Traditional processing increases biological activities of *Dendrobium officinale* Kimura et. Migo in Southeast Yunnan, China

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The orchid *Dendrobium officinale* grows throughout southeast China and southeast Asian countries and is used to treat inflammation and diabetes in traditional Chinese medicine. Tie pi feng dou is a well-known traditional Chinese medicine made from the dried *D. officinale* stems. Processing alters the physicochemical properties of TPFD; however, it is unclear how processing affects the quality and medicinal value of this plant. Here, we analyzed and compared the chemical composition of fresh stems of *D. officinale* and TPFD and explored possible explanations for the enhanced medicinal efficacy of processed *D. officinale* stems using qualitative and quantitative methods. To identify the components of FSD and TPFD, we used ultra-high-performance liquid chromatography combined with mass spectrometry in negative and positive ion modes and interpreted the data using the Human Metabolome Database and multivariate statistical analysis. We detected 23,709 peaks and identified 2352 metabolites; 370 of these metabolites were differentially abundant between FSD and TPFD (245 more abundant in TPFD than in FSD, and 125 less abundant), including organooxygen compounds, prenol lipids, flavonoids, carboxylic acids and their derivatives, and fatty acyls. Of these, 43 chemical markers clearly distinguished between FSD and TPFD samples, as confirmed using orthogonal partial least squares discriminant analysis. A pharmacological activity analysis showed that, compared with FSD, TPFD had significantly higher levels of some metabolites with anti-inflammatory activity, consistent with its use to treat inflammation. In addition to revealing the basis of the medicinal efficacy of TPFD, this study supports the benefits of the traditional usage of *D. officinale*.

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| TPD | Tipifengdou |
| FSD | Fresh stems of *D. officinale* |
| TCM | Traditional Chinese medicines |
| UHPLC-QEQO/MS | Ultra-high-performance liquid chromatography coupled with Q-Exactive plus quadrupole-Orbitrap mass spectrometry |
| HMDB | Human Metabolome Database |
| PCA | Principal component analysis |
| OPLS-DA | Orthogonal projections to latent structures discriminant analysis |
| PLS-DA | Partial least squares discriminant analysis |
| VIP | Variable importance of projection |
| ESI | Electrospray ionization |

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The therapeutic properties of traditional Chinese medicines (TCMs) are derived from the collective contributions of various chemical components, most of which are secondary metabolites and saccharides. Plants used in TCM accumulate secondary metabolites, including polysaccharides, alkaloids, amino acids, flavonoids, phenols, coumarins, terpenoids, and benzyl compounds, which enable them to adapt to diverse environmental conditions. These metabolites possess various physiological activities that underpin their medicinal value when consumed. Before the clinical use of a TCM, the raw materials are typically subjected to traditional processing methods, which change the physicochemical properties of the herbal materials. This can transform certain bioactive/toxic components, which is likely the primary way in which processing affects the therapeutic properties of TCM ingredients.

The perennial epiphytic herb *Dendrobium officinale* Kimura & Migo belongs to the Orchidaceae family and is native to China and southeast Asian countries. In TCM, this plant is used as a medicine or food to nourish “yin,” clearing heat, toning the stomach, and promoting fluid production. For over 1000 years, this herb has been processed and used by ethnic groups in southwest Yunnan, China, to treat inflammation and diabetes. According to the Pharmacopoeia of China (2020 edition), fresh stems of *D. officinale* (FSD) should be harvested, and the leaves and stem epidermis should be removed. The stem should then be semi-dried to a 45% moisture content before being twisted into a spring shape under heat. The resulting products, which have a moisture content of < 12%, are known as Xifengdou (translated as Tie pi feng dou, TPFD). Detailed records on the traditional processing of TPFD in China began in the Qing Dynasty.

Characterizing the composition of TCMs such as TPFD can inform the development of medicines and supplements with similar properties and help establish quality control standards for these biological products, which vary in activity levels. Conventional chemical research into herbal materials involves the systematic isolation of their chemical constituents, followed by qualitative and quantitative comparisons, chemical profiling, and identification of chemical markers. Metabolomics is widely used to elucidate the chemical compositions of herbal medicines. Typically performed using liquid chromatography with mass spectrometry (LC–MS), metabolomics is a powerful approach for elucidating the global profiles of complex secondary metabolites by measuring their presence, abundance, and chemical structures. LC–MS is also employed to identify marker compounds used to distinguish between raw and processed herbal medicines.

TPFD is a well-known traditional product of *D. officinale*; however, the chemical composition of *D. officinale* is complex, and conventional analytical approaches are time-consuming and have not yielded a complete chemical characterization of the major differences between FSD and TPFD. Previous studies of the chemical composition of *D. officinale* have mainly focused on elucidating the botanical, traditional use, phytochemistry, and pharmacology of *D. officinale*. While some studies have examined properties pertaining to the quality and safety of this herb and omics studies have explored the biosynthetic pathways and regulation mechanisms of the plant’s bioactive compounds, the major chemical differences between raw and processed TPFD products are largely unknown. Exploring the chemical changes that occur during *D. officinale* processing will therefore provide helpful information for understanding the therapeutic characteristics of TPFD. Here, we analyzed the chemical constituents of TPFD and FSD and explored the effect of traditional processing on the therapeutic value of *D. officinale* using a non-targeted metabolomics method and a variety of chromatographic techniques. We then examined the differences between FSD and TPFD using multivariate and univariate statistical analyses to elucidate the main chemical transformations that occur during *D. officinale* processing.

**Materials and methods**

**Regulatory statement.** The plant experiments were performed in accordance with relevant guidelines and regulations.

**Reagents and materials.** All chemicals and solvents used were of analytical or high-performance liquid chromatography grade. Water, acetonitrile, methanol, and formic acid were from Thermo Fisher Scientific (Waltham, MA, USA). L-2-chlorophenylalanine was obtained from Shanghai Hengchuang Biotechnology Co., Ltd. (Shanghai, China).

FSD and TPFD samples were obtained from the local market in Guangnan County, Wenshan Prefecture, Yunnan Province, southwest China. *D. officinale* is a National Geographic Indication Product. The raw materials for TPFD and FSD were collected from the fresh stems of *D. officinale* from the same batch in April 2021 and authenticated by Prof. Lixin Yang, Chinese Academy of Sciences. Type specimens were deposited in the Kunming Institute of Botany Herbarium (sample numbers: TPFD202110401 and FSD20210402).

**Sample preparation.** Similarly sized samples were selected for analysis. Each sample was prepared in quadruplicate. First, all samples were thoroughly ground. For the analysis, 80 mg of FSD (Sample No.: DF-1-1 to DF-1-4) and TPFD (Sample No.: DF-F-1 to DF-F-4) was transferred into a 1.5-mL microfuge tube. Twenty microliters of internal standard (L-2-chlorophenylalanine, 0.3 mg/mL; methanol), 1 mL methanol–water (V/V = 7:3), and two small steel balls were added. The samples were chilled to –20 °C for 2 min and then ground at 60 Hz for 2 min, extracted with ultrasonic waves for 30 min in an ice-water bath, and incubated at –20 °C for 20 min. The samples were centrifuged at 4 °C and 13,000 rpm for 10 min. Then, a glass syringe was used to collect 150 μL of supernatant, which was filtered through microfilters (0.22 μm). The filtrate was transferred into LC vials, which were stored at –80 °C until analysis.

