Characterization of the Major Components in Different Green Tea Dietary Supplements using HPLC and Multivariate Statistical Analysis

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Abstract

Aim

Green tea dietary supplements are sold all over the world, and consumers are buying record amounts of green tea products every year. However, the qualities of the products are poorly controlled, which may not only vary the benefit but also hurt the customer. The purpose of this paper is to compare the contents of the major components in different Green tea supplements.

Main Methods

A normalized extraction is used to extract green tea products. UPLC-MS/MS and co-elution with standard compounds were employed to identify the major components. An HPLC method is used to quantify the identified components in different supplements. PCA and HCA were used to evaluate the variability of these products.

Key Findings

The quality of green tea supplements was determined based on the recommended daily dose. Epigallocatechin, epicatechin, caffeine, epicatechin, epigallocatechin-3-gallate, galloatechin gallate, and epicatechin-3-gallate were identified as the major components in 12 different green tea supplements purchased from local store or internet. Quantitative analysis results showed that the contents of these components were highly variable across products. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) analysis revealed the 12 products used in this study can be divided into four groups based on the contents of the major components per daily dose.

Significance

This study suggested that the quality of different green tea supplements are highly variable, which most likely could lead to variable biological effects. Standardization of green tea supplement is necessary to derive more consistent potential benefits.

Keywords: Green tea; Dietary supplement; Quality control; HCA; PCA.

Introduction

Green tea has been consumed for thousands of years in Asia and is one of the most popular beverage in the world [1,2]. In vivo/ vitro experimental studies, epidemiological studies, and clinical trials have established a positive correlation between green tea consumption with beneficial effects including immune-stimulatory, anti-inflammatory, and prevention of different diseases including cardiovascular diseases, neuro-degradation, diabetes etc [3,4,5,6,7,8]. Molecular pharmacology studies showed that the green tea components can exert benefit to health through multiple mechanisms, such as anti-oxidation, modulation of signal transduction pathways, modulation of cell survival/death genes [9,10,11].

Due to the potential benefits, many kinds of green tea dietary supplements are sold all over the world, and consumers are buying record amounts of green tea products every year. It is reported that green tea extract has gained popularity as the fourth most commonly used dietary supplement in the U.S. market in recent years [12]. In addition, green tea products are also widely used in biomedical studies and numerous papers are published every year. For example, there are 6241 hits in the Pubmed search using “green tea” as the key word.

Commercial green tea products usually claim to be "standardized" for levels of polyphenols or catechins. However, the manufacturing procedures of these products are not really standardized because dietary supplement is not strictly regulated by the FDA. Consumers may not always know that the quality of the green tea dietary supplements could be highly different. Similarly, results from different biomedical studies could be highly various due to the variability of the products because most of the biological studies never paid attention to the quality of the green tea products. In addition, the biological activity related components are particularly liable to oxidation. Consequently, these components may decompose during processing or storage of the dietary supplement.

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For example, the stability of EGCG, one of the major polyphenols in green tea, is well-known to degrade as a function of increasing pH and affected by the matrix including trace levels of transition metals [13]. It is of paramount importance to accumulate solid, systemic scientific evidence to establish a qualified standard for green tea supplements.

Polyphenolic compounds together with caffeine have been identified in green tea fresh leaf or beverage [14,15,16] and there are several reports showing that the actual content of catechins or polyphenols was not consistent with the label claims [17,18]. However, there are very few studies comparing the contents of polyphenols and caffeine across green tea dietary supplement based on the recommended daily dose. We think this comparison is very important because the amount that a consumer get from the products is based on not only the contents in the products but also the daily dose recommended by the manufacture. This amount will directly affect the biological effects including potential benefits and side effect. In this paper, we established a normalized extraction method based on the daily dose and evaluate the quality difference across 12 different green tea dietary supplements by multivariate statistical analysis.

Materials and Methods

Chemical and reagents

Twelve herbal supplements were bought from US local stores or internet website (table 1). Standard compounds eallocatechin (GC), epigallocatechin (EGC), caffeine, epicatechin (EC), epigallocatechin-3-gallate (EGCG), gallocatechin gallate (GCG), and epicatechin-3-gallate (ECG) were obtained from Sigma-Aldrich (St Laws, MO, USA); Solvents were LC-MS grade and purchased from VWR (Suwanee, GA, USA). Other chemicals were used as received. The dietary supplement products were randomly labeled as A to L and kept at 4 °C until analysis. The standard compounds were kept at -20 °C.

