered to be the fundamental structures of many flavonol glycosides detected in GT. According to the literature (23), the relative contents of myricetin, quercetin, and kaempferol increased when a new oolong tea was converted into an old one due to the degradation of flavonol glycosides. In the GTDS samples, especially liquid samples, these three basic flavonol aglycones were in much higher concentration than those in the raw leaves, indicating that degradation of flavonol glycoside took place during the manufacturing and storage processes.

Peaks 37 and 42 (Table 3) with retention time at 25.2 and 26.2 min, respectively, had the same *m*/*z* (431.0968, M–H), which gave the chemical composition $C_{21}H_{20}O_{10}$. In the negative CID-MS/MS spectra, significant product ions at *m*/*z* 341, 311, and 283 were observed in keeping with the cross ring cleavage of a flavonol-*C*-monoglycoside (24–26). Based on retention times and the mass fragmentation data, these two peaks were identified as vitexin and isovitexin, respectively. These two flavonol-C-glycosides existed mainly in GTDS samples and may have been formed via the degradation product of vitexin diglycosides or isovitexin diglycosides.

Figure 2 shows the HPLC/MS profiles of all samples. Peak 16 with a retention time of 12.5 min, was high in GT samples but very low in GTDS samples. The *m*/*z* of the peak was 633.0734 $[M-H]^-$, suggesting that the molecular composition was $C_{27}H_{22}O_{18}$ (Δm = 0.10 ppm). The product ions at *m*/*z* 463 and 301 corresponded to the cleavage of gallic acid ($-C_7H_6O_5$, 170.0210 Da, Δm = 3.08 ppm) and gallic acid plus glucosyl residue ($-C_{13}H_{16}O_{10}$, 332.0728 Da, Δm = 4.66 ppm). A product ion at *m*/*z* 169 was consistent with the deprotonated ion of gallic acid. Based on literature reports (20, 21), this compound was identified as strictinin, a known component in GT.

Ten peaks (52, 53, 54, 55, 57, 59, 62, 64, 65, and 66), corresponding to the acylated flavonol *O*-glycosides in T5 and T8, were characterized. These components were not found in most of the GTDS samples except for Sample B. The acylated flavonol glycosides are considered to have a positive correlation with the GT grade (21). Hence, it can be deduced that most of the GTDS products are made from lower-grade GT.

Theaflavins

GT chemical composition may vary with the origin, harvest time, and drying conditions. However, theaflavins were seldom reported in GT leaves. Peaks 56, 58, and 61 at 41.3, 42.6, and 43.3 min, and with deprotonated ions at *m*/*z* 563, 715, and 867, respectively, of samples A, C, D, E, J, K, and L were not found in GT leaves. These three peaks were identified as theaflavins. The main MS^2 (MS/MS) product ions of *m*/*z* 563 were *m*/*z* 545, 527, 519, 501, and 407, corresponding to the loss of a H_2O, two H_2O, CO_2, CO_2 plus H_2O, and a 156 Da unit, respectively. The fragment of 156 Da was consistent with the loss of H_2O and RDA fission (138 unit). This peak was identified as theaflavin and confirmed with a reference standard. Similarly, Peaks 58 and 61 were identified as theaflavin 3-gallate and theaflavin-3,3′-digallate, respectively. As reported in the literature (1), these constituents are major theaflavins found in fermented tea, such as black tea, and are the products of catechin oxidation during the fermentation process. Therefore, it can be concluded that the oxidation occurred during the manufacturing process of GTDSs.

Additives in GTDSs

GTDS products in liquid form were all deeply dark-colored and tasted bitter except for Sample T. Despite the label claims that no sugar was added, a peak corresponding to sucrose (*m*/*z* 341.1082, $C_{12}H_{22}O_{11}$, Δm = 2.15 ppm) was a common component in liquid GTDS products. The sucrose peak was not detected in GT samples, so sugar must have been added to some of the GTDSs for taste despite the label claims.

The labels of Samples N and Q claimed that natural sweeteners Luo Han Guo and stevia leaf were added. As shown in Figure 3, the HPLC/MS profiles of Samples N and Q were obviously different from the other samples. The constituents corresponding to Luo Han Guo and stevia leaf were characterized according to the accurate MS and MS/MS measurements. From the MS/MS experiments the primary fragments of the parent ions of these natural sweeteners was the loss of glucose. Taking Peak 13 (Table 4, Figure 3A) as an example, the deprotonated ion at *m*/*z* 625.3218 suggested the molecular formula is $C_{32}H_{50}O_{12}$ (1.84 ppm). The MS/MS spectrum gave a typical fragmentation pattern of glycone glycosides with successive losses of a rhamnosyl residue and a hexosyl-rhamnosyl group (Figure 4). The product ion at *m*/*z* 317 was steviol glycones ($C_{20}H_{30}O_3$). The formula and MS/MS spectrum are consistent with the structure of esteviolbioside, which is a component from stevia leaf. In total, 17 peaks from stevia leaves and five peaks from Luo Han Guo were identified, as listed in Table 4 (27–31).

