Evaluation on quality consistency of *Ganoderma lucidum* dietary supplements collected in the United States

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*Ganoderma lucidum* is a well-known medicinal mushroom. At present, numerous *G. lucidum* products have emerged in the form of dietary supplements in the United States due to its various benefits. However, the quality consistency of these products based on their label ingredients has seldom been evaluated due to the lack of a suitable toolkit. In this study, 19 batches of products of *G. lucidum* (Red Reishi, Reishi), herbal/mushroom supplements purchased in the United States, were evaluated based on their bioactive components including triterpenes and polysaccharides by using chromatographic methods and saccharide mapping. The results showed that the measured ingredients of only 5 tested samples (26.3%) were in accordance with their labels, which suggested the quality consistency of *G. lucidum* dietary supplements in the U.S. market was poor, which should be carefully investigated.

Dietary supplements are consumed with intent to derive important health benefits. An estimated one hundred millions of Americans spend more than $28 billion on dietary supplements every year¹. However, under Dietary Supplements Health and Education Act of 1994 (DSHEA), quality of dietary supplements is not evaluated by the U.S. Food and Drug Administration (FDA)¹,². Both industry and FDA acknowledge that many products have been introduced into the market without any safety assessment¹. Alarmingly, hundreds of products marketed as supplements have been found spiked with illicit substances not listed on the products' labels, risking serious injury or even death³. Indeed, within the first three months of 2015, FDA warned about or recalled over 30 tainted sexual enhancement products containing prescription erectile-dysfunction drugs or related synthetic analogues⁴. In February 2015, the New York State Attorney General compelled major retailers – Walgreens, Walmart, Target, and GNC – to halt sales of certain herbal supplements, after deoxyribonucleic acid (DNA) barcoding results failed to detect DNA from the botanical materials listed on the label of ~80% tested products⁵. Though the absence of DNA might be an artifact caused by its destruction during the manufacturing process, it still triggered a great concern on the quality of dietary supplements in the United States. Usually, chemical characters of herbal supplements are more stable than their DNA sequences during different manufacturing processes. Therefore, taking chemical insights into the quality of herbal/mushroom supplements should be more reasonable.

*Ganoderma lucidum*, known as “Lingzhi” in China or “Reishi” in Japan, is a well-known medicinal mushroom and traditional Chinese medicine, which has been used for the prevention and treatment of a variety of diseases such as bronchitis, allergies, hepatitis, immunological disorders, and cancer⁶,⁷. Due to its various benefits in protecting human health, numerous *G. lucidum* products have emerged in the form of dietary supplements in the United States, and it is also listed in the Dietary Supplements and Herbal Medicines of USP. However, their quality consistency to label ingredients has seldom been evaluated due to the lack of a suitable toolkit to audit ingredient and adulterants. Usually, triterpenes and polysaccharides are considered as the main bioactive components in *G. lucidum* fruiting body⁷,⁸, which have been used as markers for *G. lucidum* officially recorded in Chinese Pharmacopoeia (2015) due to their anti-cancer and immunomodulatory activities.

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Figure 1. HPTLC fingerprints of triterpenes in *G. lucidum* dietary supplements. (A) Photographed under white light; (B) photographed under UV 365 nm; Sample codes were the same as in Table 1.

