Chemical profiling of root bark extract from *Oplopanax elatus* and its *in vitro* biotransformation by human intestinal microbiota

Jin-Yi Wan1,*, Jing-Xuan Wan1,*, Shilei Wang2, Xiaolu Wang1, Wenqian Guo1, Han Ma1, Yuqi Wu1, Chong-Zhi Wang3, Lian-Wen Qi2, Ping Li2, Haiqiang Yao1 and Chun-Su Yuan3

1 School of Traditional Chinese Medicine & National Institute of TCM Constitution and Preventive Medicine, Beijing University of Chinese Medicine, Beijing, China
2 State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing, China
3 Tang Center for Herbal Medicine Research & Department of Anesthesia and Critical Care, University of Chicago, Chicago, IL, USA
* These authors contributed equally to this work.

**ABSTRACT**

*Oplopanax elatus* (Nakai) Nakai, in the Araliaceae family, has been used in traditional Chinese medicine (TCM) to treat diseases as an adaptogen for thousands of years. This study established an ultra-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF/MS) method to identify chemical components and biotransformation metabolites of root bark extract from *O. elatus*. A total of 18 compounds were characterized in *O. elatus* extract, and 62 metabolites by human intestinal microbiota were detected. Two polyynes, falcarindiol and oplopandiol were recognized as the main components of *O. elatus*, whose metabolites are further illustrated. Several metabolic pathways were proposed to generate the detected metabolites, including methylation, hydrogenation, demethylation, dehydroxylation, and hydroxylation. These findings indicated that intestinal microbiota might play an essential role in mediating the bioactivity of *O. elatus*.

**INTRODUCTION**

*Oplopanax elatus* (Nakai) Nakai is the plant of genus *Oplopanax*, which belongs to the Araliaceae family. It is mainly distributed in northeast China, Korea and far east of Russia (*Dou et al., 2009; Yang et al., 2010*). As a traditional medicinal plant, *O. elatus* is being utilized as a ginseng-like herbal medicine and has been long used as an adaptogen to treat arthritis, diabetes mellitus, rheumatism, neurasthenia, and cardiovascular diseases (*Dai et al., 2016; Eom et al., 2017; Knispel et al., 2013; Moon et al., 2013; Panossian et al., 2021*). Previous studies have identified several components derived from *O. elatus*, such as the lignans, saponins, phenolic glycosides, and polyynes (*Huang et al., 2010; Shao et al., 2011*).
To date, polynyes have been chiefly reported with high contents in the root of *O. elatus* (Huang et al., 2014a). Increasing attention has been paid to two main polynyes facarindiol (FAD) and oplopandiol (OPD), because of their significant anti-tumor activities (Purup, Larsen & Christensen, 2009; Qiao et al., 2017; Sun et al., 2016). However, most studies remain focused on the pharmacological and chemical constituents of *O. elatus*, while its metabolic profiles are rather obscured.

It is widely known that human beings live in symbiotics with coevolutionary microbiota (Thursby & Juge, 2017; Yang & Lao, 2019). The human gastrointestinal tract is the primary habitat for trillions of microbes. The gut microbiota serves metabolic functions crucial for the human host (Bäckhed et al., 2004; Chen et al., 2016; Rajilić-Stojanović & de Vos, 2014) and influences the biofunctions (Barko et al., 2018; Defois et al., 2018; Pagliari et al., 2017). Like most herbal medicines, *O. elatus* products are orally administered. The multiple constituents of *O. elatus* are typically brought into contact with intestinal bacteria and subsequently transformed in the digestive tract (Gao et al., 2018; Huang et al., 2014b; Koppel, Maini Rekdal & Balskus, 2017; Shikov et al., 2014; Teschke et al., 2015). However, existing reports did not address intestinal microflora’s biotransformed metabolites of *O. elatus*. Therefore, elucidating how gut microbes treat these complex components may contribute to a complete understanding of the metabolic profiles and biological activities of *O. elatus*.

Recently, various analytical platforms are typically applied to identify metabolic profiles in the complex extracts of TCMs. Most notably, ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) is one of the powerful analytical tools (Jin et al., 2018; Wu et al., 2019; Yang et al., 2016). With the newly developed chromatographic technique, the UPLC system allows significant improvements in the resolution, analysis speed, and reduction of solvent waste (Chekmeneva et al., 2018; Du et al., 2017; Wang et al., 2008). Meanwhile, high-resolution Q-TOF/MS can give more specific and accurate mass information on characteristic molecular ions and fragment ions, providing a reliable basis for the qualitative analysis of complex samples (Li et al., 2019; Lou et al., 2015; Wewer et al., 2011). Based on these characteristics, UPLC-Q-TOF/MS was ultimately selected for fast identification of constituents in *O. elatus*.

In the present study, we focused on the metabolic behavior of human intestinal microflora on *O. elatus*. A highly selective and sensitive UPLC-Q-TOF/MS method was established to characterize the chemical and metabolic profiles of *O. elatus*. Furthermore, the proposed metabolic pathways were also summarized. This work will provide a better understanding for exploring the bioactivities of *O. elatus in vivo*.

**MATERIALS & METHODS**

**Materials and reagents**

The general anaerobic medium for bacteria culture was obtained from Shanghai Kayon Biological Technology Co. Ltd. (Shanghai, China). Formic acid and HPLC-grade acetonitrile were purchased from Merck (Darmstadt, Germany). Deionized water
(18 MΩ-cm) was supplied with a Millipore Milli-Q water system (Milford, MA, USA). All other reagents were from standard commercial sources and of analytical purity.

**Preparation of *Oplopanax elatus* extract**

Root bark of *O. elatus* was obtained from Benxi city (Liaoning, China). The voucher samples were deposited at the Tang Center for Herbal Medicine Research at the University of Chicago (Chicago, IL, USA). The air-dried root bark of *O. elatus* was pulverized into powder and sieved through an 80-mesh screen. Eight g of the powder were extracted twice by heat-reflux with 70% ethanol for 2 h. The combined extract was evaporated under vacuum and lyophilized with a yield of 28%. The samples were stored at 4 °C until use.

**Preparation of human intestinal microflora**

The Institutional Review Board approved the present study protocol at the University of Chicago (IRB protocol number: 12536). Fresh fecal samples were collected from six healthy adult volunteers (male, aged 20–55, non-smokers without antibiotic consumption for more than 6 months, and written consent was obtained). All the fecal samples were mixed for analysis. A total of five g of samples were homogenized in 30 ml cold physiological saline, and centrifuged at 13,000 rpm for 10 min to obtain the resulting fecal supernatant.

