Research Article

Rapid Identification of Chemical Constituents in *Hericium erinaceus* Based on LC-MS/MS Metabolomics

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*Hericium erinaceus* is a precious edible and medicinal fungus with high nutritional value. It has many functions, such as enhancing immunity, antitumor antioxidation, antihyperglycemic, antihyperlipidemic, and protecting gastric mucosa. However, there are few researches about the *H. erinaceus* compounds. In this paper, ultraperformance liquid chromatography tandem high-resolution mass spectrometry (UPLC-Q-exactive-MS/MS) was used to isolate and identify the compounds in *H. erinaceus*. 102 compounds were identified in *H. erinaceus* by comparing with standard databases such as MZVault, MZCloud, and BGI Library (self-built standard library by BGI Co., Ltd), including flavonoids, terpenoids, phenolic acids, phenylpropanoids, steroids, organic acids, and amino acids.

1. Introduction

*Hericium erinaceus* is a precious edible and medicinal fungus, and it is listed as one of the “Four Famous Cuisines” of China, together with bear’s paws, trepang, and shark’s fin [1]; it has been used for a long time in traditional Chinese medicine [2]. Researches showed that the chemical constituents of *H. erinaceus* include terpenoids, phenolics, steroids, pyranones, fatty acids, and alkaloids; about 80 small molecular compounds were isolated and identified from *H. erinaceus* [3]. Terpenoids in *H. erinaceus* were mainly diterpenoids with cyathane skeleton. Terpenoids in *H. erinaceus* were first isolated and identified by Kawagishi et al. from mycelia of *H. erinaceus*, named Erinacines A-C [4]. Subsequently, Kawagishi et al. isolated and identified Erinacines D-G, Erinacines J, and Erinacines K from mycelia of *H. erinaceus* [5–7]; Lee et al. isolated and identified Erinacines H and Erinacines I from mycelia of *H. erinaceus* [8]; Kenmoku et al. isolated and identified Erinacine P and Erinacine Q from mycelia of *H. erinaceus* [9, 10]. Most of these diterpenoids compounds were stimulators of nerve growth factor-synthesis. Phenolics were also main constituents in *H. erinaceus*. 8 phenolics were isolated and identified from fruiting bodies of *H. erinaceus* by the Kawagishi team between 1990 and 1993, named Hericenone A-H [11–13]. After that, three phenolics were isolated and identified by Arnone et al. [14] with 5′-carbonyl group replaced by 5′-methylene, named Hericenes A-C. Subsequently, two new phenolics were isolated from fruiting bodies of *H. erinaceus* by Ma et al. [15], named Hericenone I and Hericenone D; they have the same fatty acid chain. A new skeleton phenolic compound was isolated from fruiting bodies of *H. erinaceus* by Li et al. [16], named Erinacene D. In addition to terpenoids and phenolics compounds, *H. erinaceus* was rich in steroids, six new (erinarols A-F), and five known ergostane-type sterol fatty acid esters were isolated from the fruiting bodies of *H. erinaceus*; erinarols A and B significantly activated the transcriptional activity of PPARα, PPARγ, and PPARδ [17]. Previous pharmacological studies showed that *H. erinaceus*
had many pharmacological activities, such as regulating immunity [18, 19], neuroprotection [20, 21], antidepressant [22, 23], antioxidant [24], antitumor [25], anti-hyperglycemic [26], and antihyperlipidemic properties [27], and protecting gastric mucosa [28, 29].

High-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) technology has the advantage of rapid identification of compounds. With the maturity of LC-MS/MS technology, it is more and more widely used in the field of food and medicine [30–35]. In this study, ultraperformance liquid chromatography tandem high-resolution mass spectrometry (UPLC-Q-Exactive-MS/MS) combined with standard substance database was used to rapidly identify the chemical components of \textit{H. erinaceus}.

\section{Materials and Methods}

\subsection{Instrument.} The LC-MS experiment was carried out by ultraperformance liquid chromatography (Waters 2D UPLC, USA) and high-resolution mass spectrometry (Q Exactive, Thermo, USA). The Hypersil GOLD aQ column (100 mm×2.1 mm, 1.9 μm) was purchased from Thermo Fisher Scientific, USA. Low-temperature high-speed centrifuge (Centrifuge 5430) was purchased from Eppendorf. The vortex finder (QL-901) was purchased from Qilinbei Instrument Manufacturing Co., Ltd. The pure water meter (Milli-Q) was purchased from Integral Millipore Corporation, USA.

\subsection{Reagent.} \textit{d}_3-leucine, \textit{13C}_9-phenylalanine, \textit{d}_5-tryptophan, and \textit{13C}_3-progesterone were used as internal standards. Methanol (A454-4) and acetonitrile (A996-4) were both in mass spectral grade, which were purchased from \textit{H}ermo Fisher Scientific, USA. Ammonium formate (17843–250G) was obtained from Honeywell Fluka, USA. Formic acid (50144–50 mL) was obtained from DIMKA, USA.

\subsection{Materials.} Fruiting bodies of \textit{H. erinaceus} were obtained from Henan Longfeng Industrial Co., Ltd. The specimens (No. 2020-09-09) were saved in National Research and Development Center of Edible Fungi Processing Technology, Henan University.