| Codes | Lot.            | Monosaccharides and molar ratios* | Label ingredients | Measured ingredients |
|-------|-----------------|----------------------------------|-------------------|----------------------|
|       |                 | Ara     Fuc   Man    Glc   Gal |                   | Triterpenes | Polysaccharides |
| GL01  | X005            | —       2.4     4.0    100.0 2.6 | Fruiting body     | +/+/−d    +/+/−   |
| GL02  | Not clear       | —       —       —      100.0 4.0 | 27% polysaccharides +6% triterpenes | +/+      +/−−a  |
| GL03  | 1501901         | —       —       1.0    —      — | Water extract     | −/−−      +/−−   |
| GL04  | MD14352A        | —       —       —      1.0    — | 13.5% polysaccharide +6% triterpenes | +/+      +/−−   |
| GL05  | J0203           | 1.4     2.1     —      100.0 1.1 | Fruiting body     | +/−       +/−−   |
| GL06  | 250913-GLS      | 1.1     2.5     —      100.0  — | Fruiting body     | +/−       +/−−   |
| GL07  | 20027414        | —       —       —      1.0    — | 10% polysaccharide | −/−       +/−   |
| GL08  | 17365/4         | —       —       —      1.0    — | Fruiting body     | +/−       +/−−   |
| GL09  | REV-1.12        | —       8.9     3.8    100.0 7.0 | Water extract: ethanol extract = 2:1 | +/+      +/−   |
| GL10  | 250913-GLS      | —       —       1.0    —      — | Fruiting body     | +/−       +/−−   |
| GL11  | 142786          | —       —       1.0    —      — | Fruiting body +10% polysaccharides | +/−      +/−−   |
| GL12  | ZFPGI11110-WE   | —       —       —      100.0 1.1 | Reshi mushroom extract | ±/±+/−  ±/±/−  |
| GL13  | QYZG11403-St    | 8.5     8.5     100.0  48.0 | Reshi mushroom extract | ±/±      ±/±/±   |
| GL14  | QYZG11311-St    | 11.0    10.0    100.0  55.0 | Reshi mushroom extract | ±/+      ±/±+   |
| GL15  | XTYGI1309-St    | 3.1     4.6     100.0  11.0 | Reshi mushroom extract | ±/+      ±/±+   |
| GL16  | AMGSGI          | —       —       1.0    —      — | Reshi mushroom water extract | −/−      +/−−   |
| GL17  | FPHDG1-1114     | —       —       1.0    —      — | Reshi mycelia      | ±/−       ±/−−   |
| GL18  | PRMUSHPW16150126| —       —       1.0    —      — | Fruiting body      | +/−      +/−−   |
| GL19  | 411346          | —       —       1.0    —      — | Reshi mushroom extract | ±/−      ±/−−   |
| GL20  | —               | 2.3     4.6     100.0  12.2 | Authenticated material | +/+      +/−−   |

Table 1. Compositional monosaccharides of polysaccharides in *G. lucidum* dietary supplements and their measured ingredients. *The data was presented as average of two determinations, their relative average deviation was less than 4%; bNot detected; c labeled (+) or unlabeled (+) ingredients; d detected (+) or un-detected (−); t uncertain (±).
In this study, the quality consistency to their label of 19 batches of *Ganoderma lucidum* (Reishi, Lingzhi) dietary supplements purchased in the United States was evaluated using a reliable and scientific toolkit including colorimetric assay, high performance thin layer chromatography (HPTLC), gas chromatography coupled with mass spectrometry (GC-MS), saccharide mapping based on polysaccharide analysis using carbohydrate gel electrophoresis (PACE), and high performance size exclusion chromatography coupled with multi angle laser light scattering and refractive index detector (HPSEC-MALLS-RID) method.

**Results and Discussion**

**HPTLC fingerprints of triterpenes in *G. lucidum* dietary supplements.** HPTLC is a simple and rapid technique for the routine quality control of herbal medicines, which provides picture-like chromatograms with special colors. Indeed, HPTLC fingerprints of triterpenes in fruiting body of *G. lucidum* collected from different regions of China were roughly consistent, which exhibited with pink or red bands under white light after being colorized. Therefore, an authenticated fruiting body of *G. lucidum* (GL20) was used as the reference material. Figure 1 showed the HPTLC fingerprints of ethanol extracts from *G. lucidum* dietary supplements under white light and ultraviolet (UV) 365 nm, respectively. The results showed that HPTLC fingerprints of triterpenes (pink/red bands in Fig. 1) in *G. lucidum* dietary supplements (GL01-GL19) produced by different manufacturers were remarkably different, and triterpenes closely related to *G. lucidum* (GL20) were only detected in 8 out of 19 (42.1%) tested products based on HPTLC analysis.

**Colorimetric assay of polysaccharides in *G. lucidum* dietary supplements.** Starch-like polysaccharides, absent in fruiting body of *G. lucidum*, are frequently employed as excipients, or neutral fillers in dietary supplements. Therefore, in order to identify the presence of starch or malto-dextrin in the dietary supplements, the colorimetric assay with iodine-potassium iodide (I₂-KI) reagent was performed. Starch or malto-dextrin should be excluded for the determination of polysaccharides from *G. lucidum* dietary supplements. As shown in Fig. 2, starch-like polysaccharides were detected in 13 out of 19 (68.4%) tested products before α-amylase digestion rather than *G. lucidum* (GL20). Indeed, after the treatment with α-amylase, the positive response of starch-like polysaccharides in these products to I₂-KI reagent was disappeared (Fig. 2B), which further confirmed that starch-like polysaccharides were present in *G. lucidum* dietary supplements.