**Incubation of sample in intestinal bacteria**

Two microliters of the fecal supernatant were added with eight ml anaerobic dilution medium containing five mg of *O. elatus* extract, which were then anaerobically incubated at 37 °C for 24 h in an anaerobic workstation (Electrotek, UK). The reaction mixtures were extracted three times with water-saturated n-butanol. All the n-butanol layers were mixed and dried under a nitrogen stream and then dissolved in one ml methanol. The solutions were centrifuged at 13,000 rpm for 10 min for analysis.

**UPLC-Q-TOF/MS analysis**

Data were collected as previously described (Wang et al., 2020). The Agilent 1290 Series UPLC system (Agilent Technologies, Santa Clara, CA, USA) was applied to perform the chromatographic analysis, and a binary pump, an online degasser, an auto plate-sampler, and a thermostatically controlled column compartment were also equipped for this system. The separation was carried out on UPLC ACQUITY HSS C8 column (2.1 mm × 100 mm × 1.7 μm, Waters) with a constant flow rate of 0.4 mL/min, and the column temperature was kept at 40 °C. A gradient mobile phase system of 0.1% formic acid in water (phase A) and acetonitrile (phase B) was applied as follows: 5% B at 0–1 min, 5–20% B at 1–18 min, 20–30% B at 18–27 min, 30–35% B at 27–32 min, 35–60% B at 32–40 min, 60–95% B at 40–50 min, 95% B at 50–53 min, 95–5% B at 53–55 min. The injection volume of samples was set at 2 μL for MS mode and five μL for MS/MS mode.

The Agilent 6545 Q-TOF-MS system with a Dual electrospray ionization source was used to conduct the detection. Nitrogen (purity > 99.999%) served as a sheath gas and drying gas, and the flow velocities were set at 11 and 8 L/min. The temperatures of sheath gas and drying gas were set at 350 and 320 °C respectively. Positive and negative ion modes...
were both employed in this study. The other parameters were set as follows: nebulizer pressure, 35 psig; voltage, 3,500 V; fragmentor voltage, 175 V; mass range, m/z 100–1,700; data acquisition rate, 1.5 scans/s; MS/MS spectra collision energy, 50 eV (Wang et al., 2020).

**Data analysis**

Mass data were analyzed by the Agilent MassHunter Workstation software (Version B.06.01), based on the accurate measurements of m/z values with online databases (MassBank, etc.), to screen probable compounds. The empirical molecular formula was deduced by comparing the theoretical mass of molecular ions at the mass accuracy of less than five ppm.

**RESULTS**

**Optimization of UPLC-Q-TOF/MS conditions**

To obtain the chromatograms with better resolution and higher baseline stability of *O. elatus* extract and its primary metabolites, multiple mobile phases such as acetonitrile-water and methanol-water were detected. Acetonitrile-water was applied as the solvent, for its stronger separation ability, shorter retention time, and lower column pressure. Additionally, 0.1% formic acid added in the water as mobile phase adducts may help to achieve higher response and better peak sensitivity (Tao et al., 2016). Therefore, the optimal solvent system consisting of acetonitrile-water (0.1% formic acid), which remarkably enhanced the efficiency of ionization and satisfactory sensitivity, was ultimately selected as mobile phase with a gradient elution.

In addition, the factors related to MS performance, including ionization mode and collision energy, were further improved. The positive ion mode was ultimately employed to gain comprehensive data for structural characterization and metabolite assignment with much lower background noise. The collision energy was optimized to obtain the higher ionization efficiency and relative abundance of precursor and product ions.

**Chemical profiling of *O. elatus* extract**

In total, 18 ingredients of *O. elatus* were detected in this study, and their chemical structures are shown in Fig. 1. There are six types of compounds, including nine polyynes, three lignans, one phenylpropanoid, two sesquiterpenes, one triterpenoid, and two fatty acids. The total ion chromatogram (TIC) of *O. elatus* extract is shown in Fig. 2A in the positive ion mode by UPLC-Q-TOF-MS. Table 1 shows the detailed information, including retention time, signal intensity, molecular formula, calculated and experimental mass m/z, ppm error, and fragment ions of these 18 components (Schymanski et al., 2014; Wang et al., 2020).

Polyyynes have been found as the main constituents in the root of *O. elatus* (Yang et al., 2014). Among them, falcarindiol and oplopandiol were determined to have very high contents in the air-dried root bark. As shown in Table 1, polyynes exhibit the same elemental composition and similar MS/MS behaviors, with the characteristic fragment ions at m/z 79.05 in the positive ion mode.

For example, the typical protonated molecular ion [M+H]^+ of FAD was observed at m/z 261.1848 in the mass spectrum. The fragment ion at m/z 105.0713 was formed by the losses
Figure 1 The chemical structures of bioactive compounds detected in *Oplopanax elatus* extract. (A) Polyynes; (B) Lignans; (C) Phenylpropanoid; (D) Sesquiterpenes; (E) Triterpenoid; (F) Fatty acids.
of H$_2$O and C$_{10}$H$_{18}$ with m/z 79.0548 by further loss of C$_2$H$_2$. OPD was identified by the protonated molecular ion [M+H]$^+$ at m/z 263.2010 compared with calculated m/z 263.2006. The fragment ion at m/z 107.0459 was produced by the losses of H$_2$O and C$_{10}$H$_{18}$, and m/z 79.0563 was formed by further loss of C$_2$H$_4$.

In addition, phenylpropanoid compound 4-(3-hydroxyprop-1-en-1-yl)-2,6-dimethoxyphenyl β-D-glucopyranoside was determined to be C$_{17}$H$_{24}$O$_9$ at m/z 373.1495 ([M+H]$^+$, C$_{17}$H$_{24}$O$_9$; calculated as 373.1493). The neutral loss of 1 × Glc moiety formed fragment ion at m/z 211.1526. Similarly, three lignans were determined by the neutral loss of 1 × Glc moiety in the [M+Na]$^+$ mode.