For quality control (QC), pooled samples were prepared by mixing aliquots of all the samples.
Secondary metabolite analysis. An ultra-high-performance liquid chromatography (UHPLC, Dionex Ultimate 3000 RS) with a mass spectrometer (Q-Exactive plus quadrupole-Orbitrap) equipped with a heated electrospray ionization (ESI) source (Thermo Fisher Scientific) was used to analyze the metabolic profiles in ESI positive and negative ion modes. The column (ACQUITY UPLC HSS T3, 1.8 μm, 2.1 × 100 mm) was employed in positive and negative modes. The elution reagents were (A) water with 0.1% (v/v) formic acid and (B) acetonitrile with 0.1% (v/v) formic acid, and the gradient was as follows: 0 min, 5% B; 1 min, 5% B; 2.5 min, 30% B; 6 min, 50% B; 7 min, 70% B; 10 min, 80% B; 12 min, 100% B; 14 min, 100% B; 14.2 min, 5% B; and 16 min, 5% B at 0.35 mL/min and a column temperature of 40 °C. Samples were maintained at 4 °C during analysis. The injection volume was 5 μL.

The mass range was detected between 100 and 1200 mass-to-charge ratio (m/z). A resolution of 70,000 was used for full MS scans, and 17,500 was used for higher-energy collisional dissociation (HCD) MS/MS scans, with a collision energy of 10, 20, and 40 eV. The mass spectrometer was operated as follows: spray voltage, 3800 V(+) and 3200 V(−); sheath gas flow rate, 40 arbitrary units; auxiliary gas flow rate, 15 arbitrary units; capillary temperature, 320 °C; auxiliary gas heater temperature, 350 °C; and S-lens RF level, 55. Every four samples, a QC sample was injected to assess repeatability.

Statistical analysis. Progenesis QI V2.3 (Nonlinear Dynamics, Newcastle, UK) was used for baseline filtering, peak identification, integral retention time correction, peak alignment, and normalization of raw LC–MS
data, with 5 ppm precursor tolerance, 10 ppm product tolerance, and 5% product ion threshold. Compound identification was based on comparing the precise m/z values, secondary fragments, and isotopic distribution with the Human Metabolome Database (HMDB) for qualitative analysis.

The data were further processed by removing peaks with missing values (ion intensity = 0) in more than 50% of groups by replacing zero values with half of the minimum value and by screening according to the qualitative results of the compound. Compounds with scores below 36 (of 60) were deemed to be inaccurate and removed.

A data matrix was generated from the positive and negative ion data and used for principal component analysis (PCA) in R. Orthogonal partial least squares discriminant analysis (OPLS-DA) and partial least squares

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**Figure 2.** Model of the multivariate analysis and its cross-validation. (A): PCA for TPFD samples versus FSD samples. (B): PLS-DA for TPFD samples versus FSD samples. (C): OPLS-DA for TPFD samples versus FSD samples. (D): Response permutation testing of the model predicted by OPLS-DA.

**Figure 3.** Differentially abundant metabolites between TPFD and FSD. (A): Volcano plot of the 2352 metabolites identified. (B): Main classes of differentially abundant metabolites.
| Metabolites                                                                 | Compound ID          | m/z    | Retention time(min) | VIP  | P-value | log2(FC) |
|---------------------------------------------------------------------------|----------------------|--------|---------------------|------|---------|----------|
| Isopropyl apiosylglucoside                                               | HMBDR0041513         | 353.145| 3.952               | 2.124| 0.000   | 39.862   |
| D-erythro-D-galacto-octitol                                               | HMBDR0029953         | 281.063| 2.998               | 1.763| 0.000   | 39.372   |
| Cyclodopa glucoside                                                      | HMBDR0029833         | 380.095| 0.771               | 1.633| 0.000   | 39.126   |
| 6'-Apiosyllotaustralin                                                    | HMBDR0034207         | 416.155| 1.184               | 1.190| 0.000   | 38.195   |
| Maltobiose                                                               | HMBDR0012235         | 1013.316| 1.004              | 1.108| 0.000   | 38.010   |
| N-(1-Deoxy-1-fructosyl)alanine                                           | HMBDR0038662         | 234.097| 0.816               | 1.797| 0.000   | 15.901   |
| (E)-2-O-Cinnamoyl-beta-D-glucopyranose                                   | HMBDR0035880         | 328.139| 0.867               | 2.119| 0.000   | 13.310   |
| Tetraphyllin B                                                           | HMBDR0029914         | 310.090| 2.009               | 2.573| 0.000   | 10.850   |
| Ethyl beta-D-glucopyranoside                                             | HMBDR0029968         | 231.084| 1.385               | 1.657| 0.000   | 6.401    |
| Cyclodopa glucoside                                                      | HMBDR0036699         | 230.102| 1.268               | 2.371| 0.000   | 10.061   |
| 6'-Apiosyllotaustralin                                                    | HMBDR0034207         | 416.155| 1.184               | 1.190| 0.000   | 38.195   |
| Maltohexaose                                                             | HMBDR0012235         | 1013.316| 1.004              | 1.108| 0.000   | 38.010   |
| Isopropyl apiosylglucoside                                               | HMBDR0041513         | 353.145| 3.952               | 2.124| 0.000   | 39.862   |
| D-erythro-D-galacto-octitol                                               | HMBDR0029953         | 281.063| 2.998               | 1.763| 0.000   | 39.372   |
| Cyclodopa glucoside                                                      | HMBDR0029833         | 380.095| 0.771               | 1.633| 0.000   | 39.126   |
| 6'-Apiosyllotaustralin                                                    | HMBDR0034207         | 416.155| 1.184               | 1.190| 0.000   | 38.195   |
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| Cyclodopa glucoside                                                      | HMBDR0029833         | 380.095| 0.771               | 1.633| 0.000   | 39.126   |
| 6'-Apiosyllotaustralin                                                    | HMBDR0034207         | 416.155| 1.184               | 1.190| 0.000   | 38.195   |
| Maltobiose                                                               | HMBDR0012235         | 1013.316| 1.004              | 1.108| 0.000   | 38.010   |
Table 1. Information of organooxygen compounds with significant changes.