The label of Sample S, a GTDS, indicated its only additive was glycerin, but its HPLC-UV/MS chromatographic profile was quite different from other GTDS samples, as shown in Figure 2. Several peaks with high abundance, observed between 35 to 60 min (Figure 3), were not tea constituents. The fragmentation behavior of these peaks suggested the successive losses of hexosyl or rhamnosyl groups. For Peak 25 (Table 4), the deprotonated ion at *m*/*z* 901.4828 suggested the molecular formula is $C_{45}H_{74}O_{18}$ (−2.84 ppm). The MS/MS spectrum displayed product ions at *m*/*z* 755 $[M\text{-}H\text{-}146]^-$ and 593 $[M\text{-}H\text{-}146\text{–}162]^-$, representing the loss of methylpenosyl and hexosyl sugar units, respectively. The product ions at *m*/*z* 59, 89, 101, and 119 were consistent with the cross-ring cleavage of a terminal glycoside. Altogether, seven components with similar mass fragmentation behavior were characterized in Sample S. A library search revealed that these peaks belonged to steroidal saponins from fenugreek (*Trigonella foenum-graecum* L.; 32–34). Therefore, a fenugreek extract dietary supplement product made by the same manufacturer was purchased and analyzed (data not shown). The result verified our speculation that fenugreek extract was either added or was a contaminant during the manufacturing process for Sample S.

Similarity and Cluster Analysis

To evaluate the similarity between GTDS and tea leaf samples, Pearson's correlation coefficient was calculated for the chromatographic profiles. Chromatograms were first aligned using the method of peak alignment by fast fourier transform (35). A reference chromatogram was calculated as the average of eight GT samples. Then Pearson's correlation coefficient was calculated for the chromatogram of each sample against the reference chromatogram of GT leaf samples. As shown in Figure 5, the similarity coefficient between the GTDS samples in solid form (Samples H–L) with GT was 0.55 to 0.91, while for the GTDS samples in liquid form (Samples M–T) was 0.12 to 0.89, which suggests that the chemical variance across the GTDS samples was significant. Samples M, N, S, and T were quite different from tea samples. Samples H, I, J, K, and O, on the other hand, showed consistency with the tea reference. A hierarchical clustering analysis was used based on Pearson's correlation coefficient matrix for the measurement of similarity between the samples. The 20 GTDS samples were separated into three clusters according to their similarities to the reference chromatogram (Figure 6). Cluster I (M, N, P, Q, R, and S) contained all the liquid GTDSs. Cluster III was mainly solid GTDSs that have similar chemical composition compared with the GT reference. Sample T was classified as a separate cluster due to its poor quality. This sample could be easily picked out by cluster analysis and using the Pearson correlation coefficient.

Principal Component Analysis (PCA)

While a simple visual inspection of chromatograms may detect some obviously outlying samples, PCA was thought to be the best method for revealing chemical variance between samples. PCA involves a mathematical procedure that transforms a number of possibly correlated variables into a smaller number of non-correlated variables called principal components. It is the simplest of the true eigenvector-based multivariate analyses.

The peak list with *m/z*, retention time (Rt), and ion abundance information for PCA was generated from XCMS (Scripps Center for Mass Spectrometry, http://masspec.scripps.edu/xcms/), which is an HPLC/MS based data analysis package with nonlinear retention time alignment, matched filtration, peak detection, and peak matching (36). Sample T was excluded prior to the XCMS peak detection as almost no peaks were detected in the sample. Eighty-one variables (*m/z*, Rt) were found with the XCMS default setting. EGCG was selected as the reference peak and the other 80 peaks were normalized against EGCG, which gave a matrix of 30 samples × 80 peaks. Pareto scaling was used for data preprocessing. The application of PCA allowed the large HPLC/MS data set to be reduced to three principal components, PC1, PC2, and PC3, that accounted for 85% of the total variance with 56% predictability in the multidimensional space. The liquid GTDS samples were clearly separated from the solid samples and GT samples in the PCA score plot (Figure 7A), which suggests that the variability across liquid samples was huge, especially for Samples M and P. The cluster of solid samples was relatively tight and had a better chemical consistency than that of the liquid samples or GT leaves. The GT leaves exhibited a wide variability of the chemical composition. This is expected since the GT samples used in this study were selected to have as much chemical variance as possible based on a previous study (21). T8 is a special grade (commercially categorized) Maofeng, and only the very young leaves were used during the manufacturing process. It was not surprising that this sample was separated in the score plot from the other samples. According to the loading plot (Figure 7B), peaks 16, 27, 36, 42, and 46 (Table 3) were mainly responsible for the separation of liquid GTDSs from other kinds of samples. These five peaks were identified as (−) epicatechin gallate, strictinin, trigalloylglucose, quercetin-3-*O*-glucosylrhamnosylglucoside, and kaempferol-3-*O*-galactosyl-rhamnosylglucoside. The PCA results showed clearly the poor qualities of the

liquid GTDS samples, which is consistent with the results of the similarity and cluster analyses.