**Detection and identification of metabolites of O. elatus extract**

The control sample was prepared in parallel, which used in the dilution medium and human fecal microflora, as shown in Fig. 2B. The biotransformed O. elatus sample by
| No. | Compound | Formula | \(t_r\) (min) | Signal intensity \((\times 10^5)\) | \([M+H]^+\) or \([M+Na]^+\) \(m/z\) | Calc \(m/z\) | Diff (ppm) | Fragment ions in the positive mode with the energy 50 V CID |
|-----|----------|---------|---------------|-------------------------------|---------------------------------|------------|----------|------------------------------------------------|
| 1   | 4-(3-hydroxyprop-1-en-1-yl)-2,6-dimethoxyphenyl \(\beta\)-D-glucopyranoside | C_{17}H_{24}O_{9} | 5.24 | 2.86 ± 0.82 | 373.1495 373.1493 | -0.51 | 211.1526[M-\(\beta\)-glc+H]\(^+\), 373.1495[M+H]\(^+\) |
| 2   | 4',7-epoxy-4,9,9' trihydroxy-3,3'-dimethoxy-5',8-lignan-4,9-bis[\(\beta\)-D-glucopyranoside] | C_{32}H_{44}O_{16} | 10.92 | 1.74 ± 0.34 | 707.2521 707.2522 | 0.08 | 545.1981[M-\(\beta\)-glc+Na]\(^+\), 707.2521[M+Na]\(^+\) |
| 3   | isolariciresinol 3-O-\(\beta\)-D-glucopyranoside | C_{26}H_{34}O_{11} | 14.14 | 0.55 ± 0.17 | 545.1997 545.1993 | -0.70 | 383.1428[M-\(\beta\)-glc+Na]\(^+\), 545.1997[M+Na]\(^+\) |
| 4   | 5-methoxylariciresinol 4-O-\(\beta\)-D-glucopyranoside | C_{27}H_{36}O_{12} | 15.04 | 0.89 ± 0.30 | 575.2112 575.2099 | -2.36 | 412.1434[M-\(\beta\)-glc+Na]\(^+\), 250.0381[M-\(\beta\)-glc+Na], 575.2112[M+Na]\(^+\) |
| 5   | 2-decenoic acid | C_{10}H_{18}O_{2} | 23.41 | 5.62 ± 1.74 | 171.1378 171.1380 | 0.92 | 55.9342[M-HCOOH-C_{10}H_{10}+H]\(^+\), 171.1378[M+H]\(^+\) |
| 6   | oploxyne B | C_{18}H_{30}O_{4} | 36.91 | 2.73 ± 0.36 | 311.2212 311.2217 | 1.57 | 107.0527[M-HCOOH-C_{10}H_{10}+H]\(^+\), 79.0547[M-HCOOH-C_{10}H_{10}+C_{2}H_{4}+H]\(^+\), 311.2212[M+H]\(^+\) |
| 7   | oploxyne A | C_{17}H_{26}O_{3} | 37.62 | 2.62 ± 0.44 | 279.1957 279.1955 | -0.82 | 107.0485[M-HCOOH-C_{16}H_{18}+H]\(^+\), 79.0545[M-HCOOH-C_{16}H_{18}+C_{2}H_{4}+H]\(^+\), 279.1957[M+H]\(^+\) |
| 8   | oplopantriol B | C_{18}H_{28}O_{3} | 37.89 | 1.28 ± 1.07 | 293.2110 293.2111 | 0.41 | 107.0491[M-HCOOH-C_{17}H_{20}+H]\(^+\), 79.0538[M-HCOOH-C_{17}H_{20}+C_{2}H_{4}+H]\(^+\), 293.2110[M+H]\(^+\) |
| 9   | 9,17-octadecadiene-12,14 diyne-1,11,16-triol,1-acetate | C_{20}H_{38}O_{4} | 38.63 | 1.57 ± 1.21 | 333.2048 333.2060 | 3.72 | 105.0700[M-HCOOH-C_{18}H_{22}+H]\(^+\), 79.0546[M-HCOOH-C_{18}H_{22}+C_{2}H_{4}+H]\(^+\), 333.2048[M+H]\(^+\) |
| 10  | oplopanadiol acetate | C_{20}H_{30}O_{4} | 39.18 | 3.93 ± 1.41 | 335.2215 335.2217 | 0.56 | 107.0503[M-HCOOH-C_{18}H_{22}+H]\(^+\), 79.0545[M-HCOOH-C_{18}H_{22}+C_{2}H_{4}+H]\(^+\), 335.2215[M+H]\(^+\) |
| 11  | 6,9-octadecadienoic acid | C_{18}H_{32}O_{2} | 39.46 | 5.13 ± 1.95 | 281.2470 281.2475 | 1.81 | 65.0396[M-CH_{3}COOH-C_{16}H_{20}+H]\(^+\), 281.2470[M+H]\(^+\) |
| 12  | falcarnindiol | C_{17}H_{24}O_{2} | 40.76 | 101.47 ± 12.16 | 261.1848 261.1849 | 0.41 | 105.0713[M-HCOOH-C_{18}H_{18}+H]\(^+\), 79.0548[M-HCOOH-C_{18}H_{18}+C_{2}H_{4}+H]\(^+\), 261.1848[M+H]\(^+\) |
| 13  | oplopanadiol | C_{17}H_{26}O_{2} | 41.23 | 119.24 ± 9.28 | 263.2010 263.2006 | -1.69 | 107.0459[M-HCOOH-C_{18}H_{18}+H]\(^+\), 79.0563[M-HCOOH-C_{18}H_{18}+C_{2}H_{4}+H]\(^+\), 263.2010[M+H]\(^+\) |
| 14  | falcarninol | C_{17}H_{24}O | 42.85 | 62.85 ± 7.26 | 245.1899 245.1900 | 0.38 | 105.0699[M-C_{10}H_{18}+H]\(^+\), 79.0556[M-C_{10}H_{18}+C_{2}H_{4}+H]\(^+\), 245.1899[M+H]\(^+\) |
| 15  | oplopantriol A | C_{18}H_{28}O_{3} | 43.31 | 67.83 ± 4.40 | 291.1956 291.1955 | 0.44 | 105.0659[M-HCOOH-C_{18}H_{20}+H]\(^+\), 79.0567[M-HCOOH-C_{18}H_{20}+C_{2}H_{4}+H]\(^+\), 291.1956[M+H]\(^+\) |
| 16  | curcumene | C_{13}H_{22} | 44.92 | 4.37 ± 2.22 | 203.1797 203.1794 | -1.35 | 134.1063[M-C_{9}H_{16}+H]\(^+\), 65.0382[M-2\times C_{2}H_{4}+H]\(^+\), 203.1797[M+H]\(^+\) |
| 17  | muurolene | C_{13}H_{24} | 45.27 | 8.27 ± 3.82 | 205.1950 205.1951 | 0.38 | 65.0550[M-2\times C_{2}H_{4}+H]\(^+\), 205.1950[M+H]\(^+\) |
| 18  | oleanolic acid | C_{30}H_{46}O_{3} | 46.88 | 10.83 ± 3.28 | 479.3508 479.3496 | -2.70 | 231.1715[M-C_{16}H_{24}O_{2}+Na]\(^+\), 479.3508[M+Na]\(^+\) |
intestinal bacteria is shown in Fig. 2C. Samples were incubated, pretreated, and analyzed under the same conditions as mentioned in “Incubation of sample in intestinal bacteria”. The potential metabolites were detected from the TIC of the transformed O. elatus sample compared to the control group. All the metabolites were further confirmed by the extracted ion chromatograms (EICs) and their MS/MS corresponding fragments. A total of 62 metabolites were identified by UPLC-Q-TOF-MS in the positive mode. Table 2 shows the retention time, signal intensity, experimental and calculated mass m/z, difference between m/z and calculated m/z in ppm, and fragment ions in the MS/MS stage of these 62 metabolites (M1-M62). All these metabolites could not be observed or only in trace amounts in control samples (Schymanski et al., 2014).