| Metabolites                                              | Compound ID | m/z     | Retention time(min) | VIP  | P-value  | log2(FC) |
|---------------------------------------------------------|-------------|---------|---------------------|------|----------|----------|
| Verbasoside                                             | HMDB0039233 | 461.167 | 3.417               | 1.008| 0.000    | 0.853    |
| Linool 3,7-oxide beta-primveroside                      | HMDB0036571 | 482.259 | 4.067               | 1.773| 0.000    | 0.766    |
| Benzyl beta-primveroside                                | HMDB0041190 | 401.145 | 3.632               | 1.550| 0.002    | 0.757    |
| Myzodendrone                                            | HMDB0041273 | 387.130 | 3.310               | 1.292| 0.001    | 0.640    |
| trans-p-Menthan-1,7,8-triol 8-glucoside                 | HMDB0034784 | 373.183 | 3.403               | 4.657| 0.003    | 0.628    |
| Pteroside P                                             | HMDB0036608 | 441.176 | 3.931               | 1.233| 0.007    | 0.401    |
| 2-O-beta-D-Glucopyranonosyl-D-mannose                   | HMDB0039722 | 337.078 | 0.860               | 1.176| 0.007    | 0.384    |
| Trehalose                                               | HMDB0009975 | 387.114 | 0.804               | 7.692| 0.004    | 0.301    |
| 4-O-beta-D-Galactopyranosyl-D-xylene                    | HMDB0038864 | 357.104 | 1.084               | 1.063| 0.032    | −0.245   |
| 3,5-Dihydroxyphenyl-1-O-(6-O-galloyl-beta-D-glucopyranoside) | HMDB0039307 | 439.086 | 0.810               | 8.940| 0.000    | −0.709   |
| a-L-Arabinofuranosyl-(1→3)-[a-L-arabinofuranosyl-(1→5)]-L-arabinose | HMDB0041223 | 432.171 | 0.816               | 2.132| 0.000    | −0.894   |
| 3,4,5-Trimethoxyphenyl 2,6-digalloylglucoside           | HMDB0039312 | 631.128 | 3.696               | 1.692| 0.000    | −0.921   |
| (S)-Nerolidol 3-O-[a-L-Rhamnopyranosyl-(1→4)-a-L-rhamnopyranosyl-(1→2)]-b-D-glucopyranoside | HMDB0040846 | 721.366 | 8.316               | 5.233| 0.000    | −1.917   |
| (S)-Nerolidol 3-O-[a-L-rhamnopyranosyl-(1→4)-a-L-rhamnopyranosyl-(1→6)]-b-D-glucopyranoside | HMDB0040846 | 699.356 | 8.331               | 6.236| 0.000    | −2.019   |
| cis-p-Coumaric acid 4-[apiosyl-(1→2)]-glucoside         | HMDB0037078 | 481.132 | 4.215               | 1.852| 0.000    | −2.756   |
| Gluconasturtiin                                         | HMDB0038423 | 441.100 | 3.342               | 2.914| 0.000    | −3.586   |
| Benzyl O-[arabinofuranosyl-(1→6)]-glucoside             | HMDB0041514 | 383.135 | 4.904               | 1.221| 0.000    | −3.883   |
| 6-Phosphogluconic acid                                  | HMDB0001316 | 277.032 | 0.850               | 2.739| 0.000    | −4.135   |
| Mangalkanyl glucoside                                    | HMDB0036015 | 431.265 | 7.825               | 2.676| 0.000    | −9.586   |
| 3-O-alpha-D-Glucopyranonosyl-D-xylene                   | HMDB0039723 | 651.161 | 8.771               | 1.070| 0.000    | −37.900  |
| 2-Phospho-D-glyceric acid                               | HMDB0003391 | 230.991 | 0.916               | 1.128| 0.000    | −38.044  |

Discriminant analysis (PLS-DA) were used to identify the metabolites that differed between groups. Seven-fold cross-validation and 200 response permutation tests were used to prevent overfitting and evaluate the quality of the model.

Variable importance of projection (VIP) values from OPLS-DA were used to rank the contribution of each variable to the discrimination of groups. A two-tailed Student’s t-test was used to verify the significance of the differences in metabolite abundance between the groups. Metabolites with VIP > 1.0 and P < 0.05 were selected as differentially abundant.

Results

Identification of metabolite diversity in *D. officinale.* Typical total ion current chromatograms (TICs) of FSD samples are presented in Fig. 1A (ESI+) and Fig. 1B (ESI−), and TICs of TPFD samples are presented in Fig. 1C (ESI+) and Fig. 1D (ESI−). A total of 23,709 substance peaks were detected in FSD and TPFD samples using the UHPLC Q-Exactive plus quadrupole-Orbitrap mass spectrometer, among which 2352 metabolites were identified (976 metabolites from the negative ion model (ESI−) and 1376 metabolites from the positive ion mode (ESI+) (Fig. 1E).

FSD and TPFD samples contained all the identified metabolites, but the relative contents of individual compounds were remarkably different between the two groups (Fig. 2). However, metabolite contents were similar in the four biological replicates of an individual sample. The PCA plots between FSD and TPFD samples also showed clear differences (Fig. 2A); for example, PC1 was clearly separated between FSD and TPFD and represented 69.7% of the difference in their chemical compositions. Figure 2B presents the PLS-DA of the two groups. The R2 value of the PCA and Q value of the PLS-DA (Fig. 2) show that compound abundances between FSD and TPFD samples were statistically significantly different. OPLS-DA, another supervised method, was used to highlight the quantitative variation in the metabolites between TPFD and FSD samples (Fig. 2C). Cross-validation with 200 permutations supported the reliability of this OPLS-DA model, with R2 and Q2 intercepts of 0.932 and 0.640, respectively (Fig. 2D). These results show that TCM processing techniques lead to significant changes to the metabolite contents of *D. officinale*.

Characterization of five categories of differentially abundant metabolites. Pairwise comparisons of metabolite abundances in FSD and TPFD using the OPLS-DA model identified differentially abundant metabolites based on the VIP value. Next, all identified and annotated metabolites were screened for different abundances between FSD and TPFD samples (Fig. 3A). Using the set criteria (VIP > 1; P < 0.05), 370 metabolites were found to be significantly differentially abundant between TPFD and FSD, the majority of which were organooxygen compounds, prenol lipids, flavonoids, carboxylic acids and their derivatives, and fatty acyls (Fig. 3B).

First, organooxygen compounds were significantly more abundant in TPFD than in FSD samples. These compounds, especially carbohydrates and carbohydrate conjugates, directly contribute to the physiological activity of *D. officinale* products. Carbohydrates and carbohydrate aggregates from *D. officinale* have antioxidant,
anti-tumor, immune-enhancing, and anti-inflammatory effects; they protect the liver and nerves; and they are useful for the treatment of diabetes and the intestinal microbiome. A total of 77 organooxygen compounds were significantly differentially abundant between FSD and TPFD samples in this study, accounting for 20.8% of all the differentially abundant metabolites. Of these, 74 compounds were carbohydrates or carbohydrate aggregates. Sixty-one carbohydrates and carbohydrate aggregates were significantly more abundant in TPFD samples, while 13 carbohydrates and carbohydrate aggregates were significantly less abundant (Table 1). In particular, isopropyl apiosylglucoside, D-erythro-D-galacto-octitol, cyclodopa glucoside, 6'-apiosyllotaustralin, and maltohexaose were an average of 5.80 × 10^{11} times more abundant in TPFD, likely due to the TCM processing. By contrast, 2-phospho-D-glyceric acid and 3-O-alpha-D-glucopyranosyl-D-xylose were an average of 3.72 × 10^{-12} times less abundant in TPFD than in FSD. In addition, 44 carbohydrates and carbohydrate aggregates were at least twice as abundant in TPFD as in FSD, but only 9 compounds were half as abundant. These results suggest that TCM processing positively affects the accumulation of carbohydrates in TPFD.

Second, Prenol lipids are naturally occurring and are formed by the condensation of isoprene subunits. Prenol lipids have critical roles not only as structural components of cell membranes, but also as essential signaling molecules in various biological processes.