Conclusions

Our results showed that a wide variability of chemical composition exists between GTDS and GT samples. The nontargeted HPLC/MS approach found constituents from other botanical extracts in some GTDSs. Although there are some good GTDS products, there is no way for the consumer to know the qualities of the GTDS products from reading the labels. More importantly, the consumer may ingest other botanical extracts unintentionally. Our study demonstrated that degradation of flavonol glycosides or oxidation of catechin occurred during the manufacturing and storage processes for GTDS samples; some additives in the GTDSs were not labeled; the daily intake amount recommended by the labels varies significantly; the quality of GTDS varies significantly; and the solid GTDS products are more chemically similar to tea leaves compared to their liquid counterparts. The claim that a GTDS is a good alternative for tea leaves is questionable from a chemical composition point of view.

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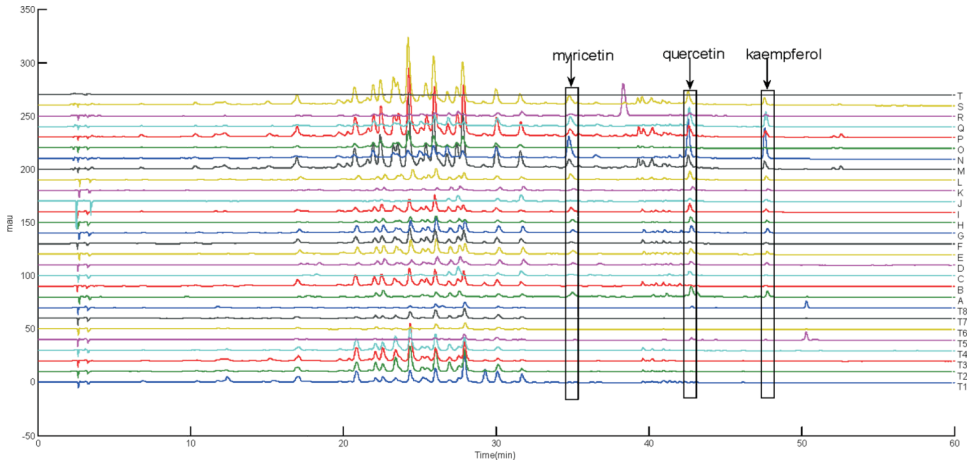

Figure 1.
The UV chromatograms detected at 350 nm of all GTDS and GT samples.

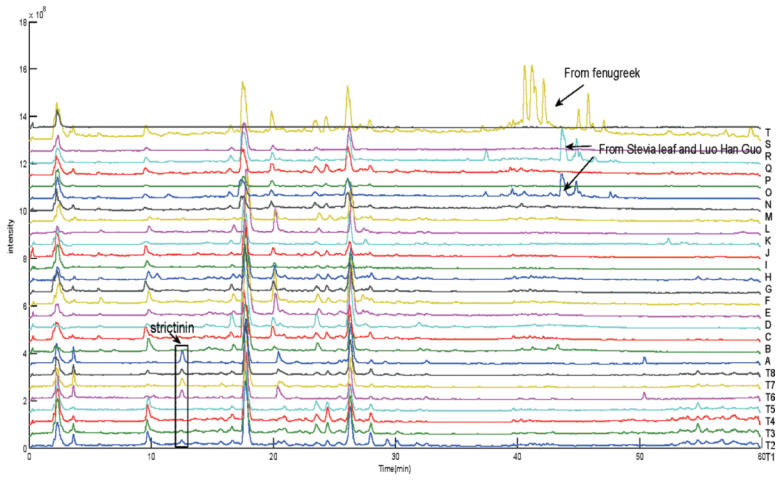

Figure 2.
HPLC/ESI-MS total ion chromatograms of all GTDS and GT samples.