**Polyynes**
A total of 46 metabolites of nine polyynes generated by the transformation of human intestinal microflora were detected and identified. For each polyyne, at least four types of metabolites were identified. Due to the high biological activities, FAD and OPD selected as the representative compounds of polyynes were stated in detail.

The EICs and MS/MS spectrums of metabolites of FAD are shown in Fig. 3. Five metabolites including M15, M17, M26, M46, and M56 were detected. M15 was assigned to be the methylation product of FAD with the molecular formula C_{18}H_{26}O_{2} at m/z 275.2008 ([M+H]^+, C_{18}H_{26}O_{2}^+; calculated as 275.2006). The fragment ion at m/z 105.0704 was generated by the neutral losses of H_{2}O and C_{11}H_{20}, and m/z 79.0548 was formed by further loss of C_{2}H_{2}. M17 was assigned to be the hydrogenation product of FAD with the characteristic fragment ions at m/z 105.0695 and 79.0535. In addition, metabolites M26, M46, and M56 were assigned as demethylation, dehydroxylation, and hydroxylation products of FAD, respectively.

Figure 4 presents the EICs and MS/MS spectrums of OPD metabolites (M27, M40, M43, and M49). M27 was assigned as the hydroxylation product of OPD, owing to the presence of [M+H]^+ at m/z 279.1958. The characteristic fragment ion at m/z 107.0496 was formed by the neutral losses of H_{2}O and C_{10}H_{18}O, and m/z 79.0541 was formed by further loss of C_{2}H_{4}. Similarly, three other metabolites like M40, M43, and M49 were supposed to be the demethylation, dehydroxylation, and methylation products of OPD.

**Lignans**
M3–M5 were the deglycosylation products of three lignans via the loss of glucose moieties. For example, the parent compound of M3 was determined to be C_{32}H_{44}O_{16} while M3 was C_{20}H_{24}O_{6}, indicating M3 was the deglycosylation product via the loss of two glucose moieties. M4 and M5 were assigned as the products by losing a glucose moiety from their corresponding parent lignan compounds.

**Phenylpropanoids**
M1 was identified as the deglycosylation metabolite of phenylpropanoid compound 4-(3-hydroxyprop-1-en-1-yl)-2,6-dimethoxyphenyl β-D-glucopyranoside. The protonated molecular ion [M+H]^+ of M1 at m/z 211.0962 was observed in the positive ion mode, providing the molecular formula of C_{11}H_{14}O_{4}.
| No. | Description | Formula | $t_\text{R}$ (min) | Signal intensity ($\times10^{6}$) | $[\text{M}+\text{H}]^+$ or $[\text{M}+\text{Na}]^+$ | Fragment ions in the positive mode with the energy 50 V CID |
|-----|-------------|---------|----------------|------------------|------------------|----------------------------------|
| M1  | deglycosylation product of 4-(3-hydroxyprop-1-en-1-yl)-2,6-dimethoxyphenyl $\beta$-D-glucopyranoside | C$_{13}$H$_{14}$O$_4$ | 9.90 | 2.83 ± 0.32 | 211.0962 211.0965 | 1.36 | 92.0582 [M-C$_2$H$_3$O-OCH$_3$×2+H]$^+$, 211.0962 [M+H]$^+$ |