**Saccharide mapping of polysaccharides in *G. lucidum* dietary supplements.** Generally, the major bioactive polysaccharide in *G. lucidum* is known as branched 1,3-β-D-glucan, a specific polysaccharide with immunostimulatory and anti-tumor activities. Indeed, PACE fingerprints of 1,3-β-D-glucanase digested fingerprints of polysaccharides in *G. lucidum* collected from different regions of China were similar. Saccharide
mapping based on PACE analysis also had advantages of high resolution and high sensitivity. Therefore, 1,3-β-D-glucanase and α-amylase were selected for enzymatic digestion of polysaccharides from *G. lucidum* dietary supplements, then the enzymatic hydrolysates were analyzed by using saccharide mapping based on PACE analysis. Figure 3A showed the PACE fingerprints of α-amylase digested polysaccharides from *G. lucidum* dietary supplements. The results showed that the positive response on α-amylase was found in most tested samples, which further confirmed that starch-like polysaccharides existed in *G. lucidum* dietary supplements.

Furthermore, Fig. 3B showed the PACE fingerprints of 1,3-β-D-glucanase digested polysaccharides in *G. lucidum* dietary supplements, which indicated that PACE fingerprints of 1,3-β-D-glucanase digested polysaccharides from GL01, GL09, GL13, GL14, and GL15 were greatly similar to that of *G. lucidum* (GL20). However, other tested samples were obviously different from that of GL20. Hierarchical cluster analysis was further performed based on the digital scanning chromatograms of PACE fingerprints of 1,3-β-D-glucanase digested polysaccharides (Fig. 4). As shown in Fig. 4B, dietary supplements including GL01, GL09, GL13, GL14, and GL15, together with GL20 could be clustered into the same group (group 1), which confirmed that polysaccharides in these samples were greatly similar.
similar. The data indicated that branched 1,3-β-D-glucan, a specific polysaccharide with immunostimulatory and anti-tumor activities in *G. lucidum*, was only detected in 5 out of 19 (26.3%) tested products.

**GC-MS fingerprints of polysaccharides in *G. lucidum* dietary supplements.** Fingerprints based on compositional monosaccharides have been successfully performed for the quality control of polysaccharides from *Ganoderma*13-15, which demonstrated that compositional monosaccharides in fruiting body of *G. lucidum* collected from different regions of China were similar. Indeed, GC-MS is an excellent technique for the analysis of monosaccharides, which has several advantages such as high resolution and high sensitivity16. Therefore, GC-MS fingerprints based on compositional monosaccharides were also used for the authentication of *G. lucidum* dietary supplements. Figure 5 showed GC-MS fingerprints of compositional monosaccharides of polysaccharides in *G. lucidum* dietary supplements. The results showed the compositional monosaccharides of polysaccharides in *G. lucidum* (GL20) were fucose (Fuc), mannose (Man), glucose (Glc), and galactose (Gal) with the molar ratios of 19.2: 4.6: 100.0: 12.2, which were in accordance with the previous study17. Indeed, GC-MS analysis showed the types of compositional monosaccharides of polysaccharides in *G. lucidum* dietary supplements including GL01, GL09, GL13, GL14, and GL15 were similar to those of GL20 (Fig. 5 and Table 1), which suggested that *G. lucidum* polysaccharides could be found in these samples. However, types of compositional monosaccharides of polysaccharides in other *G. lucidum* dietary supplements (namely, GL02 to GL08, GL10 to GL12, and GL16 to GL19) were obviously different from those of *G. lucidum* (Fig. 5 and Table 1), which indicated that *G. lucidum* polysaccharides were not detected in these tested products. Therefore, GC-MS results further supported that *G. lucidum* polysaccharides were only found in 5 out of 19 (26.3%) tested products, which were in accordance with the results from saccharide mapping based on PACE analysis.