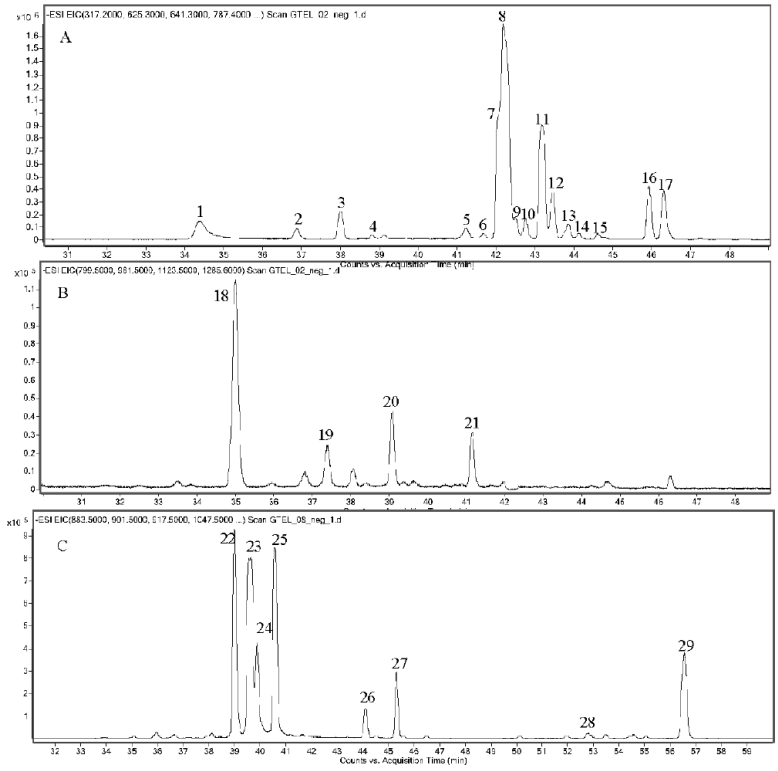

Figure 3.

Extracted ion chromatograms showing the additives identified in Supplements N, Q, and S (Table 1); constituents from (A) stevia leaves, (B) Luo Han Guo, and (C) fenugreek.

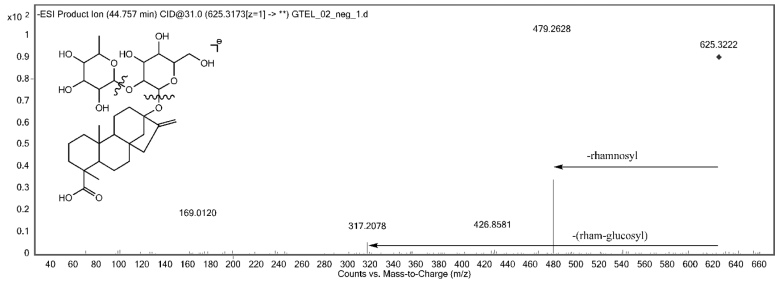

Figure 4.
The MS/MS fragmentation of esteviolbioside. The product ion with *m*/*z* 317 is the steviol aglycone.

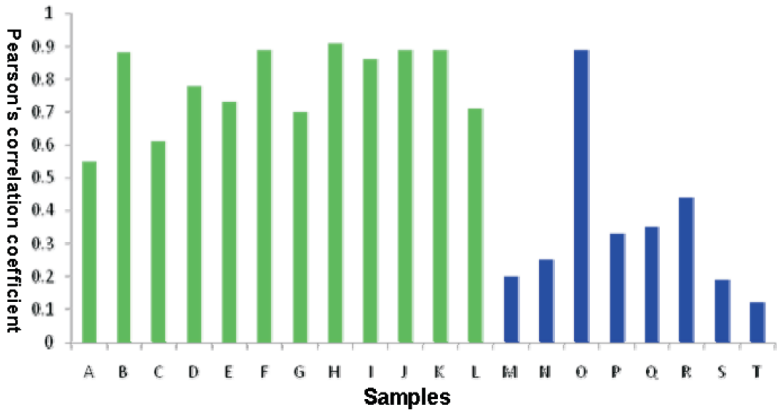

Figure 5.
The similarity of GTDS and green tea samples based on the Pearson's correlation coefficient. Green-GTDS in liquid form (samples A–L); Blue- GTDS in solid form (samples M–T).

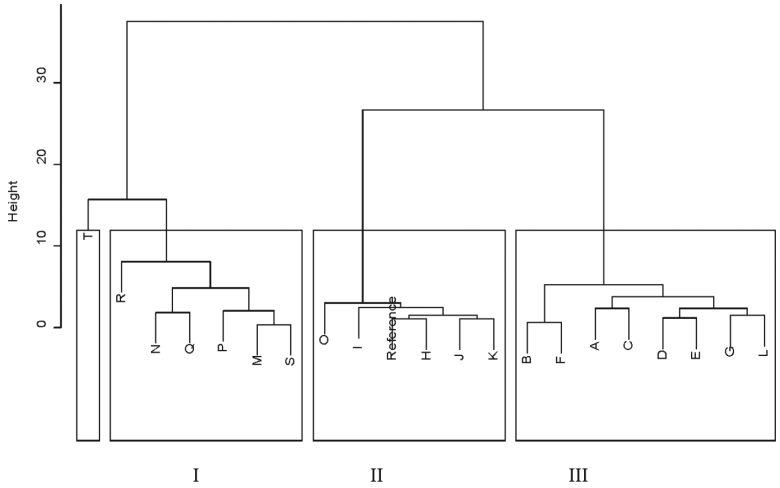

Figure 6.