| M2  | acetylation product of 9,17-octadecadiene-12,14-diene-1,11,16-triol,1-acetate | C$_{22}$H$_{30}$O$_5$ | 16.55 | 16.27 ± 2.46 | 375.2170 375.2166 | −1.07 | 79.0544 [M-C$_3$H$_2$O$_3$-C$_2$H$_2$+H]$^+$, 375.2170 [M+H]$^+$ |
| M3  | deglycosylation product of 4',7-epoxy-4,9,9'-trihydroxy-3,3'-dimethoxy-5',8'-lignan-4,9-bis[O-$\beta$-D-glucopyranoside] | C$_{26}$H$_{32}$O$_6$ | 20.06 | 11.37 ± 2.01 | 383.1470 383.1465 | −1.36 | 188.1603 [M-C$_{10}$H$_{12}$O$_4$+Na]$^+$, 383.1470 [M+Na]$^+$ |
| M4  | deglycosylation product of isolariciresinol 3-O-$\beta$-D-glucopyranoside | C$_{26}$H$_{32}$O$_6$ | 20.08 | 11.32 ± 0.98 | 383.1464 383.1465 | 0.30 | 167.4648 [M-C$_{10}$H$_{12}$O$_5$×2+H$_2$O+Na]$^+$, 383.1464[M+Na]$^+$ |
| M5  | deglycosylation product of 5-methoxylariciresinol 4-O-$\beta$-D-glucopyranoside | C$_{26}$H$_{32}$O$_7$ | 22.65 | 14.28 ± 1.42 | 413.1578 413.1571 | −1.86 | 231.8773 [M-C$_{24}$H$_{20}$O$_4$+Na]$^+$, 413.1578 [M+Na]$^+$ |
| M6  | hydroxylation product of olopandiol acetate | C$_{26}$H$_{30}$O$_5$ | 30.58 | 0.72 ± 0.43 | 351.2153 351.2166 | 3.71 | 107.8040 [M-H$_2$O-C$_{13}$H$_{12}$O$_2$+H]$^+$, 79.0547 [M-H$_2$O-C$_{13}$H$_{12}$O$_3$-C$_2$H$_4$+H]$^+$, 351.2153 [M+H]$^+$ |
| M7  | hydroxylation product of oploxyne B | C$_{18}$H$_{30}$O$_5$ | 30.64 | 3.02 ± 0.55 | 327.2163 327.2166 | 0.92 | 107.0851 [M-H$_2$O-C$_{11}$H$_{12}$O$_3$+H]$^+$, 79.0543 [M-H$_2$O-C$_{11}$H$_{12}$O$_3$-C$_2$H$_4$+H]$^+$, 327.2163 [M+H]$^+$ |
| M8  | acetylation product of oploxyne B | C$_{26}$H$_{32}$O$_3$ | 31.64 | 1.82 ± 0.63 | 353.2321 353.2323 | 0.43 | 107.0847 [M-H$_2$O-C$_{13}$H$_{22}$O$_2$+H]$^+$, 79.0545 [M-H$_2$O-C$_{13}$H$_{22}$O$_3$-C$_2$H$_4$+H]$^+$, 353.2321 [M+H]$^+$ |
| M9  | hydroxylation product of curcumene | C$_{15}$H$_{22}$O | 32.61 | 42.47 ± 4.29 | 219.1745 219.1743 | 0.73 | 63.0237[M-2×C$_3$H$_7$H$_2$O+H]$^+$, 219.1745[M+H]$^+$ |
| M10 | hydroxylation product of 9,17-octadecadiene-12,14-diene-1,11,16-triol,1-acetate | C$_{20}$H$_{28}$O$_5$ | 34.08 | 0.98 ± 0.26 | 349.1994 349.2010 | 4.45 | 105.0700[M-H$_2$O-C$_{13}$H$_{22}$O$_3$+H]$^+$, 79.0573 [M-H$_2$O-C$_{13}$H$_{22}$O$_3$-C$_2$H$_4$+H]$^+$, 349.1994[M+H]$^+$ |
| M11 | demethylation product of oploxyne B | C$_{17}$H$_{28}$O$_4$ | 34.24 | 15.83 ± 3.11 | 297.2061 297.2060 | −0.22 | 107.0508[M-H$_2$O-C$_{10}$H$_{22}$O$_2$+H]$^+$, 79.0554 [M-H$_2$O-C$_{10}$H$_{22}$O$_2$-C$_2$H$_4$+H]$^+$, 297.2061 [M+H]$^+$ |
| M12 | dehydroxylation product of 2-decenoic acid | C$_{10}$H$_{18}$O | 34.27 | 1.36±0.42 | 155.1431 155.1430 | −0.38 | 56.9427[M-CHO-C$_7$H$_{10}$+H]$^+$, 155.1431[M+H]$^+$ |
| M13 | hydroxylation product of olopantriol A | C$_{14}$H$_{26}$O$_4$ | 34.90 | 1.54 ± 0.72 | 307.1902 307.1904 | 0.61 | 105.0682[M-H$_2$O-C$_{11}$H$_{20}$O$_2$+H]$^+$, 79.0539 [M-H$_2$O-C$_{11}$H$_{20}$O$_2$-C$_2$H$_4$+H]$^+$, 307.1902 [M+H]$^+$ |