Instrumentation and condition

The HPLC conditions were as follows: Agilent 1050 with a 759 A absorbance detector (Applied Biosystem, US) detector running the ChemStation software; column, Dikma Diamonsil C18, 5 μm, 250 × 4.6 mm (Dikma Technologies, China); mobile phase A (MPA), acetonitrile, mobile phase B 0.1% formic acid in water; gradient, 0 - 15 min, 10 % MPA, 10 - 40 min 10 - 30 % MPA, 40 - 50 min 30 - 60 % MPA, 50 - 60 min 60 - 90% MPA, 60 - 65 min, 90 - 10% MPA, 65 - 70 min, 10% MPA; Detect wavelength, 230 nm; flow rate, 1 ml/mi; column temperature, 25 °C.

The UPLC-UV-MS/MS conditions were as follows: system, Waters Acqity™ with PDA detector; column, Acquity UPLC BEH C18 column (50 × 2.1 mm I.D. 1.7 μm, Waters, Milford, MA, USA); mobile phase A (MPA), 0.1% formic acid in water, mobile phase B (MPB), 100% acetonitrile, gradient, 0 - 0.5 min, 0 % MPB, 0.5 - 1.0min, 0 - 5 % MPB, 1.0 - 3.0 min, 5 - 10 % MPB, 3.0 - 4.0 min, 10 - 15 % MPB, 4.0 - 5.0 min, 18 % MPB, 5.0 - 6.0 min, 18 - 50 % MPB, 6.0 - 6.5 min, 50 - 95% MPB, 6.5 - 7.0 min 95 % MPB, 7.0 - 7.5 min, 95 - 0 % MPB, 7.5 – 8.0 min, 0 % MPB. An API 3200 QTRA triple quadruple mass spectrometer (Applied Biosystems /MDS SCIEX, CA, USA) equipped with a TurboIonspray source operating in negative or positive ion mode was used to perform the MS analysis. Ionspray voltage, -4.5 kV (+5.5 kV for caffeine analysis); ion source temperature, 400 °C; nebulizer gas (gas 1), nitrogen, 40 psi; turbo gas (gas 2), nitrogen 40 psi; curtain gas 20 psi; collision energy (CE) in MS2, -30 V (+30 V for caffeine analysis).

Sample Preparation

An amount equal to daily dose of each of the twelve green tea

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacture</th>
<th>Daily Dose (capsules/tablets)</th>
<th>Vendor</th>
<th>Lot number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>GNC Natural Brand</td>
<td>1</td>
<td>GNC</td>
<td>0989BH0014</td>
</tr>
<tr>
<td>B</td>
<td>GNC herbal plus</td>
<td>1</td>
<td>GNC</td>
<td>1875DH1990</td>
</tr>
<tr>
<td>C</td>
<td>Now</td>
<td>2</td>
<td>Internet (iherb.com)</td>
<td>4705</td>
</tr>
<tr>
<td>D</td>
<td>Safeway</td>
<td>1</td>
<td>Randalls</td>
<td>7DA0466</td>
</tr>
<tr>
<td>E</td>
<td>CVS pharmacy</td>
<td>1</td>
<td>CVS</td>
<td>9346203</td>
</tr>
<tr>
<td>F</td>
<td>Nature Made</td>
<td>4</td>
<td>Walgreen</td>
<td>RB11779</td>
</tr>
<tr>
<td>G</td>
<td>Nature’s Bounty</td>
<td>4</td>
<td>Walgreen</td>
<td>14484702</td>
</tr>
<tr>
<td>H</td>
<td>Kroger</td>
<td>1</td>
<td>Kroger</td>
<td>6PB0083</td>
</tr>
<tr>
<td>I</td>
<td>Natrol</td>
<td>1</td>
<td>Internet (Drugstore.com)</td>
<td>2031514</td>
</tr>
<tr>
<td>J</td>
<td>Whole health</td>
<td>4</td>
<td>Internet (wholehealthproduct.com)</td>
<td>70320-0</td>
</tr>
<tr>
<td>K</td>
<td>Sundown</td>
<td>4</td>
<td>Walgreen</td>
<td>010113362904</td>
</tr>
<tr>
<td>L</td>
<td>Puritan’s Pride</td>
<td>2</td>
<td>Internet (drugstore.com)</td>
<td>172428-01</td>
</tr>
</tbody>
</table>
products was suspended in equivalent volumes (30 ml) of methanol /H₂O = 1:1 (contained 0.1% of vitamin C and 0.01% of EDTA), covered by aluminum foil to avoid light exposure, and sonicated three times at low temperature (iced cold water), each time for a period of one hour. The supernatant was obtained following each sonication after centrifuge (6000 g × 15 min) and combined respectively to afford 12 extracts. The original extracts (1 ml of each) were diluted 10 times (40 times for products J and L) by 50 % methanol in water (contained 0.1% of vitamin C and 0.01% of EDTA), 200 µL of each of the diluted samples was injected in HPLC, and 10 µL was injected in UPLC-MS/MS.