\subsection{Preparation of Sample.} According to the methods commonly used by our team, dry fruiting bodies of \textit{H. erinaceus} were crushed by the grinding machine. 100 g of \textit{H. erinaceus} powder was immersed with 50% ethanol (1000 mL) for 2 times at room temperature, each time for 3 days. The filtrate was concentrated to obtain 48.6 g extract. 50 mg extract of \textit{H. erinaceus} was weighed, and then 800 μL of solution with 70% methanol: internal standard (V/V = 85 : 1) was added to the sample. Two small steel beads were added to the sample and ground in a tissue grinder (50 Hz, 5 min), ultrasound for 10 min at 4°C water, and the sample was placed for 1 h at −20°C. After that, the sample was centrifuged for 15 min at 4°C, 25000 rpm, 500 μL of supernatant was filtered through 96-well filter plate, and the filtrate was used to detect.

\subsection{Chromatographic Conditions.} The LC-MS experiment was carried out on Hypersil GOLD a Q column (100 mm×2.1 mm, 1.9 μm). The mobile phase was 0.1% formic acid-water (liquid A) and 0.1% formic acid-acetonitrile (liquid B). The elution gradient was as follows: 0−2 min 5% B; 2−22 min 5%−95% B; 22−27 min 95% B; 27.1−30 min 5% B. The flow rate was 0.3 mL/min, the column temperature was 40°C, and the injection volume was 5 μL.

\subsection{Mass Spectrometry Conditions.} The MS and MS\textsuperscript{2} data were collected by the Q Exactive mass spectrometer. The range of \textit{m/z} was 150−1500 with the MS resolution 70000, and the AGC was 1 × 10\textsuperscript{6}, and the maximum injection time was 100 ms. According to the strength of the MS ions, top3 was selected for determining MS\textsuperscript{2} fragmentation. The MS\textsuperscript{2} resolution was 35000, AGC was 2 × 10\textsuperscript{6}, the maximum injection time was 50 ms, and the fragmentation energy was set as 20, 40, and 60 eV. Ion source (ESI) parameters are as follows: sheath gas flow rate was 40, aux gas flow rate was 10, spray voltage (|KV|) of positive ion mode was 3.80, spray voltage (|KV|) of negative ion mode was 3.20, capillary temp was 320°C, and aux gas heater temp was 350°C.

\subsection{Data Analysis.} High-resolution mass spectrometer (Q Exactive, Thermo Fisher Scientific, USA) was used to collect data in positive and negative ion modes, respectively, to improve the chemical constituent coverage. Raw mass spectrum data collected by LC-MS/MS were imported into Compound Discoverer 3.1 (Thermo Fisher Scientific, USA) for data processing. It mainly includes peak extraction, retention time correction within and between groups, additive ion merging, missing value filling, background peak labeling, and metabolite identification. Finally, the molecular weight, retention time, peak area, and identification results of the compound were derived.

\section{Results}

\subsection{Total Ion Chromatogram.} The total ion chromatogram of \textit{H. erinaceus} is shown in Figure 1.

\subsection{Results of Compound Identification.} The compounds of \textit{H. erinaceus} were analyzed by LC-MS/MS; the identification of structure was based on the retention time, MS data, and MS\textsuperscript{2} data compared with the standard database. The identified compounds were classified into three grades (Level 1, Level 2, and Level 3) according to the comparison results, there into, Level 2 was confirmed on the basis of MS data, MS\textsuperscript{2} data, and properties of compounds, and Level 3 was identified on the basis of MS data and MS\textsuperscript{2} data. The credibility sequence is as follows: Level 1 > Level 2 > Level 3. Detailed results are shown in Table 1. 102 compounds were preliminarily identified in \textit{H. erinaceus} with grade of identification as Level 1 and Level 2, including 31 organic acids, 10 nucleotides and analogues, 8 amino acids, 6 carbohydrates and derivatives, 5 flavonoids, 3 unsaturated fatty acids, 3 terpenoids, 3 phenolic acids, 1 phenylpropanoid, 1
steroid, and 32 other compounds. Most flavonoids and organic acids were easily deprotonated and responded in the negative ion mode. Most nucleoside compounds were easily protonated and responded in the positive ion mode. The identification process of some compounds was as follows.

Compound 49, C_{13}H_{10}O_{6}, was easily deprotonated in the negative ion mode to produce ion $m/z$ 285 [M − H]$^-$ and then RDA cracking to get 2 fragment ions $m/z$ 133 [C_{6}H_{4}O_{2} − H]$^-$ and $m/z$ 151 [C_{7}H_{4}O_{4} − H]$^-$, Compound 49 was identified as luteolin compared to the database and references [36]. The $M^2$ spectrum is shown in Figure 3.

Compound 60 produced ion $m/z$ 283 [M − H]$^-$, then lost the methyl group, and got ion 268 [M − H − CH$_3$]$^-$, combined with retention time, MS data, and $M^2$ data, compound 60 was identified as acacetin by comparin with the database and references [37]. The $M^2$ spectrum is shown in Figure 2.

Compound 62 was easily protonated in the positive ion mode to produce ion $m/z$ 249 [M + H]$^+$, then lost the hydroxyl group, and got ion $m/z$ 231 [M + H − H$_2$O]$^+$, Compound 62 was identified as atracylenolide II by comparing with the database and references [38]. The $M^2$ spectrum is shown in Figure 4.