(Continued)
| No. | Description                           | Formula    | \(t_r\) (min) | Signal intensity \((\times 10^3)\) | \([M+H]^+\) or \([M+Na]^+\) m/z | Calc m/z | Diff ppm | Fragment ions in the positive mode with the energy 50 V CID |
|-----|--------------------------------------|------------|----------------|-----------------------------------|--------------------------------|----------|----------|---------------------------------------------------------|
| M14 | dehydroxylation product of oplopantriol A | \(C_{18}H_{36}O_2\) | 34.92          | 52.73 ± 3.40                     | 275.2003 | 275.2006 | 0.94     | 105.0698[M-H₂O\(C_9H_{18}O\)+H]⁺, 79.0541[M-H₂O-C₁₁H₂₀O-C₂H₄H⁺, 275.2003[M+H]⁺ |
| M15 | methylation product of falcariodiol  | \(C_{14}H_{26}O_2\) | 34.98          | 49.23 ± 2.49                     | 275.2008 | 275.2006 | -0.89    | 105.0704[M-H₂O-C₁₁H₂₀O-H]⁺, 79.0548[M-H₂O-C₁₁H₂₀-C₂H₄H⁺, 275.2008[M+H]⁺ |
| M16 | dehydroxylation product of oplopantriol acetate | \(C_{20}H_{30}O_3\) | 35.25          | 37.62 ± 4.21                     | 319.2256 | 319.2268 | 3.68     | 107.0525 [M-H₂O-C₁₃H₂₁O⁺H]⁺, 79.0538 [M-H₂O-C₁₃H₂₀O-C₂H₄H⁺, 319.2256[M+H]⁺ |
| M17 | hydrogenation product of falcariodiol | \(C_{17}H₂₂O₂\) | 35.89          | 1.36 ± 0.77                     | 263.2008 | 263.2006 | -0.93    | 105.0695[M-H₂O-C₁₃H₂₀O⁺H]⁺, 79.0535[M-H₂O-C₁₁H₂₀C₂H₄H⁺, 263.2008[M+H]⁺ |
| M18 | acetylation product of oplopantriol B | \(C_{20}H_{30}O_4\) | 37.06          | 12.48 ± 2.41                     | 335.2202 | 335.2217 | 4.45     | 107.0855 [M-H₂O-C₁₃H₂₁O₂O-H]⁺, 79.0543 [M-H₂O-C₁₃H₂₀O₂-C₂H₄H⁺, 335.2202[M+H]⁺ |
| M19 | hydrogenation product of oploxyne A  | \(C_{14}H₂₄O_4\) | 37.63          | 7.37 ± 2.49                     | 313.2375 | 313.2373 | -0.53    | 100.0752[M-H₂O-C₁₃H₂₁O₂-H]⁺, 79.0541 [M-H₂O-C₁₃H₂₀O₂-C₂H₄H⁺, 313.2375[M+H]⁺ |
| M20 | hydroxylation product of murolene    | \(C_{15}H₂₄O\) | 37.76          | 13.47 ± 3.57                     | 221.1901 | 221.1900 | -0.49    | 65.0374[M-2xC₆H₅O₂H⁺H]⁺, 221.1901[M +H]⁺ |
| M21 | hydrogenation product of 9,17-octadecadiene-12,14-diyn-1,11,16-triol,1-acetate | \(C_{20}H_{30}O_4\) | 37.77          | 17.38 ± 4.76                     | 335.2204 | 335.2217 | 3.85     | 105.0694[M-H₂O-C₁₃H₂₁O₂O-H]⁺, 79.0543 [M-H₂O-C₁₃H₂₀O₂-C₂H₄H⁺, 335.2204[M+H]⁺ |
| M22 | acetylation product of oplopantriol A | \(C_{20}H₂₄O₄\) | 37.90          | 2.55 ± 0.77                     | 333.2047 | 333.2060 | 4.02     | 105.0705[M-H₂O-C₁₃H₂₀O₂-H]⁺, 79.0524 [M-H₂O-C₁₃H₂₀O₂-C₂H₄H⁺, 333.2047[M+H]⁺ |
| M23 | hydrogenation product of oplopantriol acetate | \(C_{20}H₂₄O₄\) | 37.91          | 12.42 ± 3.28                     | 337.2358 | 337.2373 | 4.57     | 79.0538[M-C₁₃H₂₀O₂-C₂H₄H⁺, 337.2358[M+H]⁺ |
| M24 | hydroxylation product of 6,9-octadecadienoic acid | \(C_{14}H₂₄O₃\) | 37.92          | 17.52 ± 2.71                     | 297.2423 | 297.2424 | 0.41     | 77.0539[M-CH₃COOH-C₁₀H₂₀O-H]⁺, 297.2423[M+H]⁺ |
| M25 | hydrogenation product of oplopantriol B | \(C_{18}H₂₄O₃\) | 38.22          | 24.52 ± 3.98                     | 295.2263 | 295.2268 | 1.60     | 79.0557[M-C₁₁H₂₀O₂-C₂H₄H⁺, 295.2263[M+H]⁺ |
| M26 | demethylation product of falcariodiol | \(C_{14}H₂₂O₂\) | 38.59          | 2.26 ± 0.74                     | 247.1689 | 247.1693 | 1.45     | 105.0686[M-H₂O-C₁₃H₁₈O⁺H]⁺, 79.0528[M-H₂O-C₁₁H₁₆C₂H₄H⁺, 247.1689[M+H]⁺ |
| M27 | hydroxylation product of oplopantriol acetate | \(C_{17}H₂₆O₃\) | 38.64          | 50.12 ± 4.62                     | 279.1958 | 279.1955 | -1.18    | 107.0496[M-H₂O-C₁₀H₁₈O₁O-H]⁺, 79.0541 [M-H₂O-C₁₀H₁₆O-C₂H₄H⁺, 279.1958[M+H]⁺ |
| M28 | acetylation product of oplopantriol acetate | \(C_{22}H₂₆O₃\) | 39.27          | 4.92 ± 0.44                     | 377.2326 | 377.2323 | -0.93    | 79.0543[M-C₁₃H₂₆O₄-C₂H₄H⁺, 377.2326[M+H]⁺ |
| M29 | hydroxylation product of falcariodiol | \(C_{17}H₂₄O₂\) | 39.54          | 4.39 ± 0.58                     | 261.1851 | 261.1849 | -0.74    | 79.0538[M-C₁₀H₂₀O₂-C₂H₄H⁺, 261.1851[M+H]⁺ |
| M30 | hydroxylation product of oploxyne A  | \(C_{17}H₂₆O₄\) | 39.60          | 2.74 ± 0.94                     | 295.1902 | 295.1904 | 0.63     | 79.0557[M-C₁₀H₂₀O₃-C₂H₄H⁺, 295.1902[M+H]⁺ |
| M31 | demethylation product of falcariodiol | \(C_{19}H₂₂O\) | 39.61          | 1.02 ± 0.57                     | 231.1744 | 231.1743 | -0.25    | 79.0544[M-C₉H₁₈-C₂H₄H⁺, 231.1744[M+H]⁺ |