The cluster dendrogram of 20 GTDS samples. Cluster I (M, N, P, Q, R, S) is composed of mainly liquid GTDS samples. Cluster III is mainly solid GTDS samples that have a chemical composition similar to that of the GT reference. Sample letters are identified in Table 1.

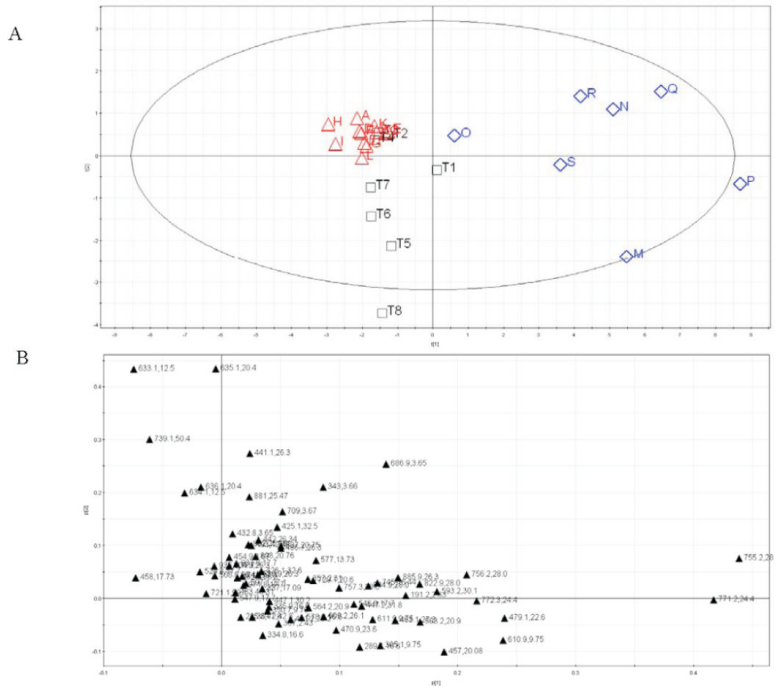

Figure 7.
PCA score (A) and loading plot (B) derived from the HPLC/MS dataset of GTDS samples and GT leaves. ▲, GTDS solid form; ◇, GTDS liquid form; □, GT samples.

Table 1

Label claims of GT extract based GTDSs

Supplement	Serving form	Labeled GT extract weight, mg/serving size	Total polyphenols	Catechins, %	EGCG, %
A	Tablet	500	—	—	35
B	Capsule	500	40%	30	14.81
C	Capsule	300	80%	40	—
D	Capsule	250	95%	75	55
E	Capsule	400	60%	40	—
F	Capsule	500	—	—	14
G	Capsule	2000	50%	—	—
H	Capsule	600	50%	—	—
I	Capsule	1000	20%	—	—
J	Capsule	250	20%	—	14
K	Tablet	600	90%	—	—
L	Capsule	600	50%	—	—
M	Liquid	2000	—	—	—
N	Liquid	100	90%	—	50
O	Liquid	2000	150 mg	—	—
P	Liquid	2000	7.5%	—	—
Q	Liquid	100	—	—	—
R	Liquid	103	—	—	—
S	Liquid	2000	150 mg	—	—
T	Liquid	—	—	—	—

Table 2

GT sample information

Sample name	Tea name	Location
T1	Mingqian green tea	Sichuan, China
T2	Japanese green tea	Japan
T3	Japanese Sancha	Japan
T4	Yinhou tea	Zhejiang, China
T5	Maojian tea	Henan Xinyang, China
T6	Tenren Pilochun	Taiwan
T7	Japanese Bancha	Japan
T8	Jiangxi Maofeng	Jiangxi, China

Table 3

Constituents identified in GTDS and green tea leaf samples with accurate mass measurement