Figure 4. Chemical structures of typical compounds from TPFD.
| Metabolites                                      | Compound ID      | m/z     | Retention time (min) | VIP   | P-value log2(FC) |
|------------------------------------------------|------------------|---------|----------------------|-------|-----------------|
| Capsianoside V                                 | HMDB0030737      | 559.276 | 4.622                | 1.176 | 0.000           | 10.228 |
| 4,11,13,15-Tetrahydrodoridentin B               | HMDB0036150      | 269.175 | 4.215                | 1.530 | 0.000           | 8.906  |
| ARLatin                                        | HMDB0035740      | 289.141 | 5.365                | 5.270 | 0.000           | 8.883  |
| (4beta,8alpha)-6-Hydroxy-7(11)-eremophilien-12,8-olide | HMDB0035148 | 251.164 | 3.569                | 1.558 | 0.000           | 6.910  |
| Artenin                                        | HMDB0034696      | 249.148 | 4.278                | 1.250 | 0.000           | 5.738  |
| Geniposidic acid                               | HMDB0034942      | 397.111 | 3.486                | 2.191 | 0.000           | 5.418  |
| Lactaronecatorin A                             | HMDB0037529      | 233.154 | 4.215                | 1.404 | 0.000           | 5.401  |
| 4-Episodeinosolide                             | HMDB0031378      | 249.148 | 3.859                | 1.018 | 0.000           | 5.304  |
| Neryl rhamnmosyl-glucoside                     | HMDB0029349      | 443.229 | 6.172                | 1.723 | 0.000           | 4.670  |

Continued
molecules; for example, vitamin K plays a key role in bone health and cardiovascular homeostasis. Similarly, vitamins E and A, as well as ubiquinones, have crucial effects on the progression of age-related diseases and chronic conditions such as inflammation and diabetes.

In this study, the number of differentially abundant prenol lipids in TPFD samples was second only to that of organooxygen compounds, accounting for 17.8% of all the differentially abundant metabolites. The relative concentrations of 46 prenol lipids were significantly higher in TPFD than in FSD samples, while 13 prenol lipids were significantly lower (Table 2). When compared with FSD samples, capsianoside V, 4,11,13,15-tetrahydroridentin, and (6beta,8alpha)-6-hydroxy-7(11)-eremophilen-12,8-olide (Fig. 4) were more abundant in TPFD samples, while ginsenoside Rg3, tanacetol B, fauronyl acetate, and nerolidyl acetate were less abundant (Fig. 4). The variation in these compounds indicates their different contributions to the final quality of TPFD products. Third, fruits and vegetables contain abundant quantities of flavonoids, which contribute to plant color and protect against microbial infection. The properties of flavonoids depend on the arrangement of hydroxyl, methoxy, and glycosidic side groups and the conjugation between the A- and B-rings. A total of 40 flavonoids, including common flavonoids such as naringin and rutin (Fig. 4), were significantly differentially abundant between TPFD and FSD samples (Table 3). After TCM processing, 26 flavonoids were significantly more abundant in TPFD than in FSD samples, while 14 flavonoids were significantly less abundant. Flavonoids possess anti-inflammatory and antioxidant activities and are considered potential therapeutic agents. The content differences of these flavonoids therefore may influence the therapeutic characteristics of TPFD.

Finally, carboxylic acids, their derivatives, and fatty acyls are also major categories of compounds that are differentially abundant between TPFD and FSD samples. Here, 35 carboxylic acids and derivatives and 29 fatty acids were significantly differentially abundant between the samples (Tables 4 and 5). Carboxylic acids and derivatives are important substances in animal and plant metabolism and are used commercially in the synthesis of pesticides, herbicides, and insect repellents. Mounting evidence suggests that carboxylic acids and derivatives also have considerable pharmacological activities; for example, pentacyclic triterpenoid carboxylic acids have strong antioxidant, anti-inflammatory, antibacterial, anti-diabetic, and anti-tumor activities. TPFD is traditionally used for the treatment of diabetes, cancer, and inflammation, among other conditions, suggesting that changes in carboxylic acids and their derivatives may determine the efficacy of TPFD. Short-chain fatty acids contribute to the flavor of D. officinale, and long-chain fatty acids can be degraded and transformed into various active flavor components through oxidation reactions. The content differences of these fatty acids may therefore affect the flavor characteristics of TPFD.

**Correlation analysis of biological activities.** The chemical composition of TPFD affects its efficacy. As mentioned above, 370 metabolites are significantly changed in the traditional processing of FSD into TPFD for TCM, the majority of which are organooxygen compounds, flavonoids, prenol lipids, fatty acids, and carboxylic acids and their derivatives. These metabolites have different activities and therefore may affect the efficacy of TPFD; therefore, future research should examine these 370 differentially abundant metabolites as potential chemical markers.

To provide visual evidence of the distinct nature of TPFD samples, the above OPLS-DA models were used to construct an S-plot and loading analysis (Fig. 5A and B), which provided a graphical projection of specific compounds. In these plots, metabolites close to the origin make a small contribution to the separation of the samples. A total of 43 metabolites (Fig. 5C) had a VIP score ≥ 4.0 in the OPLS-DA model, and a t-test revealed that they significantly differed (P < 0.05) between FSD and TPFD. In the S-plot and loading analysis, these compounds were farthest from the origin (in the positive and negative directions), indicating that they make a greater contribution to the distinction between samples. These 43 metabolites (listed in Table 6 with their activities) could therefore be used as chemical markers to assess whether the biological activity of D. officinale is altered through traditional processing.
Of the 43 chemical markers, 29 were more abundant in TPFD than in FSD samples, while 14 were less abundant. Their medicinal properties include anti-inflammatory, anti-mutagenic, analgesic, neuroprotection and anti-Alzheimer’s, anti-tumor, antibacterial, anti-toxicity, antioxidant, anti-nociceptive, anti-hypertension, anti-diabetic, anti-depressant, lipase-inhibiting, immune-enhancing, cis-diaminedichloroplatinum nephrotoxicity-preventing, cytoprotective, Fanconi syndrome–attenuating, cardiotoxicity-preventing, anti-fatigue, and anti-tyrosinase activities. Anti-inflammatory activity is the most common function of the significantly upregulated metabolites (Table 6). Among the 43 chemical markers, [6]-dehydroshogaol and capsaicin (Fig. 4) showed the greatest difference in abundance between TPFD and FSD samples, with log2 fold-change values of 13.16 and 11.88, respectively. Imm et al. established that capsaicin and [6]-dehydroshogaol inhibited the production of nitric oxide (NO) in LPS-stimulated cells in a dose-dependent manner. These chemicals are also likely to have anti-inflammatory and antioxidant effects by inactivating the eukaryotic transcription factor NF-κB.
Furthermore, the log2 fold-change values of the anti-inflammatory compounds N2-(3-hydroxysuccinoyl)arginine, naringenin, citronellyl beta-sophoroside, methyl beta-D-glucopyranoside, and hydroxysafflor yellow A (Fig. 4) were > 3 (Table 6). Thus, TPFD has better anti-inflammatory properties than FSD, which is beneficial for its applications in TCM.

In addition, the 43 marker metabolites included compounds with anti-tumor and anti-diabetic activities. Arlatin, naringenin, and methyl beta-D-glucopyranoside (Fig. 4) showed significant anti-tumor activity, while citroside A (Fig. 4) possesses significant anti-diabetic activity. The contents of all these compounds were significantly higher in TPFD than in FSD samples. These results are consistent with the reported effects of TPFD in TCM, providing scientific evidence of the efficacy of the traditional application of *D. officinale*.

We also detected some compounds in *D. officinale* with potential therapeutic effects on neurological diseases; for example, norcapsaicin (Fig. 4) has neuroprotection and anti-Alzheimer’s activities, while osmanthuside B (Fig. 4) shows anti-depressant activity. Future studies should explore the relationship between the concentrations of these compounds present and the therapeutic effects and health-promoting properties of *D. officinale* products.