**Table 2 (continued)**
Table 2 (continued)

| No. | Description                        | Formula    | $t_R$ (min) | Signal intensity ($\times 10^6$) | [M+H]$^+$ or [M+Na]$^+$ | Fragment ions in the positive mode with the energy 50 V CID |
|-----|------------------------------------|------------|-------------|----------------------------------|--------------------------|----------------------------------------------------------|
| M32 | demethylation product of oplopantriol B | C$_{17}$H$_{28}$O$_3$ | 39.69 | 33.79 ± 4.95 | 279.1965 279.1955 −3.70 | 79.0542[M-C$_{10}$H$_{20}$O$_2$-C$_2$H$_4$+H]$^+$, 279.1965 [M+H]$^+$ |
| M33 | hydrogenation product of oploxyne A  | C$_{17}$H$_{28}$O$_3$ | 40.06 | 10.32 ± 1.45 | 281.2113 281.2111 −0.64 | 79.0541[M-C$_{10}$H$_{20}$O$_2$-C$_2$H$_4$+H]$^+$, 281.2113 [M+H]$^+$ |
| M34 | hydroxylation product of oplopantriol B | C$_{18}$H$_{28}$O$_4$ | 40.07 | 5.28 ± 1.82 | 309.2062 309.2060 −0.53 | 79.0560[M-C$_{11}$H$_{22}$O$_3$-C$_2$H$_4$+H]$^+$, 309.2062 [M+H]$^+$ |
| M35 | demethoxy product of oploxyne B      | C$_{17}$H$_{28}$O$_3$ | 40.07 | 9.24 ± 1.23 | 281.2112 281.2111 −0.28 | 79.0541[M-C$_{10}$H$_{20}$O$_2$-C$_2$H$_4$+H]$^+$, 281.2112 [M+H]$^+$ |
| M36 | hydrogenation product of oplopantriol A | C$_{18}$H$_{28}$O$_3$ | 40.09 | 2.83 ± 0.75 | 293.2109 293.2111 0.76 | 79.0538[M-C$_{11}$H$_{22}$O$_2$-C$_2$H$_4$+H]$^+$, 293.2109 [M+H]$^+$ |
| M37 | demethylation product of oleanolic acid | C$_{20}$H$_{26}$O$_3$ | 40.18 | 0.54 ± 0.26 | 443.3524 443.3520 −0.97 | 165.0912[M-C$_{16}$H$_{22}$O$_2$-2×CH$_3$+H]$^+$, 443.3524[M+H]$^+$ |
| M38 | demethylation product of curcumene     | C$_{14}$H$_{20}$ | 40.18 | 2.42 ± 0.29 | 189.1636 189.1638 0.94 | 51.0229[M-2×C$_2$H$_4$+H]$^+$, 189.1636[M+H]$^+$ |
| M39 | dehydroxylation product of oploxyne A  | C$_{17}$H$_{26}$O$_2$ | 40.29 | 0.51 ± 0.23 | 263.2005 263.2006 0.22 | 79.0552[M-C$_{10}$H$_{20}$O-C$_2$H$_4$+H]$^+$, 263.2005 [M+H]$^+$ |
| M40 | demethylation product of oplopandiol  | C$_{18}$H$_{28}$O$_2$ | 40.48 | 7.12 ± 0.44 | 249.1851 249.1849 −0.78 | 107.0863[M-H$_2$O-C$_8$H$_{14}$+H]$^+$, 249.1851[M+H]$^+$ |
| M41 | hydrogenation product of falcarinol    | C$_{17}$H$_{26}$O | 40.50 | 22.15 ± 2.35 | 247.2053 247.2056 1.39 | 79.0528[M-C$_{10}$H$_{22}$-C$_2$H$_4$+H]$^+$, 247.2053 [M+H]$^+$ |
| M42 | methylation product of oplopandiol acetate | C$_{21}$H$_{32}$O$_4$ | 40.51 | 1.10 ± 0.77 | 349.2373 349.2373 0.10 | 79.0541[M-C$_{14}$H$_{26}$O$_3$-C$_2$H$_4$+H]$^+$, 349.2373 [M+H]$^+$ |
| M43 | dehydroxylation product of oplopandiol | C$_{17}$H$_{26}$O | 40.53 | 20.63 ± 3.86 | 247.2060 247.2056 −1.45 | 107.0513[M-C$_{10}$H$_{26}$+H]$^+$, 79.0528[M-C$_{16}$H$_{20}$C$_2$H$_4$+H]$^+$, 247.2060[M+H]$^+$ |
| M44 | dehydroxylation product of falcariol   | C$_{17}$H$_{24}$ | 40.53 | 4.76 ± 1.42 | 229.1953 229.1951 −0.98 | 77.0398[M-C$_{8}$H$_{14}$-C$_2$H$_4$+H]$^+$, 229.1953 [M+H]$^+$ |
| M45 | demethylation product of muurolene    | C$_{14}$H$_{22}$ | 40.55 | 4.97 ± 0.99 | 191.1796 191.1794 −0.91 | 53.0384[M-2×C$_2$H$_4$+H]$^+$, 191.1796 [M+H]$^+$ |
| M46 | dehydroxylation product of falcariolide | C$_{17}$H$_{24}$O | 41.18 | 67.22 ± 3.74 | 245.1903 245.1900 −1.26 | 105.0692[M-C$_{10}$H$_{26}$+H]$^+$, 79.0521[M-C$_{16}$H$_{20}$C$_2$H$_4$+H]$^+$, 245.1903[M+H]$^+$ |
| M47 | methylation product of oplopantriol A | C$_{19}$H$_{32}$O$_3$ | 41.41 | 0.52 ± 0.28 | 305.2111 305.2113 −0.59 | 79.0547[M-C$_{13}$H$_{26}$O$_2$-C$_2$H$_4$+H]$^+$, 305.2111 [M+H]$^+$ |
| M48 | demethylation product of oplopantriol A | C$_{17}$H$_{24}$O$_3$ | 41.42 | 53.27 ± 3.42 | 277.1800 277.1798 −0.65 | 79.0537[M-C$_{10}$H$_{20}$-C$_2$H$_4$+H]$^+$, 277.1800 [M+H]$^+$ |
| M49 | methylation product of oplopandiol    | C$_{18}$H$_{28}$O$_2$ | 41.43 | 51.35 ± 2.53 | 277.2160 277.2162 0.75 | 107.0871[M-H$_2$O-C$_{11}$H$_{20}$+H]$^+$, 79.0547[M-H$_2$O-C$_{11}$H$_{20}$-C$_2$H$_4$+H]$^+$, 277.2160[M+H]$^+$ |
| M50 | dehydroxylation product of oplopantriol B | C$_{18}$H$_{28}$O$_2$ | 41.44 | 53.87 ± 4.21 | 277.2164 277.2162 −0.70 | 79.0541[M-C$_{11}$H$_{22}$O-C$_2$H$_4$+H]$^+$, 277.2164 [M+H]$^+$ |
| M51 | methylation product of falcariol       | C$_{18}$H$_{28}$O | 41.59 | 3.75 ± 0.89 | 259.2057 259.2056 −0.23 | 79.0524[M-C$_{11}$H$_{22}$-C$_2$H$_4$+H]$^+$, 259.2057 [M+H]$^+$ |
| M52 | methylation product of oplopantriol B | C$_{19}$H$_{30}$O$_3$ | 41.69 | 1.26 ± 0.82 | 307.2262 307.2268 1.87 | 79.0543[M-C$_{13}$H$_{26}$O$_2$-C$_2$H$_4$+H]$^+$, 307.2262 [M+H]$^+$ |

(Continued)
Others
For two sesquiterpenes, M9, M38, and M58 were identified as the hydroxylation, demethylation, and hydrogenation products of curcumene, while M20 and M45 were the hydroxylation and demethylation product of muurolene, respectively. M37 and M62 were identified as the demethylation and acetylation products of oleanolic acid. In addition, for 2 fatty acids, M12 was the dehydroxylation product of 2-decenoic acid, while M24 and M59-61 were the products of 6,9-octadecenoic acid.

Proposed metabolic pathways of *O. elatus* extract
The proposed metabolic pathways of *O. elatus* extract by human intestinal microflora are presented in Fig. 5. Multiple major metabolite pathways can be observed in this study. The common pathways involved in the biotransformation of *O. elatus* extract include methylation, demethylation, hydroxylation, dehydroxylation, acetylation, hydrogenation,