**Molecular weights and contents of polysaccharides in *G. lucidum* dietary supplements.** HPSEC-MALLS-RID based on dn/dc method has been proven as a powerful and efficient technique for the determination of molecular weights and contents of polysaccharides and their fractions from herbal medicines18. Indeed, HPSEC chromatograms and molecular weights of polysaccharides in *G. lucidum* collected from different regions of China were similar19. Therefore, polysaccharides from one batch of *G. lucidum* could be used as reference standards. In order to exclude the interference from the presence of additives such as starch or malto-dextrin, polysaccharides were treated with α-amylase before HPSEC-MALLS-RID analysis based on dn/dc. Figure 6 showed the HPSEC chromatograms of polysaccharides from tested products before and after α-amylase digestion. The results confirmed that α-amylase could digest all polysaccharides into small sugars in most of tested samples. However, the tested samples of GL01, GL02, GL09, GL13, GL14, and GL15 had negative response to α-amylase, which were in accordance with the results from colorimetric assay with I2-KI reagent. Indeed, HPSEC chromatograms of polysaccharides in GL01, GL09, GL13, GL14, and GL15 were similar to that of *G. lucidum* (GL20) (Fig. 6). Due to the relatively poor resolution of SEC and co-elution of various small molecules, *M*<sub>n</sub> of peak 3 could not be accurately measured. Actually, peak 3 should be almost α-amylase hydrolysisates of starch-like polysaccharides. Therefore, the molecular weights and contents of different fractions (peaks 1 and 2) were determined and calculated. The data showed that the molecular weights of polysaccharide in *G. lucidum* (GL20) were 1.052 × 10<sup>6</sup> Da (peak 1) and 5.04 × 10<sup>4</sup> Da (peak 2), respectively. Indeed, most anti-tumor β-glucans reported in *Ganoderma* contained the fraction with an average molecular weight of about 1.0 × 10<sup>4</sup> Da. Therefore, peak 1 could be considered as a quality marker in *G. lucidum*, which could be found in tested samples of GL01, GL09, GL13, GL14, and GL15. Their varied molecular weights might be attributed to different preparation processes of manufacturers. However, although peak 1 was also found in tested samples of GL02, GL03, GL12, and GL19, their compositional monosaccharides and PACE fingerprints of 1,3-β-D-glucanase hydrolysates were significantly different from those of *G. lucidum* (Table 2).

In summary, triterpenes and polysaccharides are officially considered as major active components in *G. lucidum*. Therefore, it is very important to determine whether the products contain triterpenes and polysaccharides derived from *G. lucidum*. Due to both triterpenes and polysaccharides can be found in *G. lucidum*, its products should, at least, have chemical characters of either triterpenes or polysaccharides based on their ethanol or water extract and their obvious polarity variations. Quality consistency of the dietary supplements could be considered as desirable if their detected ingredients were in accordance with their labels. Unfortunately, the measured ingredients, only in 5 out of 19 (26.3%) tested samples, were in accordance with their labels according to the results mentioned above (Table 1), which was similar to the data of DNA barcoding test20. Although only 19 batches of products were collected and analyzed, they almost represented the *G. lucidum* dietary supplements available in USA market. Actually, ten batches are enough for evaluating the quality consistency of tested sample (China Pharmacopoeia 2015). Therefore, this study suggested the quality consistency of *G. lucidum* dietary supplements collected in USA was extremely poor, which should be carefully investigated. Furthermore, the results also suggested that saccharide mapping based on PACE analysis, and HPSEC-MALLS-RID based on dn/dc had great potential for routine quality evaluation of polysaccharides from dietary supplements.

**Materials and Methods**

**Materials and chemicals.** Nineteen batches (GL01 to GL19) of *G. lucidum* dietary supplements were purchased, in February or March 2015, directly from general e-commerce sites such as Amazon.com and eBay Inc. in the United States, and one batch of authenticated fruiting body of *G. lucidum* (GL20) was collected from Shandong Province of China (Table 1). Identity of *G. lucidum* fruiting body was confirmed by Professor Xiaolan Mao, Institute of Microbiology, China Academy of Sciences. The voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

D-glucose, α-amylase, starch (ST), and acetic anhydride were purchased from Sigma (St. Louis, MO, USA). Laminariobiose (DP2), laminaritriose (DP3), and laminartetraose (DP4), β-1,3-D-glucan (GN), and...
β-1,3-D-glucanase were purchased from Megazyme (Wicklow, Ireland), and 8-aminonaphthalene-1,3,6-trisulphonic acid (ANTS) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Silica gel 60 F254 TLC plates were obtained from Merck (Merck, Darmstadt, Germany). Polyacrylamide containing acrylamide/N, N-methylenebisacrylamide (19:1, w/w) was obtained from Bio-Rad (Hercules, CA, USA). Deionized water was prepared by a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA). All the other reagents were of analytical grade.