### Discussion

The significant differences on metabolites between FSD and TPFD. We integrated UHPLC coupled with Q Exactive plus quadrupole-Orbitrap MS in the positive and negative ion modes, combined with a HMDB and multivariate statistical analysis for qualitative analyses, to screen the different constituents of FSD and TPFD. The result of this study reveals that five type compounds including organooxygen compounds, prenol lipids, flavonoids, carboxylic acids and their derivatives, and fatty acyls show the significant differences among 370 differential metabolites which were unambiguously detected or tentatively identified. FSD and TPFD sam-

| Metabolites                          | Compound ID                  | m/z       | Retention time (min) | VIP   | P-value | log2(FC) |
|--------------------------------------|------------------------------|-----------|---------------------|-------|---------|----------|
| N-Carboxyacetyl-D-phenylalanine      | HMBDR039102                  | 232.061   | 4.016               | 1.111 | 0.000   | 37.990   |
| Agaritinal                           | HMBDR040694                  | 283.140   | 1.704               | 1.536 | 0.000   | 14.545   |
| D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose | HMBDR038663                  | 266.123   | 0.816               | 8.636 | 0.000   | 11.310   |
| (S)-2,3,4,5-Tetrahydropyridine-2-carboxylate | HMBDR012130                  | 110.060   | 0.850               | 1.034 | 0.000   | 7.307    |
| N5-Acetyl-N2-gamma-L-glutamyl-L-ornithine | HMBDR039423                  | 605.282   | 4.081               | 3.591 | 0.000   | 7.153    |
| Ustiloxin D                          | HMBDR041054                  | 475.219   | 4.470               | 2.133 | 0.000   | 6.074    |
| N2-(3-Hydroxysuccinoyl)arginine      | HMBDR032765                  | 329.084   | 1.184               | 4.284 | 0.000   | 5.518    |
| N-Acetyl-L-glutamate 5-semialdehyde | HMBDR006488                  | 156.066   | 0.906               | 1.428 | 0.000   | 2.683    |
| 2-Aminoheptanedioic acid             | HMBDR034252                  | 176.092   | 0.799               | 1.480 | 0.000   | 2.569    |
| L-gamma-Glutamyl-S-allythio-L-cysteine | HMBDR038515                  | 367.065   | 2.154               | 1.080 | 0.000   | 2.014    |
| N2-Galacturonyl-L-lysine             | HMBDR033105                  | 305.135   | 0.816               | 1.179 | 0.001   | 1.973    |
| Pyroglutamic acid                    | HMBDR000267                  | 130.050   | 1.184               | 1.414 | 0.000   | 1.853    |
| N,N'- Bis(gamma-glutamyl) cystine    | HMBDR038458                  | 499.114   | 0.886               | 1.916 | 0.004   | 0.407    |
| N-gamma-Glutamyl-S-allycysteine      | HMBDR031874                  | 279.178   | 0.810               | 1.371 | 0.000   | 0.509    |
| Isocitric acid                       | HMBDR000193                  | 191.019   | 0.865               | 4.588 | 0.017   | 0.548    |
| L-threo-Isocitric acid               | HMBDR001874                  | 191.019   | 1.197               | 4.778 | 0.008   | 0.831    |
| L-Valine                             | HMBDR000883                  | 118.086   | 0.867               | 1.908 | 0.000   | 0.929    |
| O-Acetylsertine                      | HMBDR000311                  | 130.050   | 0.867               | 1.791 | 0.000   | 1.471    |
| L-Threonine                          | HMBDR000167                  | 120.066   | 0.816               | 1.473 | 0.000   | 0.565    |
| Citric acid                          | HMBDR000094                  | 215.016   | 1.184               | 8.410 | 0.000   | 1.718    |
| (−)-Dioxibrassinin                   | HMBDR038634                  | 306.997   | 1.133               | 1.857 | 0.000   | 0.901    |
| L-Proline                            | HMBDR000162                  | 116.071   | 0.833               | 2.284 | 0.000   | 2.279    |
| Glyclyglycylglycine                  | HMBDR029419                  | 377.143   | 11.608              | 1.872 | 0.000   | 2.579    |
| N6-Acetyl-5S-hydroxy-L-lysine        | HMBDR033891                  | 205.118   | 0.850               | 1.225 | 0.000   | 2.623    |
| (S)-2-Azetidinocarboxylic acid       | HMBDR029615                  | 140.090   | 0.610               | 1.557 | 0.000   | 2.920    |
| L-2-Amino-3-(1-pyrazolyl)propanoic acid | HMBDR034267                  | 156.077   | 0.721               | 1.572 | 0.000   | 2.960    |
| L-Isoleucine                         | HMBDR000172                  | 132.102   | 1.422               | 8.430 | 0.000   | 2.970    |
| Nigellimine N-oxide                  | HMBDR033436                  | 237.123   | 3.226               | 1.033 | 0.000   | 3.139    |
| L-Dihydroorotic acid                 | HMBDR003349                  | 176.066   | 1.433               | 2.001 | 0.000   | 3.889    |
| N5-(4-Methoxybenzyl)glutamine        | HMBDR033598                  | 267.134   | 3.226               | 1.254 | 0.000   | 4.033    |
| L-Glutamine                          | HMBDR000641                  | 147.076   | 0.799               | 3.313 | 0.000   | 4.087    |
| Pyroglutamic acid                    | HMBDR000070                  | 147.113   | 0.709               | 2.144 | 0.000   | 4.142    |
| N-Perhydroxylpatic acid              | HMBDR040830                  | 327.120   | 7.595               | 1.316 | 0.004   | 4.986    |
| (2S,2'S)-Pyrosaccharopine            | HMBDR038676                  | 276.155   | 2.289               | 1.017 | 0.000   | 7.770    |
| L-2-Amino-5-hydroxypentanoic acid    | HMBDR031658                  | 172.037   | 1.683               | 1.822 | 0.006   | 15.423   |

Table 4. Information of carboxylic acids and derivatives with significant changes.
ples contained all the identified metabolites, there is a large amount of metabolites data available on FSD and TPFD under different processing. But the relative contents of individual compounds were remarkably different between the two groups.

The possible mechanism of substance and its bioactivities. Chemical structures of typical compounds and related bioactive in TPFD have been identified by OPLS-DA methods. For example, 43 metabolites were identified as chemical markers that could be used to distinguish FSD and TPFD samples according to qualitative research, and the contents of some compounds with anti-inflammatory and antioxidant, such as [6]-dehydroshogaol, capsaicin, arlatin, naringenin, methyl beta-D-glucopyranoside, and citroside A, significantly increased after processing according to quantitative research. This finding shows that traditional processing could possibly changed the contents of partial active compounds and improved the efficiency of *D. officinale*.

Anti-inflammatory and antioxidant related substance bases. 15 of 43 biomarkers screened for TPFD versus FSD, including [6]-Dehydroshogaol, N2-(3-Hydroxysuccinoyl)arginine, 4’5,8-Trihydroxyflavone, Naringenin, Citronellyl beta-sophoroside, Methyl beta-D-glucopyranoside, Hydroxysafflor yellow A, Corchoionol C 9-glucoside, Oleoside dimethyl ester, trans-p-Menthan-1,7,8-triol 8-glucoside, Acuminoside, Isocitric acid, D-threo-Isocitric acid, L-Isoleucine, Nerolidyl acetate, presented anti-inflammatory and antioxidant bio-activities. Their chemical construct show in following (Fig. 6).