| No. | Description                                      | Formula  | t<sub>R</sub> (min) | Signal intensity (×10^5) | [M+H]<sup>+</sup> or [M+Na]<sup>+</sup> | Fragment ions in the positive mode with the energy 50 V CID |
|-----|--------------------------------------------------|----------|----------------------|--------------------------|-----------------------------------------|----------------------------------------------------------|
| M53 | methylation product of oploxyne B                | C<sub>16</sub>H<sub>32</sub>O<sub>4</sub> | 41.76                 | 0.37 ± 0.20              | 325.2371 325.2373                      | 79.0540[M-C<sub>12</sub>H<sub>26</sub>O<sub>3</sub>-C<sub>2</sub>H<sub>4</sub>+H]<sup>+</sup>, 325.2371 [M+H]<sup>+</sup> |
| M54 | dehydroxylation product of 9,17-octadecadiene-12,14-diyne-1,11,16-triol,1-acetate | C<sub>20</sub>H<sub>28</sub>O<sub>3</sub> | 42.15                 | 28.46 ± 1.55             | 317.2096 317.2111                      | 79.0537[M-C<sub>13</sub>H<sub>24</sub>O<sub>2</sub>-C<sub>2</sub>H<sub>2</sub>+H]<sup>+</sup>, 317.2096 [M+H]<sup>+</sup> |
| M55 | dehydroxylation product of oploxyne B            | C<sub>18</sub>H<sub>30</sub>O<sub>3</sub> | 42.16                 | 56.82 ± 2.47             | 295.2273 295.2268                       | 79.0550[M-C<sub>13</sub>H<sub>24</sub>O<sub>2</sub>-C<sub>2</sub>H<sub>4</sub>+H]<sup>+</sup>, 295.2273 [M+H]<sup>+</sup> |
| M56 | hydroxylation product of falcarindiol            | C<sub>17</sub>H<sub>24</sub>O<sub>3</sub> | 42.20                 | 0.42 ± 0.28              | 347.2213 347.2217                      | 79.0541[M-C<sub>14</sub>H<sub>30</sub>O<sub>3</sub>-C<sub>2</sub>H<sub>2</sub>+H]<sup>+</sup>, 347.2213 [M+H]<sup>+</sup> |
| M57 | methylation product of 9,17-octadecadiene-12,14-diyne-1,11,16-triol,1-acetate | C<sub>21</sub>H<sub>30</sub>O<sub>4</sub> | 42.52                 | 0.07 ± 0.03               | 347.2213 347.2217                      | 79.0541[M-C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>-C<sub>2</sub>H<sub>4</sub>+H]<sup>+</sup>, 347.2213 [M+H]<sup>+</sup> |
| M58 | hydrogenation product of curcumene               | C<sub>16</sub>H<sub>24</sub> | 42.65                 | 70.36 ± 4.29             | 205.1953 205.1951                       | 67.0540[M-2×C<sub>2</sub>H<sub>4</sub>+H]<sup>+</sup>, 205.1953 [M+H]<sup>+</sup> |
| M59 | demethylation product of 6,9-octadecenoic acid   | C<sub>17</sub>H<sub>30</sub>O<sub>2</sub> | 42.75                 | 0.48 ± 0.32              | 267.2327 267.2319                      | 65.0390[M-CH<sub>3</sub>CHO-C<sub>11</sub>H<sub>20</sub>+H]<sup>+</sup>, 267.2327[M+H]<sup>+</sup> |
| M60 | dehydroxylation product of 6,9-octadecenoic acid | C<sub>18</sub>H<sub>32</sub>O<sub>2</sub> | 43.40                 | 65.77 ± 5.21             | 265.2536 265.2526                       | 69.0688[M-CH<sub>3</sub>CHO-C<sub>11</sub>H<sub>20</sub>+H]<sup>+</sup>, 265.2536 [M+H]<sup>+</sup> |
| M61 | hydrogenation product of 6,9-octadecenoic acid   | C<sub>18</sub>H<sub>34</sub>O<sub>2</sub> | 43.50                 | 128.46 ± 8.42            | 283.2636 283.2632                      | 65.0389[M-CH<sub>3</sub>COOH-C<sub>11</sub>H<sub>22</sub>+H]<sup>+</sup>, 283.2636[M+H]<sup>+</sup> |
| M62 | acetylation product of oleanolic acid            | C<sub>32</sub>H<sub>50</sub>O<sub>4</sub> | 48.23                 | 2.48 ± 1.63              | 521.3606 521.3601                      | 220.0842[M-C<sub>18</sub>H<sub>34</sub>O<sub>2</sub>-C<sub>2</sub>H<sub>2</sub>O+Na]<sup>+</sup>, 521.3606[M+Na]<sup>+</sup> |
Figure 3 Metabolites of falcarindiol using UPLC-TOF/MS in the positive ion mode. (A) Extracted ion chromatograms (EICs); (B) MS/MS spectra and structural elucidation.
demethoxylation, and deglycosylation. Among them, polyynes were undoubtedly the most important compounds, as 46 out of 62 metabolites originated from polyynes. By comparing the signal intensity of metabolites, we could find that methylation, dehydroxylation and hydroxylation are major metabolic pathways of polyynes. Moreover, four metabolites of lignans and phenylpropanoid were produced by the loss of glucose.

Figure 4 Metabolites of oplopandiol using UPLC-TOF/MS in the positive ion mode. (A) EICs; (B) MS/MS spectra and structural elucidation. DOI: 10.7717/peerj.12513/fig-4
The other metabolites were generated from one triterpenoid and two fatty acids. This indicated that polyynes of *O. elatus* generated comprehensive biotransformation and were more readily metabolized than other compounds under the same conditions.
In summary, the main metabolic pathways of *O. elatus* refer to hydrolytic and reductive reactions by gut microorganisms. Because of the complexity of active ingredients or constituent concentrations, *in vivo* exposure, and individual differences, the metabolic profiles of *O. elatus* might be affected by several factors.

**DISCUSSION**

In this study, a UPLC-Q-TOF-MS/MS method was developed to screen and identify the chemical composition and metabolites from a traditional Chinese herb, the air-dried root bark of *O. elatus*. A total of 18 ingredients and 62 metabolites biotransformed by human intestinal microflora were characterized from *O. elatus* in UPLC-Q-TOF/MS positive ion mode. Two polyynes, falcarnidiol and oplopandiol, as the main components of *O. elatus* and their metabolites by human intestinal microflora are mainly illustrated. It could be noted that the major metabolic pathways of *O. elatus* refer to methylation, dehydroxylation, and hydroxylation. Studies on the chemical and metabolic profiling of *O. elatus* by human intestinal microflora will be helpful for the understanding of mechanism research on the active components and further *in vivo* investigation.

**ADDITIONAL INFORMATION AND DECLARATIONS**

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**Competing Interests**

The authors declare that they have no competing interests.

**Author Contributions**

- Jin-Yi Wan performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Jing-Xuan Wan performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Shilei Wang performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Xiaolu Wang performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Wenqian Guo analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
• Han Ma analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
• Yuqi Wu analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
• Chong-Zhi Wang conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Lian-Wen Qi performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Ping Li analyzed the data, prepared figures and/or tables, and approved the final draft.
• Haiqiang Yao conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Chun-Su Yuan conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Human Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

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Data Availability
The following information was supplied regarding data availability:

The raw measurements are available in the Supplementary Files.

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