Preparation of Standard Curves and Quality Control Sample for HPLC quantitative analysis

The standard curve for HPLC analysis was prepared in 50 % MeOH (contained 0.1% of vitamin C and 0.01% of EDTA) from the stock solution (10mg/ml, in DMSO/EtOH=1:4) of each of the individual standard compounds. Predetermined volumes of the stock solutions were mixed in 50% MeOH (contained 0.1% of vitamin C and 0.01% of EDTA), which was further diluted by the same solvent to afford the standard curve samples. The injection volume was 200 µL. Coumarin was also selected as internal standard. A 10 µl of 0.25 mM coumarin in methanol was added to 30 µl of samples.

Method Validation for HPLC Quantitative Analysis

Calibration curve: Calibration curves were prepared as described in section 2.4. The linearity of each calibration curve was determined by plotting the peak area ratio of analytes to I.S. Least-squares linear regression method was used to determine the slope, intercept and correlation coefficient of linear regression equation.

Precission and extraction reproducibility: The precisions for HPLC analysis were determined by injecting the sample from products A. The original extract of product A was diluted 10, 50, and 100 times by 50% of methanol (0.1% of Vc and 0.01% of EDTA) in water to obtain a high, medium, and low concentration for injection precision study. Peak areas of the major peaks were compared to determine the precision of HPLC quantitative analysis.

To determine the extraction reproducibility, three daily doses of product A, D, and K were extracted five times respectively according to the normal extraction procedure described previously. Samples were analyzed by HPLC. The relative peak areas of the six compounds at different concentration are compared and RSD was calculated to indicate the sample stability.

4.5.3 Stability: The stability was determined using sample from product A. The original extract were diluted by 50% of methanol (0.1% of Vc and 0.01% of EDTA) in water high (dilute 5 times), medium (dilute 10 times), and low concentration (dilute 100 times) and injected at day 1. The samples were kept at room temperature for 8 hours for the second injection. The relative peak areas of the identified compounds were compared to evaluate sample stability.

Data Analysis

Hierarchical cluster analysis: Hierarchical cluster analysis (HCA), a multivariate analysis approach, was employed to distinguish the similarity across different green tea dietary supplements. Ward's method as the amalgamation rule and the squared Euclidean distance were used to establish clusters. The HCA calculation was based on the contents of the major compounds identified in the green tea dietary supplements. SPSS 19.0 was used to perform the HCA cluster analyses.

Principal component analysis: Principal component analysis (PCA) was carried out on the contents of the major identified compounds in green tea dietary supplements. The data set was organized in a matrix with 12 lines corresponding to different products and 7 columns corresponding to the different compounds. The data was autoscaled (scaling by Pareto) and the PCA calculation was performed using Markerviewer® 1.2.1 (AB Sciex, CA, USA).

Results

Extraction method set up

Different extract solvents, including H₂O, 50 % MeOH in water, 100% MeOH, 50% DCM in MeOH, 100 % DCM, were evaluated to extract the products. The 50% MeOH was finally selected as extraction solvent because more peaks were observed in the HPLC analysis. For the extraction procedure, the extraction capability has no different between sonicated for 1 hour in ice cold water and shake overnight at room temperature. The 0.1% Vc and 0.01% EDTA in solvent and foil to avoid light exposure, which is considered to be a common way to prevent oxidation via free radicals, were considered to prevent the compounds from being oxidized during the extraction [19, 20].

Optimization of chromatographic conditions in HPLC analysis

Different solvents, including methanol, acetonitrile, formic acid in water, and ammonium acetate in water at different pH value were tested as mobile phases. To obtain the highest resolution and sensitivity, 0.1% formic acid in acetonitrile and 0.1% formic acid in water were selected as the mobile phases and the detection wave length was set at 230 nm. The shapes of the major peaks were symmetrical and were in the middle of the chromatogram (figure 1A) using this HPLC condition. Coumarin was used as internal standard because it is not detected in the products and eluted at the end of the chromatograph.