4. Discussion and Conclusion

In this study, 102 compounds were preliminarily identified in $H$. erinaceus, including organic acids, nucleotides and analogues, amino acids, carbohydrates and derivatives, flavonoids, unsaturated fatty acids, terpenoids, phenolic acids, phenylpropanoid, and steroid. It revealed that the compounds in $H$. erinaceus were diverse. Previous studies showed that the chemical constituents of $H$. erinaceus include terpenoids, phenolics, steroids, pyranones, fatty acids, and alkaloids; thereinto, reports about terpenoids and phenolics in $H$. erinaceus were more [3–16]. Terpenoids and phenolics were less in this study; the reasons may be different material parts, different origins, different varieties, different extraction methods, and so on. In addition, nucleotides were also the main constituents in $H$. erinaceus. Yan et al. [39] studied the content of five nucleosides in $H$. erinaceus from different habitats by high-performance liquid chromatography; the results showed that different origins of $H$. erinaceus had the same nucleoside components, such as cytosine, inosine, and adenosine, and the content of inosine and adenosine in $H$. erinaceus was higher. In this study, 10 nucleotides and analogues were identified, including adenosine, adenine, guanosine, guanine, and uridine.

Most of the compounds in $H$. erinaceus had a good biological activity; researches about polysaccharide were more, which had a wide variety of pharmacological functions such as antimicrobial, antiabetic, and antihypertension ones [1]. Small molecular compounds isolated from $H$. erinaceus also had many biological activities. Diterpenoids in $H$. erinaceus could promote the synthesis of nerve growth factors, such as Erinacines A, Erinacines B, Erinacines C, Erinacines D, Erinacines E, Erinacines F, Erinacines G, Erinacines H, and Erinacines I [4–6]. Phenolics compounds in $H$. erinaceus could also promote the synthesis of nerve growth factors, such as Hericenone C, Hericenone D, Hericenone E, and Hericenone H [12, 13]. Hericenones A and B showed cytotoxicity against HeLa cells [11]. In addition, Hericenone B was found to be a potential antiplatelet aggregation agent [40]. Steroids in $H$. erinaceus could activate the transcriptional activity of PPARs, PPAR, and PPARy, such as erinarols A and erinarols B. In addition, nucleosides and flavonoids may be the main active components of $H$. erinaceus. Nucleoside components have many biological activities, such as antitumor, antivirus, and gene therapy. Studies have shown that adenosine, inosine, and guanosine have many pharmacological effects such as regulating immunity, neuroprotection, and treatment of cardiovascular diseases [41]. Flavonoids also have many pharmacological effects, such as neuroprotection, antimyocardial ischemia, hypotension, improved learning and memory, antigastric ulcer, protection of reproductive tissue, anti-inflammatory, and antitumor [42].