Figure 5. GC-MS profiles of methylated hydrolysates of polysaccharides from *G. lucidum* dietary supplements. Ara, arabinose; Fuc, fucose; Man, mannose; Glc, Glucose; Gal, Galactose; IS, internal standard. Sample codes were the same as in Table 1.
Preparation of triterpenes and polysaccharides. Powder of each sample (1.0 g) was immersed in 20.0 mL of ethanol and refluxed in a Syncore parallel reactor (Büchi, Flawil, Switzerland) for 30 min at 78 °C according to previous report [10]. Then the extract was centrifuged at 4,000 \( \times \) g for 10 min (Allegra X-15R centrifuge; Beckman Coulter, Fullerton, CA, USA). The supernatant was evaporated to dryness under vacuum using rotary evaporator (Büchi, Flawil, Switzerland), and the residue was dissolved in 2.0 mL of methanol. After filtration through a 0.22 \( \mu \)m membrane filter, the extract was used for HPTLC analysis. Finally, ethanol extracted residues were dried under vacuum at 45 °C and further used for polysaccharides preparation.

Microwave assisted extraction was used for polysaccharides extraction according to a previously reported method with modification [12]. Briefly, dried ethanol extracted residues (~1.0 g) were suspended in 25.0 mL of...
deionized water and extracted with microwave assisted extraction (Multiwave 3000, Anton paar GmbH, Graz, Austria). The microwave irradiation program was performed at 900 W and 90 °C for 7 min. Then the extract was centrifuged, and the supernatant (~25.0 mL) was evaporated to about 10.0 mL under vacuum using rotary evaporator. Then three times of solution volume of ethanol (95%, w/v) were added for the precipitation of crude polysaccharides. After the solution was kept at 4 °C for 12 h, centrifugation (4,500 × g for 15 min) was performed. The supernatant was collected and the powder of the supernatant was obtained by freeze-drying.

**HPTLC analysis of triterpenes.** Determination of triterpenes in *G. lucidum* and its dietary supplements was performed according to a previously reported method with minor modification. Briefly, all samples (5 μL) were applied on a 20 × 10 cm silica TLC plate with an AS30 HPTLC Applicator (Desaga GmbH, Germany). The bands, at 10 mm from the bottom edge, were 8 mm wide and 13 mm between two bands. The plate was developed to a distance of 90 mm with dichloromethane/ethyl acetate/petroleum ether/formic acid/ethanol, 8:3:9:0.8:0.5 (v/v/v/v/v) as mobile phase at room temperature. Finally, the developed plates were colorized with 10% (v/v) H2SO4 in ethanol, and heated at 110 °C for 10 min on a YOKO-XR plate heater (Wuhan YOKO technology Ltd., China). Then the plate was covered with transparent glass and photographed under white light and UV 365 nm, respectively.

**Analysis of polysaccharides.** Colorimetric assay with I2-KI reagent. All samples (5 mg/mL, 200 μL) were mixed with 20 μL of I2-KI reagent, and then photographed under white light. Water and soluble starch were used as negative and positive control, respectively. Furthermore, all samples (5 mg/mL, 200 μL) were treated with α-amylase at a final concentration of 20 U/mL, and digested overnight (12 h) at 40 °C. Then, colorimetric assay was also performed.

**Saccharide mapping based on PACE analysis.** Saccharide mapping based on PACE analysis was performed according to the previous method with minor modification. Briefly, polysaccharides of each sample (30.0 mg) were dissolved in 5.0 mL of hot water (60 °C). Then the compounds with molecular weights less than 3 kDa were removed by centrifugation (4000 × g, 25 min) with an ultra centrifugal filter (molecular weight cutoff: 3 kDa, Millipore, Billerica, MA, USA) for seven times. The polysaccharide solution (1.0 mL) of each sample was mixed with α-amylase and 1,3-β-D-glucanase (the final concentration of 20.0 U/mL and 2.0 U/mL, respectively), and digested overnight (12 h) at 40 °C. The hydrolysates were dried, and then used for the derivatization with ANTS for PACE analysis. Polysaccharide solutions without enzymatic digestion were used as blank control after the treatment as described above. Reference polysaccharides including 1,3-β-D-glucan (5 mg/mL, 100 μL) and starch (5 mg/mL, 100 μL) treated with the corresponding enzymes, respectively, were used as positive control.