Peak No.	Rt	Formula	[M−H]−	Main product ions	Δm, ppm	Identification
1	2.59	$C_7H_{14}N_2O_3$	175.1086[a]	158, 129, 84, 70	−5.06	Theanine
2	2.75	$C_7H_{12}O_6$	191.0578	85, 127	−8.79	Quinic acid
3	2.81	$C_{13}H_{16}O_{10}$	331.0687	169, 191, 271, 89	−4.81	Galloylglucose
4	3.41	$C_{30}H_{26}O_{14}$	609.1259	347, 191, 305, 271, 423	−1.50	Theasinensin C
5	3.53	$C_{14}H_{16}O_{10}$	343.0656	191, 169, 125	4.27	3-*O*-galloylquinic acid
6	3.67	$C_{14}H_{16}O_{10}$	343.0651	191, 169, 125	5.73	5-*O*-galloylquinic acid
7	3.77	$C_{14}H_{16}O_{10}$	343.0655	191, 169, 125	4.56	4-*O*-galloylquinic acid
8	3.97	$C_7H_6O_5$	169.0121	125, 81	12.63	Gallic acid
9	5.01	$C_7H_8N_4O_2$	181.0707[a]	163, 138	7.23	Theobromine
10	5.69	$C_{15}H_{14}O_7$	305.0650	125, 167, 251, 219, 165, 209, 261, 139, 137	5.48	Gallocatechin
11	9.81	$C_7H_8N_4O_2$	181.0725[a]	163, 135, 107, 153, 145	−2.77	Theophylline
12	9.96	$C_{37}H_{39}O_{18}$	761.1355	609, 591, 453, 471	0.15	Theasinensin B or E
13	10.47	$C_{16}H_{18}0_8$	337.0914	289, 173, 163, 119, 93	4.41	3-*p*-Coumaroylquinic acid
14	12.04	$C_{15}H_{14}O_6$	289.0703	245, 205, 109, 125, 203, 151, 123,137	5.04	Catechin
15	12.24	$C_8H_{10}N_4O_2$	195.0731[a]	138,110	−0.01	Caffeine
16	12.53	$C_{27}H_{22}O_{18}$	633.0734	301, 463, 275, 249, 169	−0.10	Strictinin
17	13.53	$C_{30}H_{26}O_{12}$	577.1331	407, 289, 125, 245, 451	3.55	(Epi)catechin(epi)catechin
18	14.51	$C_{30}H_{26}O_{12}$	577.1334	407, 289, 125, 245, 451	3.03	Procyanidin B2
19	15.09	$C_{37}H_{30}O_{17}$	745.1391	423, 305, 593, 125	2.58	Gallocatechin catechingallate
20	15.54	$C_{44}H_{34}O_{22}$	913.1477	632, 494, 806	−0.88	Theasinensin A or D
21	16.68	$C_{15}H_{14}O_6$	289.071	245, 205, 109, 125, 203, 151, 123, 137	2.63	Epicatechin
22	16.99	$C_{16}H_{18}0_8$	337.091	289, 173, 163, 119, 93	5.59	5-*p*-Coumaroylquinic acid
23	17.75	$C_{22}H_{18}O_{11}$	457.0774	305, 169, 125, 161, 331	0.51	Epigallocatechin gallate
24	18.49	$C_{37}H_{30}O_{16}$	729.1444	169, 125, 305, 423,577	2.34	Procyanidin B2 3′-*O*-gallate
25	19.49	$C_{37}H_{30}O_{16}$	729.1447	407, 289, 169, 125, 575, 593, 305, 271	1.93	Procyanidin B3-3-*O*-gallate
26	19.94	$C_{22}H_{18}O_{11}$	457.0775	169, 125, 305	0.29	Epigallocatechin gallate
27	20.32	$C_{27}H_{22}O_{18}$	635.0876	465, 483, 313, 169, 125	0.46	Trigalloylglucose
28	20.67	$C_{37}H_{30}O_{16}$	729.146	169, 305, 635, 465, 407, 577, 125, 687, 595	0.15	Procyanidin B3-3-*O*-gallate or isomer