In a word, $H$. erinaceus has high nutritional value and medicinal value. At present, it has been developed into a variety of functional foods, including health wine, health drinks, healthy yogurt, tea, cans, and health vinegar [2], which was of great significance to study the chemical composition of $H$. erinaceus. In this study, 102 compounds were preliminarily identified to provide reference for the follow-up study of $H$. erinaceus.
| Number | Retention time (min) | Molecular formula | Relative molecular weight (Da) | The measured values (m/z) | Adduct ions | Error (ppm) | Peak | Compound name | Compound type | mzVault best match | mzCloud best match | Level |
|--------|---------------------|-------------------|-----------------------------|--------------------------|-------------|-------------|------|---------------|---------------|----------------|----------------|-------|
| 1      | 0.743               | C₁₇H₂₃N₃O₁₇P₂    | 607.08113                   | 606.07379                | [M – H]⁻    | -0.72       | 143833617.1 | Udp-α-acetylglucosamine | Nucleotides and analogues | NA       | 95.8       | Level 2 |
| 2      | 0.826               | C₁₇H₂₃NO₃       | 161.10508                   | 162.12124                | [M + H]^+   | -0.70       | 454543107.3 | L(−)-Carnitine | Others         | 77.9       | 85.7       | Level 2 |
| 3      | 0.869               | C₅H₁₀O₅         | 134.02147                   | 133.01422                | [M – H]⁻    | -0.41       | 2352956792.8 | DL-Malic acid | Organic acids | NA       | 98.8       | Level 2 |
| 4      | 0.87                | C₃H₆O₃          | 116.011                     | 115.00369                | [M – H]⁻    | 0.33        | 1998652749.7 | Fumaric acid | Organic acids | 90.7       | 98.1       | Level 1 |
| 5      | 0.883               | C₅H₁₀O₅         | 129.04263                   | 128.03534                | [M – H]⁻    | 0.31        | 1198916725.1 | 4-Oxoprolipine | Amino acids | NA       | 88         | Level 2 |
| 6      | 0.928               | C₅H₁₀O₅         | 152.06831                   | 151.06111                | [M – H]⁻    | -1.07       | 533909840.4 | Ribitol | Carbohydrates and derivatives | 90.6       | 98.1       | Level 1 |
| 7      | 0.939               | C₆H₁₀O₆         | 182.07893                   | 181.07164                | [M – H]⁻    | -0.61       | 328836918.0 | D(-)-Mannitol | Carbohydrates and derivatives | 93.0       | 98.4       | Level 2 |
| 8      | 0.94                | C₁₂H₂₂O₁₂       | 358.1107                    | 357.10416                | [M – H]⁻    | -1.19       | 58626917.89 | Lactobionic acid | Carbohydrates and derivatives | 87.8       | NA         | Level 1 |
| 9      | 1.026               | C₆H₁₀O₇         | 182.07914                   | 183.0865                 | [M + H]^+   | 0.55        | 272725138.5 | Galactitol | Carbohydrates and derivatives | 85.6       | 93.8       | Level 1 |
| 10     | 1.061               | C₉H₁₃N₅O₅       | 243.08565                   | 244.07914                | [2M + H]^+  | 0.55        | 156519671.1 | Cytarabine | Nucleotides and analogues | 79.0       | 85.6       | Level 2 |
| 11     | 1.066               | C₉H₁₃N₅O₅       | 181.07393                   | 182.08116                | [M + H]^+   | 0.21        | 226778893.2 | L-Tyrosine | Amino acids | 86.5       | 91.3       | Level 2 |
| 12     | 1.079               | C₆H₁₀O₇         | 222.07377                   | 221.06636                | [M – H]⁻    | -0.81       | 939035522.5 | Ethyl-β-d-glucuronide | Others | NA       | 96.2       | Level 2 |
| 13     | 1.08                | C₆H₁₀O₇         | 244.0691                    | 243.0618                 | [M – H]⁻    | -1.77       | 270847192.6 | Uridine | Nucleotides and analogues | 87.9       | 86.2       | Level 2 |
| 14     | 1.084               | C₆H₁₀O₇         | 192.02693                   | 191.01961                | [M – H]⁻    | -0.38       | 261106825.7 | Citric acid | Organic acids | 92.1       | 96.7       | Level 1 |
| 15     | 1.088               | C₆H₁₀O₇         | 148.03713                   | 147.02985                | [M – H]⁻    | -0.31       | 355626048.3 | D-α-hydroxyglutaric acid | Organic acids | NA       | 91.2       | Level 2 |
| 16     | 1.094               | C₆H₁₀O₇         | 174.0163                    | 173.00929                | [M – H]⁻    | -0.82       | 116011083.2 | Trans-aconitic acid | Organic acids | 43.7       | 81.9       | Level 2 |
| 17     | 1.095               | C₇H₁₁N₅O₅       | 189.06359                   | 188.05672                | [M – H]⁻    | -0.67       | 91397041.99 | N-acyl-dl-glutamic acid | Amino acids | NA       | 87.1       | Level 2 |
| 18     | 1.099               | C₆H₁₀O₇         | 178.04763                   | 177.04068                | [M – H]⁻    | -0.59       | 177666674.9 | Δ-Gluconic acid δ-lactone | Carbohydrates and derivatives | NA       | 90.6       | Level 2 |
| 19     | 1.106               | C₆H₁₀O₇         | 164.04747                   | 165.03914                | [M + NH₄]^+ | 0.74        | 246283181.7 | 2-Hydroxycinnamic acid | Phenylpropanoids | 77.2       | 86.1       | Level 2 |
| 20     | 1.134               | C₆H₁₀O₇         | 118.02667                   | 117.01939                | [M – H]⁻    | 0.54        | 319467862.4 | Succinic acid | Organic acids | 91.9       | 91         | Level 1 |
| 21     | 1.14                | C₁₀H₁₂N₃O₄      | 267.09628                   | 321.09454                | [M + FA – H]⁻ | -1.76       | 302054111.9 | Adenosine | Nucleotides and analogues | 86.1       | 85.2       | Level 2 |
| 22     | 1.142               | C₇H₁₀O₇         | 206.0424                    | 205.03548                | [M – H]⁻    | -1.25       | 118715221.1 | 3-Hydroxy-3- (methoxy carbonyl) pentanedioic acid | Others | 4.8       | 87.8       | Level 2 |
| 23     | 1.143               | C₇H₉N₃         | 135.05446                   | 134.04736                | [M – H]⁻    | -0.25       | 111025841.7 | Adenine | Nucleotides and analogues | 76.2       | 81.1       | Level 1 |
| 24     | 1.15                | C₁₀H₁₃N₅O₅      | 283.0917                    | 284.09888                | [M + H]^+   | 0.12        | 843230783.7 | Guanosine | Nucleotides and analogues | 84.8       | NA         | Level 2 |
| 25     | 1.153               | C₅H₉N₃O       | 151.04949                   | 152.05672                | [M + H]^+   | 0.53        | 291864775.2 | Guanine | Nucleotides and analogues | 80.0       | 91.6       | Level 1 |