Twelve different green tea dietary supplements were extracted and analyzed by HPLC. Each of these products displayed a distinctive HPLC fingerprint chromatogram (figure 1A). Seven major peaks were observed in these chromatograms. The HPLC profiles were similar across these 12 products indicating that the UV-visible components contained in green tea products were essentially identical.
Identification of the major components in green tea dietary supplements

The major components were identified by UV spectra and MS/MS in the UPLC-UV-MS/MS analysis and confirmed by co-elution with standard compounds in LC-UV. In the UPLC-UV-MS analysis (figure 1B, table 3), the MS was detected at both positive and negative mode. In the negative ESM scan, an ion of m/z 441, further produced fragments ion at m/z 288.8, 169.1, 124.9 in MS² experiment, was assigned for ECG according to the references [21]. Similarly, the ion of m/z 289 with fragment ion of m/z 244.8, 205.3, 151.2, 49.1, 125.2 in MS² experiment was identified as EC.

Two peaks showed up for ion of m/z 457 at 3.18 and 3.39 min with fragment ions of 305, 169, 125 and two peaks at 1.10 and 2.29 min for ion of m/z 305 with fragment ions of m/z 221, 165, 125 were identified as EGCG, GCG and EGC, GC respectively by comparing the retention times with standard compounds. In the positive ESM scan, an ion of m/z 195 with fragment of 138, 110, and 69 was identified as caffeine [22]. To confirm the identification, standard compounds were added in the mixed sample (mix product A to L) and injected in HPLC. The chromatograms of before and after add in were compared. Thus, peaks 1-7 were clearly identified as GC (1), EGC (2), caffeine (3), EC (4), EGCG (5), GCG (6), and ECG (7) respectively (figure 2).

Method validation

The precision of the HPLC method was determined by randomly using extract of product A as the tested sample. The injection precision was determined by injecting the same sample six times in
the same day. The relative standard deviations (R.S.D.s) of the peak areas were lower than 15% suggesting that the precision of this HPLC method was in acceptable range (Table 2).

The repeatability of extraction was evaluated by five individual extractions of the product A, D, and K. The relative standard deviations (R.S.D.s) of retention time and relative peak areas of the seven identified peaks were lower than 15.0 % indicating that the extraction method was reproducible (Table 2).

The stability was determined by injecting samples prepared from product A at different times. The relative standard deviations (R.S.D.s) of peak areas were less than 15 % revealing that the sample was stable at the experimental condition (Table 2).

Quantification of the identified compounds in the products
The identified compounds in green tea dietary supplement were quantified using the validated HPLC method. The results showed that the contents of these identified peaks were highly variable (Table 3). The amount per daily dose varied from 11.7 to 87.7 mg (7.5 folds) for GC, from 11.4 to 259.0 mg (22.6-folds) for EGC, from 12.9 to 388.8 mg for caffeine, from 3.6 to 89.2 mg (24.6-folds), from 32.9 to 1090.6 mg (33-folds) for EGCG, from 4.5 to 70.1 mg (15.5-folds) for GCG, and from 7.1 to 280.9 mg (39.8-folds) for ECG. The total amount of polyphenols per daily dose ranged from 72.6 to 1810.6 mg (24.9-folds).

Data Analysis
Hierarchical Clustering Analysis (HCA): HCA is a common data analysis tool to assign a set of samples into groups by converting the observed data into statistical structures. The aim of HCA is to provide a better alternative for visual representation of high-dimensional data. HCA groups the analyte vectors according to their inter-vector spatial distances in their full dimensional vector space. Clusters are generated during the HCA calculation, which is correlated to the levels of dissimilarity: the smallest distance indicates the highest degree of similarity. A dendrogram is usually used to present the distance across sample clusters and the distance pattern allows the observation of sample profiles through simple interpretation.

In this study, the results of HCA showed that the 12 products were separated into four clusters according to their distance in the analysis, which indicates the similarity of the contents of those analytes. Cluster I contains products A and E, II contains products C and K, III contains products G, I, D, H, F, B, and IV contains products J and L (Figure 3A). The qualities of the green tea dietary supplements are similar within a group, while across groups, the qualities are various. For example, the qualities of product C and
The 12 green tea dietary supplements used in this study can be divided into four groups based on the contents of the major compounds (Figure 3). The qualities of the products are similar in the same group, however, across groups, the qualities are highly different. The high variability means that consumer cannot obtain the same amount of these compounds by taking different green tea products, even if they correctly followed the manufacturers’ recommendations. For example, by taking product A, a consumer will take 103.3 mg of total polyphenols, including 18.09 mg of GC, 26.29 mg of EGC, 6.51 mg of EC, 39.41 mg of EGCG, 4.51 mg of GCG, and 8.60 mg of ECG. However, if this consumer takes a product from product J, he/she will take 1810.58 mg (17.5-folds of product A) of total polyphenols including 26.29 mg of EGC, 6.51 mg of EC, 39.41 mg of EGCG, 4.51 mg of GCG, and 8.60 mg of ECG.