This study found that 40 flavonoids were significantly different in TPFD and FSD samples, of which 26 flavonoids more abundant in TPFD than in FSD samples, while 14 flavonoids were significantly less abundant. Flavonoids as anti-inflammatory and antioxidant substances, two key substances, Naringenin and

| Metabolites | Compound ID | m/z | Retention time (min) | VIP | P-value | log2(FC) |
|-------------|-------------|-----|----------------------|-----|---------|----------|
| 1-(3-Methyl-2-butenoyl)-6-apiosylglucose | HMDB003952 | 417.137 | 4.173 | 3.630 | 0.000 | 6.660 |
| 3-Hydroxy-methylsalicylic acid | HMDB000355 | 523.098 | 1.283 | 1.028 | 0.001 | 5.356 |
| (3S,7E,9R)-4,7-Megastigmadiene-3,9-diol [apiosyl-(1→6)-glucoside] | HMDB0029766 | 549.255 | 3.696 | 1.583 | 0.000 | 4.962 |
| Eriosiposide B | HMDB0038029 | 499.252 | 4.533 | 1.946 | 0.000 | 4.694 |
| (3S,7E,9S)-7-Hydroxy-4,7-megastigmadien-3-one-9-glucoside | HMDB0036822 | 415.198 | 4.298 | 2.168 | 0.000 | 4.055 |
| 9-Hydroxy-7-megastigmen-3-one glucoside | HMDB0040701 | 417.213 | 4.059 | 1.208 | 0.000 | 2.878 |
| 3-Hydroxy-4,6-heptadiyne-1-yl 1-glucoside | HMDB0038688 | 395.204 | 4.321 | 2.859 | 0.000 | 2.553 |
| Blumenol C glucoside | HMDB0040688 | 501.208 | 4.622 | 2.504 | 0.000 | 2.171 |
| Eriosiposide A | HMDB0038028 | 501.208 | 4.622 | 2.504 | 0.000 | 2.171 |
| (3S,5R,6S,7E,9x)-7-Megastigmene-3,6,9-triol 9-glucoside | HMDB0041176 | 435.224 | 3.696 | 1.929 | 0.000 | 2.102 |
| Isopentyl gentiobioside | HMDB0041512 | 393.177 | 3.503 | 1.885 | 0.001 | 2.010 |
| 13-Oxo-9, 11-tridecadienoic acid | HMDB0034564 | 207.138 | 3.673 | 1.543 | 0.000 | 1.779 |
| Corchoionol C 9-glucoside | HMDB0029772 | 431.192 | 3.674 | 4.472 | 0.000 | 1.724 |
| Betulaluside A | HMDB0035634 | 355.173 | 4.278 | 3.904 | 0.000 | 1.246 |
| Premil apiosyl-(1→6)-glucoside | HMDB0031956 | 379.161 | 3.546 | 1.250 | 0.000 | 1.196 |
| (2E,4E,7R)-2,7-Dimethyl-2,4-octadiene-1,8-diol 8-O-b-D-glucopyranoside | HMDB0038747 | 355.173 | 3.901 | 7.401 | 0.000 | 1.187 |
| [6]-Gingerdiol 5-O-beta-D-glucopyranoside | HMDB0036123 | 503.250 | 4.990 | 2.347 | 0.000 | 1.064 |
| 3,7-Dimethyl-5-octene-1,7-diol 1-glucoside | HMDB0034771 | 357.188 | 3.962 | 1.340 | 0.000 | 0.761 |
| 6-Feruloylglycose 2,3,4-trihydroxy-3-methylbutylglycoside | HMDB0036214 | 497.163 | 4.363 | 1.441 | 0.002 | −0.443 |
| Angelic acid | HMDB0029608 | 118.086 | 1.184 | 1.483 | 0.000 | −2.146 |
| Avenolic acid | HMDB0029978 | 279.232 | 9.311 | 1.333 | 0.000 | −2.399 |
| Mangiferic acid | HMDB0029800 | 263.237 | 8.565 | 1.810 | 0.000 | −2.355 |
| 5a,6a-Epoxy-7E-megastigmente-3b,9e-diol 9-glucoside | HMDB0038306 | 389.218 | 9.883 | 1.387 | 0.000 | −3.445 |
| 4,8,12,15-Octadecatetraenoic acid | HMDB0032672 | 277.216 | 9.709 | 1.684 | 0.000 | −3.581 |
| Stearidonic acid | HMDB0006547 | 277.216 | 8.799 | 2.397 | 0.000 | −4.694 |
| 12-Hydroxy-8, 10-octadecadienoic acid | HMDB0029998 | 297.242 | 8.437 | 2.989 | 0.000 | −4.897 |
| 9, 10-DHOME | HMDB0004704 | 313.238 | 8.414 | 2.529 | 0.000 | −5.173 |
| (R)-1-O-[b-D-Glucopyranosyl-(1→6)-b-D-glucopyranoside]-1,3-octanediol | HMDB0032799 | 488.271 | 3.569 | 2.465 | 0.000 | −5.512 |
| Corchorifatty acid F | HMDB0035919 | 309.207 | 8.814 | 1.747 | 0.000 | −5.921 |

Table 5. Information of fatty acyls with significant changes.