Peak No.	Rt	Formula	[M−H]−	Main product ions	Δm, ppm	Identification
29	20.89	$C_{26}H_{28}O_{14}$	563.1397	169, 305, 353, 125	1.65	Apigenin 6-C glucosyl-8-C-arabinoside or apigenin 6-C-arabinoyl-8-C-glucoside
30	21.76	$C_{20}H_{34}O_{22}$	625.144	316, 317, 465, 169, 125	4.63	Myricetin 3-*O*-rhanmosylglucoside
31	22.16	$C_{21}H_{20}O_{13}$	479.0816	316, 317, 125	3.15	Myricetin 3-*O*-galactoside
32	22.57	$C_{21}H_{20}O_{13}$	479.0822	316, 317, 127, 431, 341, 97	1.90	Myricetin 3-*O*-glucoside
33	23.44	$C_{33}H_{40}O_{21}$	771.1991	301, 169, 125	−0.22	Quecetin 3-*O*-galactosylrutinoside
34	23.68	$C_{27}H_{30}O_{15}$	593.1489	293, 413, 473, 169	3.86	4″-*O*-glucosylvitexin
35	23.59	$C_{23}H_{20}O_{11}$	471.092	305, 183, 161, 125, 139	2.72	(−)-Epigallocatechin-3-*O*-(3-*O*-methyl)gallate
36	24.45	$C_{33}H_{40}O_{21}$	771.1997	301, 169, 125	−0.99	Qucercetin 3-*O*-glucosylrutinoside
37	25.22	$C_{21}H_{20}O_{10}$	431.0968	311, 283, 169, 341	3.63	Vitexin
38	25.58	$C_{27}H_{30}O_{14}$	577.1546	413, 293, 169, 353, 457	2.90	Vitexin-2″-*O*-rhamnoside
39	25.35	$C_{44}H_{30}O_{20}$	881.1565	169, 289, 441	0.64	ECG dimer
40	25.58	$C_{27}H_{30}O_{14}$	577.1546	413, 293, 169, 353, 457	2.90	Vitexin-2″-*O*-rhamnoside
41	26.07	$C_{27}H_{30}O_{16}$	609.1461	300, 311, 463, 271, 169	0.01	Quercetin 3-*O*-rhamnosylgalactoside or rutin
42	26.24	$C_{21}H_{20}O_{10}$	431.0968	311, 283, 169, 341	3.63	Isovitexin
43	26.27	$C_{22}H_{18}O_{10}$	441.0827	289, 169, 245	0.05	Epicatechin gallate
44	27.15	$C_{21}H_{20}O_{12}$	463.0862	300, 301, 169	4.31	Quercetin 3-*O*-glucoside
45	27.22	$C_{22}H_{18}O_{10}$	441.0816	289, 169, 245	2.53	Catechin gallate
46	27.97	$C_{33}H_{40}O_{20}$	755.2045	285, 169	−0.64	Kaempferol 3-*O*-glucosylrutinoside
47	30.04	$C_{27}H_{30}O_{15}$	593.1497	285, 169, 97, 125, 305	2.51	Kaempferol 3-*O*-rhamnosylgalactoside
48	31.72	$C_{21}H_{20}O_{11}$	447.0921	284, 285, 357, 169	2.64	Kaempferol 3 -*O*-glucoside
49	31.94	$C_{23}H_{20}O_{11}$	455.0970	289, 183, 245, 125, 205	3.00	Methoxyepiafzelechine gallatea
50	32.47	$C_{22}H_{18}O_{9}$	425.0866	273, 169, 125, 341, 229	2.83	Epiafzelechin gallatea
51	33.80	$C_{15}H_{10}O_{8}$	317.0285	179, 151, 257, 289, 299, 193, 165, 163	5.63	Myricetin
52	39.76	$C_{47}H_{54}O_{27}$	1049.2785	301, 903, 431	−0.50	Quercetin 3-*O*-acylglycoside
53	40.36	$C_{49}H_{42}O_{17}$	901.2373	285, 755, 737, 615, 145, 113	−2.63	Quercetin 3-*O*-*p*-coumaroyl-pentosyl-rhamnosylhexoside
54	40.54	$C_{41}H_{44}O_{22}$	887.2241	301, 300, 741, 169, 723	1.18	Quercetin 3-*O*-*p*-coumaroyl-pentosyl-rhamnosylhexoside
55	40.66	$C_{41}H_{44}O_{22}$	887.2239	301, 300, 285, 741, 431, 113, 163, 145	2.30	Quercetin 3-*O*-*p*-coumaroyl-pentosyl-rhamnosylhexoside
56	41.30	$C_{29}H_{24}O_{12}$	563.1178	545, 527, 519, 501	3.01	Theaflavin
57	41.91	$C_{49}H_{42}O_{17}$	901.2397	—	−5.29	Quercetin 3-*O*-p-coumaroyl-pentosyl-rhamnosylhexoside
58	42.61	$C_{36}H_{28}O_{16}$	715.1302	545, 563, 527, 671	0.36	Theaflavin 3-gallate
59	42.67	$C_{42}H_{46}O_{21}$	885.2450	285, 431, 739, 575, 145	1.00	Kaempferol 3-*O*-*p*-coumaroyl dirhamnosylhexoside
60	43.10	$C_{15}H_{10}O_{7}$	301.0334	151, 179, 273, 107	6.54	Quercetin

Peak No.	Rt	Formula	[M−H]−	Main product ions	Δm, ppm	Identification
61	43.28	$C_{43}H_{32}O_{20}$	867.1398	715, 697, 679, 527, 545, 559, 565	1.86	Theaflavin-3,3'-digallate
62	44.60	$C_{30}H_{26}O_{13}$	593.1285	285, 145, 447	2.63	Kaempferol 3-*O*-*p*-coumaroyl glucoside
63	47.70	$C_{15}H_{10}O_6$	285.0390	151, 179	5.11	Kaempferol
64	50.50	$C_{39}H_{32}O_{15}$	739.1652	285, 453, 145, 163, 593, 575	2.22	Kaempferol 3-*O*-di-*p*-coumaroyl glucoside
65	50.92	$C_{39}H_{32}O_{15}$	739.1657	285, 453, 145, 163, 593, 575	1.55	Kaempferol 3-*O*-di-*p*-coumaroyl glucoside
66	51.52	$C_{39}H_{32}O_{15}$	739.1640	285, 453, 145, 163, 593, 575	3.84	Kaempferol 3-*O*-di-*p*-coumaroyl glucoside

[a] Identified by $[M + H]^+$ and positive MS/MS data.