### Discussion

Twelve different green tea dietary supplements were obtained from the most popular local stores or internet (Table 1). A normalized extraction method, together with a stable and reproducible HPLC quantification method, is set up. The major peaks in the HPLC chromatogram are identified and the uniformity of the major components in these products is analyzed (Figure 2, Table 3). The results showed that the products used in this study can be divided into four groups based on the daily dose (Figure 3). In the same group, the amounts of the major components are similar but across group, the amounts are highly various. The variability across products could affect bioavailability and biological effects.

In this study, the total variance of the data explained by the PCA calculation was 94.1%, with 89.1% from PC1 and 5.7% from PC2. According to the PC1 scores, which indicates the similarity of the contents of these analytes, these products can be divided into four groups: group I, products A and E; group II, products B, D, H, G, F, I; group III, products C and K, and group IV, products L and J (Figure 3B). This grouping is identical to that of analysis by HCA. The loading plot (Figure 3C) suggested that the EGCG contributes the greatest influence on the scores and GC contributes the smallest influence. Caffeine, ECG, EGCG also affected the scores of the loading plot. Thus, the distinction of green tea dietary supplements should be evaluated by both polyphenols, especially EGCG, as well as caffeine.

### Principal component analysis (PCA)

PCA is a well-known exploratory data analysis approach to group the samples according to their qualities. The aim of PCA is to determine underlying information from multivariate data by transforming and reducing the dimensions of the original data matrix into two matrices, scores (T) and loadings (P). The results are presented in the forms of scores which shows the variability across samples, and loading which indicates the influence over the difference groups of samples. PC1 accounts for the greatest variance in the data, and other PCs indicate smaller variability of data.
## Table 3: Quantification and identification of the major compounds in different products (mg/daily dose)*

<table>
<thead>
<tr>
<th></th>
<th>GC</th>
<th>EGC</th>
<th>Caffeine</th>
<th>EC</th>
<th>EGC</th>
<th>GCG</th>
<th>ECG</th>
<th>Total polyphenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>18.09±0.91</td>
<td>26.19±2.06</td>
<td>16.07±1.19</td>
<td>6.50±0.48</td>
<td>39.41±1.65</td>
<td>4.51±0.57</td>
<td>8.60±0.34</td>
<td>103.32</td>
</tr>
<tr>
<td>B</td>
<td>21.06±107</td>
<td>82.08±4.14</td>
<td>58.22±0.99</td>
<td>24.13±0.43</td>
<td>39.33±9.74</td>
<td>6.61±0.29</td>
<td>74.34±1.80</td>
<td>547.55</td>
</tr>
<tr>
<td>C</td>
<td>47.38±3.48</td>
<td>118.43±7.97</td>
<td>63.07±1.73</td>
<td>49.86±2.10</td>
<td>461.54±15.45</td>
<td>55.12±3.83</td>
<td>102.12±2.29</td>
<td>834.40</td>
</tr>
<tr>
<td>D</td>
<td>14.97±0.41</td>
<td>43.10±2.87</td>
<td>119.40±10.19</td>
<td>24.72±1.95</td>
<td>284.20±13.57</td>
<td>18.47±1.92</td>
<td>86.32±5.02</td>
<td>471.83</td>
</tr>
<tr>
<td>E</td>
<td>11.73±0.32</td>
<td>11.44±0.15</td>
<td>12.90±0.22</td>
<td>3.62±0.10</td>
<td>32.89±1.37</td>
<td>5.81±0.79</td>
<td>7.05±0.38</td>
<td>72.57</td>
</tr>
<tr>
<td>F</td>
<td>36.21±3.04</td>
<td>71.07±3.60</td>
<td>120.10±9.03</td>
<td>32.25±2.64</td>
<td>288.21±17.29</td>
<td>26.34±1.54</td>
<td>87.10±8.06</td>
<td>541.23</td>
</tr>
<tr>
<td>G</td>
<td>20.07±1.34</td>
<td>38.10±1.53</td>
<td>86.54±4.55</td>
<td>12.89±0.42</td>
<td>167.07±8.90</td>
<td>15.76±0.42</td>
<td>42.59±1.87</td>
<td>296.50</td>
</tr>
<tr>
<td>H</td>
<td>16.77±0.79</td>
<td>41.59±2.90</td>
<td>116.29±6.62</td>
<td>18.19±0.90</td>
<td>266.52±13.47</td>
<td>16.65±0.65</td>
<td>68.07±3.15</td>
<td>427.79</td>
</tr>
<tr>
<td>I</td>
<td>28.56±1.24</td>
<td>76.46±5.60</td>
<td>114.94±7.34</td>
<td>24.33±1.63</td>
<td>188.05±12.34</td>
<td>15.11±0.33</td>
<td>50.79±3.48</td>
<td>383.30</td>
</tr>
<tr>
<td>J</td>
<td>87.72±6.35</td>
<td>258.96±22.63</td>
<td>388.79±25.50</td>
<td>89.10±5.93</td>
<td>1062.31±77.48</td>
<td>70.10±1.93</td>
<td>242.30±16.49</td>
<td>1810.58</td>
</tr>
<tr>
<td>K</td>
<td>48.33±3.65</td>
<td>120.15±8.67</td>
<td>202.91±10.55</td>
<td>42.42±2.67</td>
<td>392.42±28.74</td>
<td>41.42±1.68</td>
<td>104.08±7.80</td>
<td>748.82</td>
</tr>
<tr>
<td>L</td>
<td>37.04±1.04</td>
<td>101.20±8.97</td>
<td>171.92±8.20</td>
<td>75.60±6.17</td>
<td>1090.55±79.92</td>
<td>37.90±2.85</td>
<td>280.91±16.54</td>
<td>1623.20</td>
</tr>
</tbody>
</table>

