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

Yang G1,2, Jian W1, Xu B1, Yin T1, Ma Y1, Hu M1,2 and Gao S1,2*

1 Hubei University of Medicine, 30 Remin South Road, Shiyan, China 420000
2Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, The University of Houston, 1441 Moursund Street, Houston, TX, 77030, USA

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 varies 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. Epicatechin, epigallocatechin, caffeine, epicatechin, epigallocatechin-3-gallate, gallocatechin 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 Asian 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 labile to oxidation. Consequently, these components may decompose during processing or storage of the dietary supplement.

Sub Date: August 10 2015, Acc Date: September 1 2015, Pub Date: September 2 2015

Citation: Gao S, Yang G, Jian W, Xu B, Yin T, et al. (2015) Characterization of the Major Components in Different Green Tea Dietary Supplements using HPLC and Multivariate Statistical Analysis. BAOJ Pharm Sci 1: 008.

Copyright: © 2015 Song Gao, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 C_{18}, 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/min; column temperature, 25 °C.

The UPLC-UV-MS/MS conditions were as follows: system, Waters Acquity™ with PDA detector; column, Acquity UPLC BEH C_{18} 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 - 1.0 min, 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 1. Manufactures of green tea dietary supplement

| Product | Manufacture          | Daily Dose (capsules/tablets) | Vendor                      | Lot number       |
|---------|----------------------|------------------------------|-----------------------------|------------------|
| A       | GNC Natural Brand    | 1                            | GNC                         | 0989BH0014       |
| B       | GNC herbal plus      | 1                            | GNC                         | 1875DH1990       |
| C       | Now                  | 2                            | Internet (iherb.com)        | 4705             |
| D       | Safewy               | 1                            | Randalls                    | 7DA0466          |
| E       | CVS pharmacy         | 1                            | CVS                         | 9346203          |
| F       | Nature Made          | 4                            | Walgreen                    | RB11779          |
| G       | Nature’s Bounty      | 4                            | Walgreen                    | 14484702         |
| H       | Kroger               | 1                            | Kroger                      | 6PB0083          |
| I       | Natrol               | 1                            | Internet (Drugstore.com)    | 2031514          |
| J       | Whole health         | 4                            | Internet (wholehealthproduct.com) | 7D320-0 |
| K       | Sundown              | 4                            | Walgreen                    | 010113362904     |
| L       | Puritan’s Pride       | 2                            | Internet (drugstore.com)    | 172428-01        |
products was suspended in equivalent volumes (30 ml) of methanol
\( /H_2O = 1:1 \) (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.

Precession 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 H_2O, 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 fragment ions at \( m/z \) 288.8, 169.1, 124.9 in MS\(^2\) 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 MS\(^2\) 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 precession 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...
K are similar as they are in the same group (Figure 3A), while the quality of product C is different with that of G, I, D, H, F, B, then different with A, E and highly different with J and K according to the distance in the dendrogram. This observation is understandable by analyzing the contents of the compounds in Table 3. The contents of totally polyphenols in product C (834.40 mg/daily) is only 1.1 folds of product K (748.82 mg/daily) but 0.5 folds of product J. For EGCG, product C is 1.1 folds of product K and 0.4-fold of product J. For product from product J, he/she will take 1810.58 mg (17.5-folds of GCG, and 8.60 mg of ECG. However, if this consumer takes a 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 K are similar as they are in the same group (Figure 3A), while the quality of product C is different with that of G, I, D, H, F, B, then different with A, E and highly different with J and K according to the distance in the dendrogram. This observation is understandable by analyzing the contents of the compounds in Table 3. The contents of totally polyphenols in product C (834.40 mg/daily) is only 1.1 folds of product K (748.82 mg/daily) but 0.5 folds of product J. For EGCG, product C is 1.1 folds of product K and 0.4-fold of product J. The HCA result clearly shows the similarity across products.

**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.

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 those 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, EG 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.