| Code  | Peak 1 | Peak 2 |
|-------|--------|--------|
|       | Mw × 10^3 kDa | Mw/Mn | Content (%) |
| GL01  | 0.232 | 1.4 | 1.39 | 0.218 | 1.2 | 5.56 | 6.95 |
| GL02  | 0.096 | 1.3 | 4.42 | 0.303 | 1.1 | 15.88 | 20.30 |
| GL03  | 2.740 | 2.5 | 0.62 | 0.256 | 1.8 | 13.10 | 13.72 |
| GL04  | ND     | ND   | 0    | 0.725 | 1.2 | 1.48 | 1.48 |
| GL05  | ND     | ND   | 0    | 0.950 | 1.4 | 0.70 | 0.70 |
| GL06  | ND     | ND   | 0    | 1.139 | 1.3 | 0.56 | 0.56 |
| GL07  | ND     | ND   | 0    | 1.201 | 1.3 | 1.33 | 1.33 |
| GL08  | ND     | ND   | 0    | 0.922 | 1.1 | 0.12 | 0.12 |
| GL09  | 1.872 | 2.1 | 0.93 | 1.351 | 1.1 | 1.88 | 2.81 |
| GL10  | ND     | ND   | 0    | 0.899 | 1.1 | 0.50 | 0.50 |
| GL11  | ND     | ND   | 0    | 1.051 | 1.6 | 0.69 | 0.69 |
| GL12  | 0.881 | 2.2 | 1.09 | 0.476 | 1.1 | 6.67 | 7.84 |
| GL13  | 0.196 | 1.3 | 1.21 | 0.257 | 1.1 | 1.84 | 3.05 |
| GL14  | 0.502 | 1.4 | 1.40 | 0.388 | 1.1 | 2.09 | 3.49 |
| GL15  | 1.375 | 1.2 | 0.70 | 0.654 | 1.5 | 7.58 | 8.28 |
| GL16  | ND     | ND   | 0    | 0.407 | 1.4 | 1.20 | 1.20 |
| GL17  | ND     | ND   | 0    | 0.291 | 1.5 | 0.29 | 0.29 |
| GL18  | ND     | ND   | 0    | 0.347 | 2.4 | 0.72 | 0.72 |
| GL19  | 2.297 | 1.6 | 0.55 | 0.228 | 1.9 | 13.04 | 13.59 |
| GL20  | 2.105 | 1.8 | 1.61 | 0.504 | 1.3 | 0.54 | 2.15 |

Table 2. Molecular weights and contents of polysaccharides in *G. lucidum* dietary supplements after α-amylase digestion. aContent of polysaccharides in the raw material, all data were the average of two measurements with coefficient of variation <5%; bNot detected in the sample; Peaks were the same as in Fig. S4; Sample codes were the same as in Table 1.
All samples (1–3 μL) were separated using a vertical slab gel electrophoresis apparatus, Mini-Protean Tetra System (Bio-Rad, Hercules, CA, USA). Electrophoresis on 30% (w/v) polyacrylamide as the resolving gel with a stacking gel of 8% (w/v) polyacrylamide was used for the separation of enzymatic hydrolytes. Samples were electrophoresed firstly at 200 V for 10 min and then at 700 V for 45 min. All runs were performed at least two times. Gels were imaged using an InGenius LHR CCD camera system (Syngene, Cambridge, UK) under UV 365 nm.

**GC-MS analysis.** Compositional monosaccharides of polysaccharides in *G. lucidum* fruiting dietary supplements were investigated by using GC-MS analysis according to previous report with minor modification. Briefly, the sample (3.0 mg) was hydrolyzed with 2.0 M TFA (1.0 mL) at 95 °C in a sealed tube for 10 h. Then the hydrolysates were washed with methanol and evaporated to dryness before derivation with hydroxylamine hydrochloride and acetic anhydride at 90 °C for 30 min. Furthermore, the derivatives of mixed monosaccharide standards (1.0 mg/mL of Ara, Fuc, Gal, Glc, and Man, respectively) were prepared as described above. The derivatives were analyzed by using an Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA). A capillary column (30 m × 0.25 mm, i.d.) coated with 0.25 μm film 5% phenyl methyl siloxane was used for separation. High purity helium was used as carried gas with a flow rate of 1.0 mL/min. The column temperature was set at 165 °C and held for 7 min for injection, then programmed at 5 °C/min to 185 °C and held for 5 min, then at 4 °C/min to 200 °C, and finally at 20 °C/min to 280 °C, and held for 2 min.