| 26     | 1.181               | C₁₀H₁₃N₃O₃      | 251.1088                    | 252.10921                | [M + H]^+   | 0.17        | 597456561.5 | 2′-Deoxyadenosine | Nucleotides and analogues | 84.5       | 93.6       | Level 2 |
| Number | Retention time (min) | Molecular formula | Relative molecular weight (Da) | The measured values (m/z) | Adduct ions | Error (ppm) | Peak | Compound name | Compound type | mzVault best match | mzCloud best match | Level |
|--------|---------------------|-------------------|-------------------------------|--------------------------|-------------|-------------|------|----------------|----------------|-----------------|-----------------|-------|
| 27     | 1.187               | C₆H₁₂O₆           | 180.0632                      | 161.0457                 | [M – H – H₂O]⁻ | -0.40       | 136214268.5 | L-Sorbose       | Carbohydrates and derivatives  | 66.2            | 89.4            | Level 1 |
| 28     | 1.47                | C₇H₁₃N₂O₄S       | 289.0893                     | 289.0962                 | [M + H]⁺     | -0.82       | 82256337.5  | 2′-o-Methyladenosine thymidinosine | Others         | NA              | 85.2            | Level 2 |
| 29     | 3.116               | C₇H₁₃N₂O₄        | 204.08972                    | 203.08231               | [M – H]⁻     | -0.75       | 46680672.16 | 1-Tryptophan | Amino acids | 92              | 92.9            | Level 1 |
| 30     | 3.123               | C₁₁H₁₂N₂O₄       | 260.13022                    | 260.13786               | [M + H]⁺     | 0.17        | 228758811.1 | 2′-o-Methyladenosine thymidinosine | Others         | NA              | 93              | Level 2 |
| 31     | 3.29                | C₇H₁₃N₂O₄S       | 297.08932                    | 297.09625               | [M + H]⁺     | -0.82       | 82256337.5  | 2′-o-Methyladenosine thymidinosine | Others         | NA              | 93              | Level 2 |
| 32     | 4.218               | C₇H₁₂O₃           | 126.03166                    | 125.02444               | [M – H]⁻     | -0.27       | 23877907.5  | Pyrogallol      | Phenolic acids | 85.7            | 69.8            | Level 2 |
| 33     | 4.221               | C₇H₁₂O₃           | 170.02147                    | 169.01414               | [M – H]⁻     | -0.33       | 71859123.5  | Gallic acid     | Phenolic acids | 85.5            | 84.6            | Level 2 |
| 34     | 4.236               | C₇H₁₂O₃           | 160.07347                    | 159.06624               | [M – H]⁻     | -0.56       | 770557559.2 | 3-Methyladipic acid | Organic acids | 87.1            | 96.9            | Level 2 |
| 35     | 4.352               | C₁₂H₁₃N₂O₄       | 216.08996                    | 215.08209               | [M + H]⁺     | 0.37        | 179365585.2 | 2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid | Organic acids | NA              | 92.9            | Level 2 |
| 36     | 4.734               | C₆H₁₂O₃           | 132.07867                    | 131.07133               | [M – H]⁻     | 0.21        | 111408164.4 | 2-Hydroxyacproic acid | Organic acids | NA              | 89.2            | Level 2 |
| 37     | 5.007               | C₆H₁₂O₃           | 173.10533                    | 172.10225               | [M + H]⁺     | 0.77        | 49085835.4  | N-Acetyl-L-leucine | Amino acids | 84.2            | 88.9            | Level 2 |
| 38     | 5.273               | C₆H₁₂O₃           | 166.06292                    | 165.05562               | [M – H]⁻     | -0.47       | 71202767.9  | L(-)-3-Phenylactic acid | Others         | 79.8            | 92.4            | Level 2 |
| 39     | 5.495               | C₆H₁₂O₃           | 210.13664                    | 210.13735               | [M + H]⁺     | -0.87       | 148811351.4 | Cyclo(leucylprolyl) | Amino acids | 86.6            | 86.6            | Level 2 |
| 40     | 5.832               | C₆H₁₂O₃           | 174.08912                    | 173.08185               | [M – H]⁻     | -0.50       | 361132054.6 | Suberic acid | Organic acids | 89.2            | 92.8            | Level 2 |
| 41     | 5.85                | C₆H₁₂O₃           | 207.08931                    | 206.08209               | [M – H]⁻     | -1.14       | 138547712.2 | N-Acetyl-L-phenylalanine | Amino acids | 89.4            | 88.5            | Level 2 |
| 42     | 6.629               | C₆H₁₂O₃           | 186.08906                    | 185.08185               | [M – H]⁻     | -0.78       | 68946334.8  | 1-(Carboxymethyl)cyclohexanecarboxylic acid | Organic acids | NA              | 89.1            | Level 2 |
| 43     | 6.977               | C₆H₁₂O₃           | 242.08046                    | 241.08263               | [M + H]⁺     | 0.35        | 161472292.4 | Lumichrome | Others         | 89.6            | 39.1            | Level 2 |
| 44     | 7.755               | C₁₁H₁₇N₂O₄        | 210.10987                    | 210.11719               | [M + H]⁺     | -0.38       | 89473999.72 | 2-Hydroxyacproic acid | Organic acids | 93.2            | 96.7            | Level 1 |
| 45     | 7.998               | C₁₁H₁₇N₂O₄        | 202.12036                    | 201.13111               | [M – H]⁻     | -0.74       | 435644695.3 | 3-Tartaric acid | Organic acids | NA              | 87.5            | Level 2 |
| 46     | 8.296               | C₁₁H₁₇N₂O₄        | 286.04722                    | 285.04053               | [M – H]⁻     | -1.80       | 17137849.28 | Luteolin      | Flavonoids | 94.3            | 86.7            | Level 2 |
| 47     | 9.458               | C₁₁H₁₇N₂O₄        | 270.05241                    | 269.04538               | [M – H]⁻     | -1.52       | 9772253.50  | Apigenin      | Flavonoids | 96.2            | 86.7            | Level 1 |
| 48     | 9.756               | C₁₁H₁₇N₂O₄        | 300.06343                    | 301.07077               | [M + H]⁺     | 0.15        | 3178963.77  | Diosmetin     | Flavonoids | 78              | 88.5            | Level 2 |
| 49     | 9.757               | C₁₁H₁₇N₂O₄        | 300.06297                    | 299.05612               | [M – H]⁻     | -1.39       | 100200736.4 | Hispidulin     | Flavonoids | 93              | 79.8            | Level 1 |
| 50     | 9.785               | C₁₁H₁₇N₂O₄        | 248.14096                    | 249.14859               | [M + H]⁺     | -1.14       | 76530068.2  | (3-ar,8r,8ar,9ar)-8-Hydroxy-8a-methyl-3,5-bis(methylene) decahydrobenzofuran-2(3H)-one | Others         | 81.7            | 80.9            | Level 2 |
| 51     | 10.161              | C₁₁H₁₇N₂O₄        | 194.13062                    | 195.13806               | [M + H]⁺     | -0.32       | 139186228.7 | Sedanolide     | Others         | NA              | 88.1            | Level 2 |