Table 4

Constituents identified from additives in supplements N, Q, and S, identified in Table 1

Peak No.	Formula	[M−H]⁻	Theoretical value	Identification	Error, Δm ppm	Main product ions, *m/z*
1	$C_{20}H_{30}O_3$	317.2107	317.2117	Steviol[a]	4.77	—
2	$C_{44}H_{70}O_{23}$	965.4231	965.4235	Rebaudioside E[a]	−0.43	—
3	$C_{50}H_{80}O_{28}$	1127.4785	1127.4763	Rebaudioside D[a]	−1.92	965, 856, 803, 641, 169
4	$C_{44}H_{70}O_{22}$	949.4306	949.4286	Rebaudioside C[a] or isomer	−2.11	641, 806, 479, 169
5	$C_{38}H_{60}O_{18}$	803.3712	803.3707	Stevioside or rebaudioside B isomer[a]	−0.64	641, 479, 317, 161
6	$C_{50}H_{80}O_{28}$	1127.4785	1127.4763	Rebaudioside D[a] or isomer	−1.92	965, 641, 169
7	$C_{44}H_{70}O_{23}$	965.4252	965.4235	Rebaudioside A[a]	−1.75	463, 441
8	$C_{38}H_{60}O_{18}$	803.3700	803.3701	Stevioside[a]	0.86	641, 479, 317, 161
9	$C_{44}H_{70}O_{23}$	965.4242	965.4235	Rebaudioside E or isomer[a]	−0.71	—
10	$C_{38}H_{60}O_{18}$	803.3712	803.3701	Rebaudioside B[a]	−0.64	641, 479, 169
11	$C_{43}H_{68}O_{22}$	935.4143	935.4124	Rebaudioside F[a]	−1.44	773, 611
12	$C_{44}H_{70}O_{22}$	949.4306	949.4286	Rebaudioside C[a]	−2.11	787, 625, 479
13	$C_{32}H_{50}O_{12}$	625.3218	625.3230	Esteviolbioside[a]	1.84	625, 641, 479, 317, 169
14	$C_{38}H_{60}O_{18}$	803.3700	803.3707	Stevioside or rebaudioside B[a]	0.86	641, 479
15	$C_{44}H_{70}O_{22}$	949.4280	949.4286	Rebaudioside C[a]	0.63	806, 787, 625
16	$C_{44}H_{70}O_{23}$	965.4230	963.4280	Rebaudioside A[a]	−0.53	463, 441
17	$C_{32}H_{50}O_{13}$	641.3169	641.3173	Steviobioside or rubusoside[a]	1.50	479, 317, 113
18	$C_{60}H_{102}O_{29}$	1285.8471	1285.6434	Mogroside *V*[b]	2.88	1123, 179, 961, 799
19	$C_{54}H_{92}O_{24}$	1123.5904	1123.5906	Mogroside IV[b]	0.16	961, 799, 641, 169
20	$C_{48}H_{82}O_{19}$	961.5369	961.5378	Mogroside III[b]	0.58	805, 641, 543, 169, 479
21	$C_{42}H_{72}O_{14}$	799.4845	799.4849	Mogroside II E[b]	0.54	641, 683, 521, 479
22	$C_{57}H_{94}O_{28}$	1225.5904	1225.5894	Trigoneoside XIIIa[c]	−0.82	1063, 901, 755, 89
23	$C_{51}H_{84}O_{23}$	1063.5371	1063.5331	Trigoneoside IVa[c]	−3.79	901, 755, 1047, 89, 119, 59, 101
24	$C_{51}H_{84}O_{22}$	1047.5375	1047.5381	Trigonelloside C[c]	0.62	901, 755, 89, 119, 101
25	$C_{45}H_{74}O_{18}$	901.4828	901.4837	Trigofoenoside A or Glycoside D[c]	−2.84	755, 89, 119, 59, 101
26	$C_{51}H_{82}O_{22}$	1045.5254	1045.5225	Graecunin E[c]	0.09	883, 737
27	$C_{45}H_{72}O_{17}$	883.4702	883.4697	Graecunin G[c]	−0.59	737, 293, 89, 119, 59, 101, 163, 179, 203
28	$C_{61}H_{94}O_{27}$	1257.5903	1257.5910	Foliatheasaponin III[c]	0.53	1091, 1125, 89, 191, 205, 247, 119
29	$C_{45}H_{72}O_{17}$	883.4705	883.4697	Graecunin G or isomer	−0.93	721, 575, 89, 119, 179, 205, 289

[a] From stevia leaf.

[b] From Luo Han Guo.

[c] From fenugreek.