**HPSEC-MALLS-RID based on the dn/dc analysis.** Contents and molecular weights of polysaccharides and their fractions in *G. lucidum* dietary supplements were simultaneously determined using HPSEC-MALLS-RID based on dn/dc method according to our previous report with minor modification.

Firstly, polysaccharide solutions (~5.0 mg/mL) of *G. lucidum* dietary supplement were treated with α-amylase at a final concentration of 20.0 U/mL in a total volume of 1.0 mL for 24 h at 40 °C to completely hydrolyzed additives (such as soluble starch, dextrin, and pullulan) into small sugars. Polysaccharide solutions without α-amylase, and α-amylase without polysaccharide solutions treated as described above, were used as blank control. Secondly, HPSEC-MALLS-RID measurements were carried out on a MALLS (DAWN HELEOS, Wyatt Technology Co., Santa Barbara, CA, USA) equipped with columns of TSK-Gel G5000PWXL (300 mm × 7.8 mm, i.d.) and TSK-Gel G3000PWXL (300 mm × 7.8 mm, i.d.) in series at 35 °C. The MALLS instrument was equipped with a He-Ne laser (λ = 658 nm). An Optilab rEX refractometer (DAWN EOS, Wyatt Technology Co., Santa Barbara, CA, USA) was simultaneously connected. The *M*ₜ was calculated by the Zimm method of static light scattering based on the basic light scattering equation as follows:

\[
Kc = \frac{1}{M \alpha} \left( 1 + \frac{16\pi^2(\frac{S}{3})^2}{3\lambda^2\sin^2\left(\frac{\theta}{2}\right)} \right) + 2A_2 + \ldots
\]

where *K* = an optical constant equal to [\(4\pi^2n^2(\text{dn/dc})^2\)]/(\(N_a\lambda^4\)), *C* = polysaccharide concentration; *R*ₚ = Rayleigh ratio; *λ* = wavelength; *n* = refractive index of the solvent (0.9% NaCl aqueous solution); *dn/dc* = refractive index increment of polysaccharides in 0.9% NaCl aqueous solution, which is recommended as 0.15 mL/g according to our previous study; *Nₐ* = Avogadro’s number; *A₂* = second virial coefficient.

Contents of polysaccharides were calculated based on the refractive index difference with dn/dc value according to the following equation:

\[
C_i = \frac{\alpha(V_i - V_{i,\text{baseline}})}{dn/dc}
\]

where *Cᵢ* is the concentration of polymers; *α* is the RID calibration constant (in RI units per volt); *Vᵢ* and *Vᵢ, baseline* are the RID voltages of sample and baseline, respectively; *dn/dc* is the specific refractive index increment.

**Data analysis.** The digital scanning chromatograms of PACE fingerprints were generated using Quantity-One software (version 4.6.2, Bio-Rad, Hercules, USA), and hierarchical cluster analysis of PACE fingerprints was also analyzed using Quantity-One with average linkage method.