**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.

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 EGCG, 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 difference comparing with product A) of total polyphenols including...
Table 3: Quantification and identification of the major compounds in different products (mg/daily dose)*

|    | GC   | EGC  | Caffeine | EC   | EGCG | GCG  | ECG  | Total polyphenols |
|----|------|------|----------|------|------|------|------|------------------|
| A  | 18.09±0.91 | 26.19±2.06 | 16.07±1.19 | 6.50±0.48 | 39.41±1.65 | 4.51±0.57 | 8.60±0.34 | 103.32 |
| B  | 21.06±107 | 82.08±4.14 | 58.22±0.99 | 24.13±0.43 | 339.33±9.74 | 6.61±0.29 | 74.34±1.80 | 547.55 |
| C  | 47.38±3.48 | 118.43±7.97 | 63.07±1.73 | 49.86±2.10 | 461.54±15.45 | 55.12±3.83 | 102.12±2.29 | 834.40 |
| D  | 14.97±0.41 | 43.10±2.87 | 119.40±10.19 | 24.72±1.95 | 284.20±13.57 | 18.47±1.92 | 86.32±5.02 | 471.83 |
| E  | 11.73±0.32 | 11.44±0.15 | 12.90±0.22 | 3.62±0.10 | 32.89±1.37 | 5.81±0.79 | 7.05±0.38 | 72.57 |
| F  | 36.21±3.04 | 71.07±3.60 | 120.10±9.03 | 32.25±2.64 | 288.21±17.29 | 26.34±1.54 | 87.10±8.06 | 541.23 |
| G  | 20.07±1.34 | 38.10±1.53 | 86.54±4.55 | 12.89±0.42 | 167.07±8.90 | 15.76±0.42 | 42.59±1.87 | 296.50 |
| H  | 16.77±0.79 | 41.59±2.90 | 116.29±6.62 | 18.19±0.90 | 266.52±13.47 | 16.65±0.65 | 68.07±3.15 | 427.79 |
| I  | 28.56±1.24 | 76.46±5.60 | 114.94±7.34 | 24.33±1.63 | 188.05±12.34 | 15.11±0.33 | 50.79±3.48 | 383.30 |
| J  | 87.72±6.35 | 258.96±22.63 | 388.79±25.50 | 89.10±5.93 | 1062.31±77.48 | 70.10±1.93 | 242.30±16.49 | 1810.58 |
| K  | 48.33±3.65 | 120.15±8.67 | 202.91±10.55 | 42.42±2.67 | 392.42±28.74 | 41.42±1.68 | 104.08±7.80 | 748.82 |
| L  | 37.04±1.04 | 101.20±8.97 | 171.92±8.20 | 75.60±6.17 | 1090.55±79.92 | 37.90±2.85 | 280.91±16.54 | 1623.20 |

|    | HPLC Retention time (min) | UPLC Retention time (min) | UV λmax (nm) | ESI-MS ([M-H]- m/z) | ESI-MS2 m/z |
|----|--------------------------|--------------------------|-------------|-------------------|------------|
| A  | 9.23                     | 1.52                     | 205.2, 270.4 | 305.3             | 221.2, 165.0, 125.0 |
| B  | 16.24                    | 2.23                     | 205.6, 270.4 | 305.1             | 221.0, 165.3, 125.1 |
| C  | 18.56                    | 2.76                     | 200.3, 273.5 | 195.2 ([M+H]+)    | 138.1, 110.1, 69.2 |
| D  | 26.2                     | 3.66                     | 206.5, 272.4 | 289.1             | 244.8, 205.3, 151.2, 125.2 |
| E  | 28.25                    | 3.83                     | 222.0, 274.7 | 457.2             | 331.4, 305.2, 169.3, 125.3 |
| F  | 30.9                     | 4.49                     | 208.8, 274.1 | 456.7             | 305.1, 168.8, 125.0 |
| G  | 37.21                    | 5.68                     | 221.0, 276.0 | 441               | 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

Figure 3: Dendrogram of 12 different green tea dietary supplements calculated by HCA based on the contents of the major compounds (A). PCA score plot (B) and Loading plots (C) of the 12 different green tea dietary supplements based on the contents of the major compounds.
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.