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Figure 5. Orthogonal projections of latent structures discriminant analysis (OPLS-DA) and cluster analysis. (A): S-plots of the OPLS-DA model for the TPFD versus FSD sample comparison. (B): Loading of the OPLS-DA model for TPFD samples versus FSD samples. (C): Heatmap for TPFD samples versus FSD samples.
| Metabolites                                      | Compound ID      | Class                               | VIP  | log2(FC) | P-value | Activities                          | Reference |
|-------------------------------------------------|------------------|-------------------------------------|------|----------|---------|-------------------------------------|-----------|
| [6]-Dehydroshogaol                              | HMDB0033090      | Cinnamic acids and derivatives       | 4.859| 13.161   | 0.000   | Anti-inflammatory Anti-oxidant       | 34,35,36  |
| Capsaicin                                       | HMDB0002227      | Phenols                             | 4.465| 11.882   | 0.000   | Anti-inflammatory; Analgesia         | 37,38,39  |
| D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose    | HMDB0038663      | Carboxylic acids and derivatives     | 8.636| 11.310   | 0.000   | N/A                                | N/A       |
| Norcapsaicin                                    | HMDB0036327      | Phenols                             | 4.355| 9.189    | 0.000   | Neuroprotection; Anti-Alzheimer      | 40,41,42  |
| Arlatin                                         | HMDB0035740      | Prenol lipids                        | 5.270| 8.883    | 0.000   | Anti-tumor                          | 43        |
| N2-(3-Hydroxy succinoyl)arginine                | HMDB0032765      | Carboxylic acids and derivatives     | 4.284| 5.518    | 0.000   | Anti-inflammatory                   | 44        |
| (4R,5S,7R,11S)-11,12-Dihydroxy-1(10)-spirovetiven-2-one 11-glucoside | HMDB0033150 | Prenol lipids                        | 4.807| 4.462    | 0.000   | N/A                                | N/A       |
| Maltol                                          | HMDB0030776      | Pyrans                              | 7.266| 4.351    | 0.001   | Anti-bacterial, Anti-toxicity        | 45,46     |
| 4',5,8-Trihydroxyflavonone                      | HMDB0031824      | Flavonoids                          | 10.133| 4.266    | 0.000   | Anti-mutagenic                      | 47        |
| Naringenin                                      | HMDB0002670      | Flavonoids                          | 5.399| 4.219    | 0.000   | Anti-bacterial                      | 48,49     |
| Citronellyl beta-sophoroside                    | HMDB0032839      | Prenol lipids                        | 8.101| 3.354    | 0.000   | Anti-inflammatory                   | 50        |
| Methyl beta-D-glucopyranoside                   | HMDB0029965      | Organooxygen compounds               | 5.375| 3.525    | 0.000   | Anti-tumor, Anti-bacterial, Anti-nociceptive, Anti-inflammatory | 51,52     |
| Hydroxysafflor yellow A                         | HMDB0040677      | Cinnamic acids and derivatives       | 6.967| 3.302    | 0.000   | Anti-bacterial, Anti-inflammation   | 53        |
| Apigenin 7-[(galactosyl)-(1->4)-mannoside]       | HMDB0037852      | Flavonoids                          | 5.394| 3.037    | 0.000   | N/A                                | N/A       |
| 5a,6a-Epoxy-7E-megastigmen-3a,9e-diol 3-glucoside| HMDB0031676      | Organooxygen compounds               | 4.134| 2.426    | 0.000   | N/A                                | N/A       |
| Foeniculoside VIII                             | HMDB0033009      | Organooxygen compounds               | 4.373| 2.381    | 0.001   | N/A                                | N/A       |
| Kiwisonoside                                    | HMDB0038691      | Prenol lipids                        | 4.578| 2.217    | 0.000   | N/A                                | N/A       |
| Icariside B8                                   | HMDB0036846      | Prenol lipids                        | 4.161| 1.975    | 0.000   | N/A                                | N/A       |
| Cochoinosol C 9-glucoside                      | HMDB0029772      | Fatty Acyls                         | 4.472| 1.724    | 0.000   | N/A                                | N/A       |
| Citroside A                                     | HMDB0030370      | Prenol lipids                        | 4.761| 1.723    | 0.000   | Hypertension, Anti-inflammatory     | 54        |
| 6Z,8-Hydroxygeraniol 8-O-glucoside              | HMDB0035925      | Prenol lipids                        | 5.028| 1.480    | 0.000   | N/A                                | N/A       |
| beta-D-Galactopyranosyl-(1->3)-beta-D-galactopyranosyl-(1->6)-D-galactose | HMDB0038853 | Organooxygen compounds               | 5.111| 1.441    | 0.000   | N/A                                | N/A       |
| Oleoside dimethyl ester                        | HMDB0031350      | Prenol lipids                        | 4.651| 1.346    | 0.000   | Anti-oxidant                        | 55        |
| 6-Kestose                                       | HMDB0033673      | Organooxygen compounds               | 4.641| 1.289    | 0.000   | N/A                                | N/A       |
| (2E,4E,7R)-2,7-Dimethyl-2,4-octadiene-1,8-diol 8-O-b-D-glucopyranoside | HMDB0038747 | Fatty Acyls                         | 7.401| 1.187    | 0.000   | N/A                                | N/A       |
| Linalool oxide D 3-[(apiosyl)-(1->6)-glucoside]  | HMDB0031367      | Organooxygen compounds               | 4.502| 0.881    | 0.000   | N/A                                | N/A       |
| trans-p-Menthan-1,7,8-triol 8-glucoide          | HMDB0034784      | Organooxygen compounds               | 4.657| 0.628    | 0.003   | N/A                                | N/A       |
| Osmanthuside B                                 | HMDB0038749      | Cinnamic acids and derivatives       | 4.202| 0.567    | 0.001   | Anti-depressant, Inflammatory, Anti-oxidant, Lipase inhibition | 56        |
| Trehalose                                       | HMDB0000975      | Organooxygen compounds               | 7.692| 0.301    | 0.004   | Anti-depressant                     | 57        |
| Acuminoside                                     | HMDB0029347      | Prenol lipids                        | 4.358| -0.294   | 0.031   | Immune enhancer abd Anti-inflammatory | 58        |
| Isocitric acid                                  | HMDB000193       | Carboxylic acids and derivatives     | 4.588| -0.548   | 0.017   | Surfactants, detergents, ion chelators and biologically active, Anti-oxidant | 59,60     |

Continued
4',5,8-Trihydroxyflavanone of flavonoids' compounds, have outstanding changed (Table 3) so that TPSD can better exert its anti-inflammatory and antioxidant effects (Table 6).

In order to promote further research into FSD and TPFD, attentions should be paid to the following work in the future: (1) Although current metabolites technologies have been used to study TPFD, there are limitedly comprehensive metabonomics studies. Obviously, the negative or positive ion model method such as metabolomics cannot satisfy the necessary deep research into D. officinale. (2) it is important to comprehensively investigate why differences exist in active compounds between FSD and TPFD which can provide propitiate conditions for metabolite accumulation. (3) its TPFD applications has been rarely described, and TPFD improvements are still required for its industrial applications. (4) the functions of most these compositions need to confirm through functional investigation.

### Conclusion

For thousands of years, D. officinale has been processed to enhance its medicinal value for use in TCM. The chemical composition of this material after processing is key to its efficacy. Traditional processing of D. officinale produces a difference in the contents of key metabolites. Moreover, combining metabolomics and multivariate statistical analysis methods can accurately identify markers that differentiate processed and raw materials. Thus, we revealed the basis of the improvement in efficacy of TPFD compared with FSD, enabling the identification of active substances with functions not included in the traditional use of TPFD. These results indicate the need for the further exploration of TPFD in the treatment of additional, previously untested conditions. This systematic study of FSD and TPFD provides a useful analytical strategy for rapidly screening and identifying the constituents of other TCMs and TCM formulas. In addition, the results of this research provide a theoretical basis for quality control.

### Table 6. 43 biomarkers screened for TPFD vs FSD and their biological activities.

| Metabolites                                                                 | Compound ID          | Class                                | VIP   | log2(FC) | P-value | Activities                                                                 | Reference |
|----------------------------------------------------------------------------|----------------------|--------------------------------------|-------|----------|---------|----------------------------------------------------------------------------|-----------|
| L-Histidinol                                                              | HMDB0003431          | Organonitrogen compounds             | 6.333 | -0.585   | 0.000   | Against CDDP, Nephrotoxicity, cytoprotective and attenuate fanconi syndrome, Anti-tumour activity and cardiotoxicity | 73, 74    |
| 3,5-Dihydroxyphenyl 1-O-(6-O-galloyl-beta-D-glucopyranoside)               | HMDB0039307          | Organooxygen compounds               | 8.940 | -0.709   | 0.000   | Anti-oxidant                                                              | 76        |
| D-threo-Isocitric acid                                                    | HMDB0001874          | Carboxylic acids and derivatives     | 4.778 | -0.831   | 0.001   | Anti-inflammatory                                                          | 77        |
| cis-Resveratrol 3-sulfate                                                 | HMDB0041712          | Stilbenes                           | 7.225 | -0.906   | 0.000   | N/A                                                                        | N/A       |
| Citric acid                                                              | HMDB0000094          | Carboxylic acids and derivatives     | 8.410 | -1.718   | 0.000   | Anti-oxidant, Anti-fatigue                                                 | 72        |
| (5)-Nerolidol 3-O-[a-L-Rhamnopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-b-D-glucopyranoside] | HMDB0040845          | Organooxygen compounds               | 5.223 | -1.917   | 0.000   | N/A                                                                        | N/A       |
| (5)-Nerolidol 3-O-[a-L-Rhamnopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->6)-b-D-glucopyranoside] | HMDB0040846          | Organooxygen compounds               | 6.236 | -2.019   | 0.000   | N/A                                                                        | N/A       |
| 3,4-Dihydro-2H-1-benzopyran-2-one                                         | HMDB0036626          | 3,4-dihydrocoumarins                 | 8.459 | -2.892   | 0.000   | Anti-tyrosinase                                                           | 80        |
| L-Isoleucine                                                              | HMDB0000172          | Carboxylic acids and derivatives     | 8.430 | -2.970   | 0.000   | Anti-oxidant                                                              | 81        |
| 1-O-Cinnamoyl-(6-arabinosyl-glucose)                                      | HMDB0030294          | Cinnamic acids and derivatives       | 11.458 | -3.554   | 0.000   | N/A                                                                        | N/A       |
| 3,8-Dihydroxy-9-methoxycoumestan                                          | HMDB0030562          | Isoflavonoids                        | 4.619 | -4.279   | 0.000   | N/A                                                                        | N/A       |
| Nerolidyl acetate                                                         | HMDB0039630          | Prenol lipids                        | 4.241 | -7.130   | 0.000   | Anti-inflammatory                                                          | 82        |
Figure 6. Chemical constructure of 15 significant increased compounds.