**References**

1. Cohen, P. A. Assessing Supplement Safety - The FDA’s Controversial Proposal. *N. Engl. J. Med.* 366, 389–391 (2012).
2. Cohen, P. A. Hazards of Hindsight - Monitoring the Safety of Nutritional Supplements. *N. Engl. J. Med.* 370, 1277–1280 (2014).
3. Voolker, R. FDA Expands Warning on Diet Products. *JAMA* 301, 1646–1646 (2009).
4. Kapoor, A. & Sharfstein, J. M. Breaking the gridlock: Regulation of dietary supplements in the United States. *Drug Test Anal.* 8, 424–430 (2016).
5. AO, C. New York attorney general targets supplements at retailers http://well.blogs.nytimes.com/2015/2002/2003/new-york-attorney-general-targets-supplements-at-major-retailers (2015).
6. Nie, S., Zhang, H., Li, W. & Xie, M. Current development of polysaccharides from *Ganoderma* Isolation, structure and bioactivities. *Bioactive Carbohydrates and Dietary Fibre* 1, 10–20 (2013).
7. Russell, R. & Paterson, M. *Ganoderma* - A therapeutic fungal biofactory. *Phytochemistry* 67, 1985–2001 (2006).
8. Xu, Z. T., Chen, X. U., Zhong, Z. F., Chen, L. D. & Wang, Y. T. *Ganoderma lucidum* polysaccharides: Immunomodulation and potential anti-tumor activities. *Am. J. Chin. Med.* 39, 15–27 (2011).
9. Yan, Y. Z., Xie, P. S., Lam, W. K., Chui, E. & Yu, Q. X. Study on triterpenoic acids distribution in *Ganoderma* mushrooms by automatic multiple development high performance thin layer chromatographic fingerprint analysis. *J. AOAC Int.* 93, 1384–1389 (2010).
10. Yang, J. et al. Quality evaluation of different products derived from *Ganoderma*. *J. Med. Plants Res.* 6, 1969–1974 (2012).
11. Liao, S. F. et al. Immunization of fucose-containing polysaccharides from Reishi mushroom induces antibodies to tumor-associated Globo H-series epitopes. Proc. Natl. Acad. Sci. USA 110, 13809–13814 (2013).
12. Wu, D. T., Xie, J., Hu, D. J., Zhao, J. & Li, S. P. Characterization of polysaccharides from Ganoderma spp. using saccharide mapping. Carbohydr. Polym. 97, 398–405 (2013).
13. Di, X., Chan, K. V. K. C., Leung, H. W. & Hui, C. W. Fingerprint profiling of acid hydrolyzates of polysaccharides extracted from the fruiting bodies and spores of Lingzhi by high-performance thin-layer chromatography. J. Chromatogr. A 1018, 85–95 (2003).
14. Sun, X. M. et al. Fingerprint analysis of polysaccharides from different Ganoderma by HPLC combined with chemometrics methods. Carbohydr. Polym. 114, 432–439 (2014).
15. Xie, J. et al. Comparison of polysaccharides from two species of Ganoderma. Molecules 17, 740–752 (2012).
16. Li, S. P., Wu, D. T., Lv, G. P. & Zhao, J. Carbohydrates analysis in herbal glycomics. Trac-Trend Anal. Chem. 52, 155–169 (2013).
17. Meng, L. Z. et al. A comparative study on immunomodulatory activity of polysaccharides from two official species of Ganoderma (Lingzhi). Natl. Cancer 66, 1124–1131 (2014).
18. Cheong, K.-L., Wu, D.-T., Zhao, J. & Li, S.-P. A rapid and accurate method for the quantitative estimation of natural polysaccharides and their fractions using high performance size exclusion chromatography coupled with multi-angle laser light scattering and refractive index detector. J. Chromatogr. A 1400, 98–106 (2015).
19. Wu, D.-T. et al. Characterization and discrimination of polysaccharides from different species of Cordyceps using saccharide mapping based on PACE and HPTLC. Carbohydr. Polym. 103, 100–109 (2014).
20. Wu, D.-T. et al. An evaluation system for characterization of polysaccharides from the fruiting body of Hericium erinaceus and identification of its commercial product. Carbohydr. Polym. 124, 201–207 (2015).
21. Lv, G. P. et al. Decoding glycome of Astragalus membranaceus based on pressurized liquid extraction, microwave-assisted hydrolysis and chromatographic analysis. J. Chromatogr. A 1409, 19–29 (2015).
22. Zimm, B. H. Apparatus and methods for measurement and interpretation of the angular variation of light scattering: preliminary results on polystyrene solutions. J. Chem. Phys. 16, 1099–1116 (1948).
23. Wang, J. G. et al. Structure and chain conformation of water-soluble heteropolysaccharides from Ganoderma lucidum. Carbohydr. Polym. 86, 844–851 (2011).
24. Liu, Y. F. et al. Physicochemical characterization of a high molecular weight bioactive beta-D-glucan from the fruiting bodies of Ganoderma lucidum. Carbohydr. Polym. 101, 968–974 (2014).
25. Podzimek, S. In Light Scattering, Size Exclusion Chromatography and Asymmetric Flow Field Flow Fractionation 37–98 (John Wiley & Sons, Inc., 2011).

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Author Contributions
S.P.L., J.Z., and A.B. conceived and designed the research. D.T.W., Y.D., and L.X.C conducted the experiments. D.T.W., Y.D., and L.X.C. performed the experiments. D.T.W., Y.D., and L.X.C conducted the experiments. D.T.W., Y.D., and L.X.C. analyzed the data. S.P.L. and D.T.W. drafted the manuscript, and S.P.L. and A.B. corrected the manuscript. All authors reviewed the manuscript.

Additional Information
Competing Interests: The authors declare that they have no competing interests.

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