Table 1: Continued.
| Number | Retention time (min) | Molecular formula | Relative molecular weight (Da) | The measured values (m/z) | Adduct ions | Error (ppm) | Peak | Compound name | Compound type | mzVault best match | mzCloud best match | Level |
|--------|----------------------|-------------------|------------------------------|--------------------------|-------------|------------|------|---------------|--------------|----------------|----------------|-------|
| 55     | 10.163               | C_{18}H_{34}O_{5} | 330.24027                    | 329.23309                | [M − H]^-  | −1.08      | 4975697767   | (15z)-9,12,13-Trihydroxy-15-octadecenoic acid | Organic acids | NA             | 90.2            | Level 2 |
| 56     | 10.459               | C_{12}H_{20}O_{4} | 230.15155                    | 229.1442                 | [M − H]^-  | −1.13      | 18453143.37  | Dodecanedioic acid | Organic acids | NA             | 90.1            | Level 2 |
| 57     | 11.508               | C_{20}H_{30}O_{4} | 334.21425                    | 335.22144                | [M + H]^+   | −0.48      | 288459427.2  | 2-Hydroxy-4,5′,8a′-trimethyl-1′-oxo-4-vinylcyclohexyl-1′-h-spiro[cydopentane-1,2′-naphthapolene]-5′-carboxylic acid | Organic acids | 68.1            | 85.2            | Level 2 |
| 58     | 11.573               | C_{16}H_{20}O_{5} | 188.14116                    | 187.13385                | [M − H]^-  | −0.46      | 612620120.09 | 10-Hydroxydecanoic acid Bis(methylbenzylidene) sorbitol | Organic acids | NA             | 85.5            | Level 2 |
| 59     | 11.73                | C_{22}H_{32}O_{6} | 386.17282                    | 387.17999                | [M + H]^+   | 0.27       | 16230645.1   | Acetatin | Flavonoids | 94.6 | 83.2 | Level 2 |
| 60     | 11.791               | C_{16}H_{20}O_{5} | 284.06798                    | 283.06070                | [M − H]^-  | −1.75      | 1982609.94   | Dodecanedioic acid | Organic acids | NA             | 90.1            | Level 2 |
| 61     | 11.863               | C_{15}H_{20}O_{5} | 246.12566                    | 247.13295                | [M + H]^+   | 0.27       | 4367209.94   | Argabin | Terpenoids | 65 | 86.7 | Level 2 |
| 62     | 12.006               | C_{12}H_{18}O_{4} | 248.14096                    | 249.14842                | [M + H]^+   | −1.14      | 1670639.17   | Atractylenolide III | Terpenoids | 92.9            | 92.4            | Level 2 |
| 63     | 12.437               | C_{14}H_{18}O_{4} | 258.18284                    | 257.17584                | [M − H]^-  | −1.03      | 3550240.87 | Tetradecanedioic acid | Organic acids | NA             | 88.7            | Level 2 |
| 64     | 12.818               | C_{20}H_{32}O_{5} | 320.23487                    | 321.24219                | [M + H]^+   | −0.37      | 240249419.6  | 3-Methyl-5-[(1s,2r,4ar)-1,2,4a,5-tetramethyl-7-oxo-1,2,3,4,4a,7,8,8a-octahydro-1-naphthol[enyl] pentanoic acid | Organic acids | 78.1            | 90.5            | Level 2 |
| 65     | 12.858               | C_{20}H_{32}O_{2} | 304.23998                    | 305.24768                | [M + H]^+   | −0.83      | 6899745.63 | Arachidonic acid Bis(4-ethylbenzylidene) sorbitol | Organic acids | 61.9            | 89.3            | Level 2 |
| 66     | 12.934               | C_{21}H_{30}O_{5} | 414.20413                    | 415.21118                | [M + H]^+   | 0.24       | 150755385.4 | Ethyl paraben | Others | NA             | 94.9            | Level 2 |
| 67     | 12.976               | C_{19}H_{28}O_{3} | 166.06299                    | 167.07022                | [M + H]^+   | −0.02      | 92932147.44 | 4-Ethoxy ethylbenzoate | Others | NA             | 84             | Level 2 |
| 68     | 12.977               | C_{19}H_{28}O_{5} | 194.09427                    | 195.10172                | [M + H]^+   | −0.12      | 215420117.3 | Ethyl paraben | Others | 69             | 90.6           | Level 2 |
| 69     | 13.247               | C_{18}H_{28}O_{4} | 314.24521                    | 313.23795                | [M − H]^-  | −1.60      | 106403352.7 | 4-Hydroxy-12z-octadecenoic acid | Organic acids | 94.9            | Level 2 |
| 70     | 13.379               | C_{13}H_{26}O_{2} | 266.15457                    | 265.14728                | [M − H]^-  | −2.31      | 919909720.2 | Dodecyl sulfate | Organic acids | 94.3            | Level 2 |