| HPLC Retention time (min) | 9.23 | 16.24 | 18.56 | 26.2 | 28.25 | 30.9 | 37.21 |
| UPLC Retention time (min) | 1.52 | 2.23 | 2.76 | 3.66 | 3.83 | 4.49 | 5.68 |
| UV λmax (nm) | 205.2, 270.4 | 205.6, 270.4 | 200.3, 273.5 | 206.5, 272.4 | 220.0, 274.7 | 208.8, 274.1 | 221.0, 276.0 |
| ESI-MS ([M-H]) m/z | 305.3 | 305.1 | 195.2 ([M+H]+) | 289.1 | 457.2 | 456.7 | 441 |
| ESI-MS' m/z | 221.2, 165.0, 125.0 | 221.0, 165.3, 125.1 | 138.1, 110.1, 69.2 | 244.8, 205.3, 151.2, 125.2 | 331.4, 305.2, 169.3, 125.3 | 305.1, 168.8, 125.0 | 288.8, 169.1, 124.9 |

* Quantification data were based on three individual experiments
87.72 mg of GC (4.9-folds), 288.96 mg of EGC (9.9-folds), 89.19 mg of EC (13.7-folds), 1062.31 mg of EGCG (27.0-folds), 70.19 mg of GCG (15.5-folds), and 242.30 mg of ECG (28.2-folds) (table 3). Not only the amount but also the pattern of the major components can be highly different by taking different products.

The lack of quality control for green tea dietary supplement reveals that consumers will get different amounts of components by taking different products with the exact same product name. Consequently, the pharmacological effect, no matter is benefit or harm, could be different. For example, it is reported that the protection of hepatotoxicity from green tea extract followed dose dependent manner: a low and media dose (0.01% and 0.1% in drinking water), green tea extract can suppress serum AST (aspartate aminotransferase) and ALT (Alanine transaminase) levels induced by DSS (dextran sulfate sodium), however, this hepatic protection effect was not observed at high dose (1% in drinking water).
drinking water) [23]. Thus the pharmacological effect could be different by taking different products as the contents of the major potential benefit components are highly various across products (table 3, figure 3). Another concern is that the bioavailability of the components could be various due to the variability of the contents across different products. For example, we found in our previously study that the matrix of the dietary supplement could affect drug transporters (e.g., MRP2) [20]. The bioavailability of the components in the products could be most likely different by taking different products because polyphenol usually undergoes phase II metabolism and the metabolites are substrate of efflux transporters. Taken together standardization will be necessary to ensure safe and efficacious use of supplements such as green tea dietary supplement.

Conclusion
We conclude that standardization of herbal supplement is very important, as long as the ultimate goal is to make alternative herbal medicine such as green tea as an attractive alternative to the conventional Western medical care.

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References