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References
1. Ngo, L. T., Okogun, J. I. & Folk, W. R. 21st century natural product research and drug development and traditional medicines. Nat. Prod. Rep. 30, 584–592 (2013).
2. Li, S. P., Wu, D. T., Lv, G. P. & Zhao, J. Carbohydrates analysis in herbal glycomics. Trac-Trends Anal. Chem. 52, 155–169 (2013).
3. Lei, H. B. et al. A comprehensive quality evaluation of Fuzi and its processed product through integration of UPLC-QTOF/MS combined MS/MS-based mass spectral molecular networking with multivariate statistical analysis and HPLC-MS/MS. J. Ethnopharmacol. 266, 113455 (2021).
4. Zhao, Z. Z. et al. A unique issue in the standardization of Chinese materia medica: Processing. Planta Med. 76, 1975–1986 (2010).
7. Xu, M.
9. Zhao, Y.J., Han, B.X., Peng, H.S. & Peng, D.Y. Research on evolution and transition of quality evaluation of Shihu.
12. Wan, J.Y.
15. Hu, C. & Xu, G. Metabolomics and traditional Chinese medicine.
14. Sumner, L.W., Lei, Z., Nikolau, B.J. & Saito, K. Modern plant metabolomics: Advanced natural product gene discoveries, improved technologies, and future prospects. Nat. Prod. Rep. 32, 212–229 (2015).

16. Zhang, A. et al. Metabolomics: Towards understanding traditional Chinese medicine. Planta Med. 76, 2026–2035 (2010).
17. Sun, H. et al. Metabolomics study on Fuzi and its processed products using ultra-performance liquid-chromatography/electrospray-argon ionization synapt high-definition mass spectrometry coupled with pattern recognition analysis. Analyst 137, 170–185 (2012).
18. Wang, X. et al. Metabolomics study on the toxicity of aconite root and its processed products using ultraperformance liquid-chromatography/electrospray-argon ionization synapt high-definition mass spectrometry coupled with pattern recognition approach and ingenuity pathways analysis. J. Proteome Res. 11, 1284–1301 (2012).
19. Geng, L. et al. Discrimination of raw and vinegar-processed Genkwa Flos using metabolomics coupled with multivariate data analysis: A discrimination study with metabolomics coupled with PCA. Fitoterapia 84, 286–294 (2013).

20. Chen, H. et al. Traditional uses, phytochemistry, pharmacology, and quality control of Dendrobium officinale Kimura et. Migo. Front Pharmacol. 12, 726528 (2021).
21. Wang, Y., Tong, Y., Adejobi, O.I., Wang, Y.H. & Liu, A.Z. Research advances in multi-omics on the traditional Chinese Herb Dendrobium officinale. Front. Pharm. Sci. 12, 808288 (2022).
22. Yue, H., Zeng, H. & Ding, K. A review of isolation methods, structure features and bioactivities of polysaccharides from Dendrobium species. Chin. J. Nat. Med. 18, 1–27 (2020).
23. Fahy, E. et al. Update of the LIPID MAPS comprehensive classification system for lipids. J. Lipid Res. 50, S9–S14 (2009).
24. Surmacz, I. & Swieszewska, E. Polysaccharides−secondary metabolites or physiologically important superlipids?. Biochimie Biophys. Res. Commun. 407, 627–632 (2011).
25. Kidd, P. Vitamin D and K as pleiotropic nutrients: Clinical importance to the skeletal and cardiovascular systems and preliminary evidence for synergy. Altern. Med. Rev. 15, 199–222 (2010).
26. Jiang, Q. Natural forms of vitamin E: Metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic. Biol. Med. 72, 76–90 (2014).
27. Bayat, P., Farshchi, M., Yousefian, M., Mahmoudi, M. & Yagdian-Robati, R. Flavonoids, the compounds with anti-inflammatory and immunomodulatory properties, as promising tools in multiple sclerosis (MS) therapy: A systematic review of preclinical evidence. Int. Immunopharmacol. 95, 107562 (2021).
28. Heim, K.E., Tagliaferro, A.R. & Bobilya, D.J. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13, 572–584 (2002).
29. Aquila, S., Giner, R.M., Recio, M.C., Spegazzini, E.D. & Rios, J.L. Anti-inflammatory activity of flavonoids from Cayaponia tayuya roots. J. Ethnopharmacol. 121, 333–337 (2009).
30. Sharma, H., Kumar, P., Deshmukh, R.R., Bisahaye, A. & Kumar, S. Pentacyclic triterpenes: New tools to fight metabolic syndrome. J. Agric. Food Chem. 57, 4467–4477 (2009).
31. Privitera, R. & Anand, P. Capsaicin 8% patch Quteza and other current treatments for neuropathic pain in chemotherapy-induced peripheral neuropathy (CIPN). Care Open Support. Palliat. Care 15, 125–131 (2021).
32. Vila, D.L., Nunes, N., Almeida, P., Gomes, J., Rosa, C., & Alvarez-Leite, J. Signaling targets related to antiobesity effects of capsicain: A scoping review. Adv. Nutr. 1–12 (2021).
33. Simpson, D.M., Estaniela, L., Brown, S.J. & Sampson, J. An open-label pilot study of high-concentration capsaicin patch in painful HIV neuropathy. J. Pain Symptom Manag. 35(3), 299–306 (2008).
34. Westphal, C., Cermax, J., Cole, R.O., Shortt, G.F., Perni, R., & Ponduru, S. Formulations and use of TRP channel activators in treatment of nervous system disorders. PCT Int. Appl. WO 2015160843 A1 (2015).
35. Chen, C.L., Mao, C., Zhang, J. Application of vaniloid receptor agonist to prevent anti-Alzheimer's medical products. Faming Zhouzi Shengying. CN 1736485 A (2006).
36. Ando, R., Sakurada, S., Kisara, K., Takahashi, M. & Ohnawa, K. Effects of intra-arterially administered capsainoids on vocalization in guinea pigs and medial thalamic neuronal activity in cats. Nippon Yakurigaku Zasshi 79, 275–283 (1982).
37. Chen, M. et al. Ligustrum robustum (Roxb.) blume extract modulates gut microbiota and prevents metabolic syndrome in high-fat diet-fed mice. J. Ethnopharmacol. 268, 11695 (2021).
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Author contributions
L.Y. designed the project, coordinated the entire study, and revised the manuscript. D.Z. performed all the experiments and the data analysis. Y.Z. and Z.C. conducted the field investigation and data collation. X.Y. analyzed the data, and revised the manuscript. Y.Z. and L.G. wrote and revised the final manuscript. All authors have read and agreed to the published version of the manuscript.

Competing interests
The authors declare no competing interests.

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