Acknowledgement

Funding for the study was provided by The Gustavus and Louise Pfeiffer Research Foundation to MH and by a training grant from Hubei University of Medicine to GYY.

References

1. Hasan M, Siddiqui F, Al- Ajmi M (2008) FNA diagnosis of adrenal myelolipoma : a rare entity. Cytopathol Diagn 36: 925-926.
2. Page DL, DeLellis RA, Hough AJ (1986) Tumours of the adrenal. Atlas of tumor pathology, 2nd series, Fascicle 23. Washington: Forces Institute of Pathology.
3. Meaglia JP, Schmidt JD (1992) Natural history of an adrenal myelolipoma. J Urol Apr 147 (4): 1089-1090.
4. Pareja Megía MJ, Barrero Candau R, Medina Pérez M, Valero Puerta JA (2005) Giant adrenal myelolipoma. Arch Esp Urol 58: 362-365.
5. Ide H, Terado Y, Nakagawa T, Saito K, Kamiyama Y, et al. (2007) Incidentally discovered adrenal myelolipoma Associated with hyperthyroidism. Int J Clin Oncol 12: 379-81.
6. Hans Selye, Helen Stone (1950) Hormonally Induced into Myeloid Transformation Of Adrenal Tissue. Am J Pathol. March 26 (2): 211-233.
7. Chang KC, Chen PI, Huang ZH, Lin YM, Kuo PL (2002) Adrenal myelolipoma with translocation (3; 21) (q25, p11). Cancer Genet Cytogenet 134: 77-80.
8. Hsu SW, Shu K, Lee WC, Cheng YT, Chiang PH (2012) Adrenal myelolipoma: A 10 -year single -center experience and literature review. Kaohsiung Journal of Medical Sciences 28: 377-382.
9. García PX, Your BA, Faba RO, Ruiz GI, Palou RJ, Villavicencio H (2007) Mavrich. Mielolipoma extraadrenal perirenal: aportación de un caso y revisión de la literatura. Actas Urol Esp 31 (8): 932-934.
10. Goldman HB, Howard RC, Patterson Al (1996) Spontaneous retroperitoneal hemorrhage from giant adrenal myelolipoma. J Urol 155: 639-644.
11. Alexopoulos E, Kirmizis E, Visvards G, Grollios G, Leontsini M, et al. (2003) Focal Segmental Glomerulosclerosis in a Patient wit Large Bilateral Asymptomatic Adrenal Myelolipomas. Ren Fail 25: 1051-1056.
12. Banik S, Hasleton PS, Lyon RL (1984) An unusual variant of multiple endocrine neoplasia syndrome: a case report. Histopathology 8: 135-144.
13. Tamidari H, Mishra AK, Gupta S, Agarwal A (2006) Catecholamine secreting adrenal myelolipoma. Indian J Med Sci 60: 331-333.
14. Butoria N, Guya F, Collin F, Benet C, Causseret S, et al. (2012) Retroperitoneal extra- adrenal myelolipoma: Appearance in CT and MRI. Diagnostic and Interventional Imaging 93: 204-207.
15. Vara Castrodeza A, Madrigal Rubiales B, Veiga González M, Cuesta Varela F, Sales Fernández C, et al. (2005) Adrenal gland myelolipoma : radiological view. Arch Esp Urol (1): 73-76.
16. Meyer A, Behrend M (2005) Presentation and therapy of myelolipoma. Int J Urol 12: 239-43.
17. Lopez Martin L, Garcia Cardoso JV, Gomez Munoz J, Gonzalez Enguita C (2010) Mielolipoma suprarrenal. Aportación de un caso y revisión de la literatura. Arch Esp Urol, 63 (10): 880-883.
18. Castillo OA, Vitagliano G, Cortes O, Sanchez -Salas R, Arellano L (2007) Laparoscopic adrenalectomy for adrenal myelolipoma. Arch Esp Urol 60 (1): 217-221.