| 71     | 13.427               | C_{15}H_{26}O_{2} | 300.20881                    | 301.21606                | [M + H]^+   | −0.39      | 260138250.1 | Isotretinoin | Organic acids | 85.1            | Level 2 |
| 72     | 13.908               | C_{18}H_{34}NO_{2} | 297.26671                    | 298.27426                | [M + H]^+   | −0.22      | 57608549.56 | 2-Aminoacetad-4-yne-1,3-diol | Others | NA             | 92.4            | Level 2 |
| 73     | 14.152               | C_{16}H_{30}O_{2} | 292.20372                    | 293.21088                | [M + H]^+   | −0.42      | 477279239   | 9,6,13r-12z-Oxophytoendioic acid | Organic acids | NA             | 92.4            | Level 2 |
| 74     | 14.574               | C_{12}H_{20}O_{2} | 192.11508                    | 193.12238                | [M + H]^+   | 0.28       | 134018825.8 | Senkunolide A | Others | 85.9 | NA | Level 2 |
| 75     | 14.763               | C_{11}H_{4}NO_{2} | 453.28511                    | 452.27756                | [M − H]^-  | −0.95      | 115696629.3 | Glycerophospho-n-palmitoyl ethanolamine | Others | NA             | 93.5            | Level 2 |
| 76     | 14.975               | C_{18}H_{30}O_{2} | 278.22423                    | 279.23184                | [M + H]^+   | −1.24      | 3057598727 | A-Eleostearic acid | Unsaturated fatty acids | 91 | 96.7 | Level 2 |
| Number | Retention time (min) | Molecular formula | Relative molecular weight (Da) | The measured values (m/z) | Adduct ions | Error (ppm) | Peak | Compound name | Compound type | mzVault best match | mzCloud best match | Level |
|--------|----------------------|-------------------|--------------------------------|---------------------------|-------------|-------------|------|---------------|--------------|-------------------|-------------------|-------|
| 77     | 15.058               | C₁₈H₃₆O₅S        | 326.19129                      | 325.18396                 | [M − H]⁻    | −0.84       | 934129316.5 | 4-Dodecylbenzenesulfonic acid (3s)-3-Methyl-5-[(1s,8ar)-2,5,5,8a-tetramethyl-4-oxo-1,4,4a,5,6,7,8,8a-octahydro-1-naphthalenyl] pentanoic acid | Others | NA | 92.4 | Level 2 |
| 78     | 15.149               | C₂₀H₄₂O₃         | 320.23482                      | 321.24207                 | [M + H]⁺    | −1.02       | 654199927.6 | Organic acids | 64.3 | 89.1 | Level 2 |
| 79     | 15.292               | C₁₈H₃₆O₂          | 278.22423                      | 279.23172                 | [M + H]⁺    | −1.26       | 1402325459 | Pinolenic acid (r)-3-Hydroxy myristic acid | Organic acids | 90.2 | 95.8 | Level 2 |
| 80     | 15.332               | C₁₈H₃₆O₃          | 244.20347                      | 243.19621                 | [M − H]⁻    | −1.52       | 22659032.03 | Organic acids | NA | 88.9 | Level 2 |
| 81     | 15.832               | C₁₈H₃₆O₂          | 294.2195                       | 295.22685                 | [M + H]⁺    | 0.003       | 5846063601 | 9-Oxo-10(e),12(e)-octadecadienoic acid | Organic acids | NA | 97.6 | Level 2 |
| 82     | 16.357               | C₁₉H₃₈O₄          | 470.33934                      | 471.34756                 | [M + H]⁺    | −0.57       | 30986480.74 | 18-β-glycyr rhetic acid Ginkgolic acid 9(2),11(1),13(3)-octadecatrienoic acid methyl ester | Terpenoids | 87.3 | 90.6 | Level 2 |
| 83     | 17.175               | C₂₀H₄₂O₃          | 320.23482                      | 321.24222                 | [M + H]⁺    | −1.02       | 129134534.2 | Ginkgolic acid 9(2),11(1),13(3)-octadecatrienoic acid methyl ester | Phenolic acids | 66 | 86.1 | Level 2 |
| 84     | 17.422               | C₁₈H₃₆O₂          | 292.23998                      | 293.24725                 | [M + H]⁺    | −0.87       | 84449956.04 | Organic acids | NA | 94 | Level 2 |
| 85     | 17.727               | C₂₀H₃₄O₃          | 306.25582                      | 307.26297                 | [M + H]⁺    | −0.21       | 59379266.82 | Linolenic acid ethyl ester | Others | 91.1 | 96.2 | Level 2 |
| 86     | 17.935               | C₂₁H₄₈O₄          | 354.27685                      | 355.28397                 | [M + H]⁺    | −0.44       | 469393052.8 | 1-Linoleoyl glycerol | Others | NA | 94.4 | Level 2 |
| 87     | 18.154               | C₁₆H₃₈O₃          | 272.23457                      | 271.22726                 | [M − H]⁻    | −2.12       | 232231687.4 | 16-Hydroxyhexadecanoic acid 3-Methyl-5-(5,5,8a-trimethyl-2-methylene-7-oxodecacydro-1-naphthalenyl) pentyl acetate | Organic acids | NA | 93.3 | Level 2 |
| 88     | 18.184               | C₂₂H₅₄O₃          | 348.26628                      | 349.2735                 | [M + H]⁺    | −0.47       | 79521502.69 | Others | NA | 85 | Level 2 |
| 89     | 18.301               | C₂₀H₃₄O₃          | 318.21935                      | 319.22678                 | [M + H]⁺    | −0.46       | 87070678.64 | 11-α-hydroxy-17-methyltestosterone | Steroids | 74.9 | 85.5 | Level 2 |
| 90     | 18.528               | C₁₆H₃₂O₂          | 254.22444                      | 255.23161                 | [M + H]⁺    | −0.54       | 93625271.65 | Palmitoleic acid | Organic acids | 79.8 | 97.2 | Level 2 |
| 91     | 18.532               | C₁₆H₃₂O₂          | 254.22421                      | 253.21733                 | [M − H]⁻    | −1.45       | 75050753.73 | Δ2-Trans-hexadecenoic acid | Unsaturated fatty acids | NA | 86.4 | Level 2 |
| Number | Retention time (min) | Molecular formula | Relative molecular weight (Da) | The measured values (m/z) | Adduct ions | Error (ppm) | Peak | Compound name | Compound type | mzVault best match | mzCloud best match | Level |
|--------|---------------------|-------------------|-------------------------------|---------------------------|-------------|-------------|------|---------------|--------------|-------------------|-------------------|-------|
| 92     | 18.608              | C₁₈H₃₇NO₂         | 299.28227                     | 300.28955                 | [M + H]<sup>+</sup> | −0.52       | 60691604.46 | Palmitoyl ethanolamide | Others          | NA                | 95.2             | Level 2 |
| 93     | 18.618              | C₂₀H₃₉NO₂         | 325.29787                     | 326.30499                 | [M + H]<sup>+</sup> | −0.64       | 205900632.2 | Oleoyl ethanolamide 5-((3z)-5-Hydroxy-3-methyl-3-penten-1-yl)-1,4a-dimethyl-6-methylenedecahydro-1-naphthalencarboxylic acid | Others          | NA                | 94.2             | Level 2 |
| 94     | 18.94               | C₂₀H₃₂O₃          | 320.23475                     | 319.228                   | [M − H]<sup>−</sup> | −1.22       | 11930213.5  | 9(z),11(e)-Conjugated linoleic acid | Organic acids    | 80.5              | 86.2             | Level 2 |
| 95     | 18.948              | C₁₈H₃₅NO          | 280.23944                     | 279.23248                 | [M − H]<sup>−</sup> | −2.81       | 1954067053 | Unsaturated fatty acids | Others          | 85.8              | 87.3             | Level 2 |
| 96     | 19.741              | C₁₈H₃₅NO          | 281.27169                     | 282.27902                 | [M + H]<sup>+</sup> | −0.61       | 800775254.3 | Oleamide | Others          | NA                | 97.3             | Level 2 |
| 97     | 20.138              | C₁₈H₃₅NO          | 255.25604                     | 256.26334                 | [M + H]<sup>+</sup> | −0.68       | 144475650.8 | Hexadecanamide | Others          | NA                | 90.2             | Level 2 |
| 98     | 20.821              | C₂₀H₃₄O₂          | 306.25582                     | 307.26343                 | [M + H]<sup>+</sup> | −0.21       | 6347388.901 | Linolenic acid ethyl ester | Others          | 90.2              | 96.2             | Level 1 |
| 99     | 20.977              | C₂₀H₄₂O₄          | 358.30821                     | 359.31543                 | [M + H]<sup>+</sup> | −0.27       | 26828479.9 | 1-Stearoylglycerol | Others          | 91.3              | 89.1             | Level 2 |
| 100    | 21.524              | C₂₀H₄₂O₂          | 282.25578                     | 283.26306                 | [M + H]<sup>+</sup> | −0.34       | 21237622.49 | Ethyl palmitoleate | Others          | 64.8              | 93.9             | Level 2 |
| 101    | 22.225              | C₁₈H₃₇NO          | 283.28734                     | 284.29741                 | [M + H]<sup>+</sup> | −0.61       | 133000171.1 | Stearamide | Others          | NA                | 90.6             | Level 2 |
| 102    | 22.746              | C₂₀H₃₄O₂          | 310.28707                     | 311.29428                 | [M + H]<sup>+</sup> | −0.37       | 22068099.07 | Ethyl oleate | Others          | 57.8              | 96.3             | Level 2 |
Data Availability

The data used to support this study are available within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding this study.

Acknowledgments

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