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Phytochemistry and Antioxidant Activities of the Rhizome and Radix of Millettia speciosa Based on UHPLC-Q-Exactive Orbitrap-MS

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Abstract: The root of Millettia speciosa Champ. (MSCP) is used in folk medicine and is popular as a soup ingredient. The root is composed of the rhizome and radix, but only the radix has been used as a food. Thus, it is very important to compare the chemical components and antioxidant activities between the rhizome and radix. The extracts were analyzed by UHPLC-Q-Exactive Orbitrap-MS and multivariate analysis, and the antioxidant activities were evaluated by 2,2-azinobis(3-ethylbenzothiazo-line-6-sulfonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. Ninety-one compounds were detected simultaneously and temporarily identified. Ten compounds were identified as chemical markers to distinguish the rhizome from the radix. The antioxidant activities of the radix were higher than the rhizome. Correlation analysis showed that uvaol-3-caffeate, 3-O-caffeoyloleanolic acid, and khrinone E were the main active markers for antioxidant activity, which allowed for the rapid differentiation of rhizomes and the radix. Therefore, it could be helpful for future exploration of its material base and bioactive mechanism. In addition, it would be considered to be used as a new method for the quality control of M. speciosa.

Keywords: antioxidants; chemical constituent; Millettia speciosa; multivariate analysis

1. Introduction
Millettia speciosa Champ. belongs to the Leguminosae family. It is a well-known food and medicine ingredient that is mainly found in Guangxi, Guangdong, and Hainan Provinces of China [1]. MSCP is called Niudali in southern China, which means that it has a strong power, like a bull. The MSCP is commonly used to make soups with pig bones, which can strengthen the functions of the immune system and promote anti-inflammatory and anti-tumor effects. It is also made into various functional products, such as MSCP powder, MSCP tea, and MSCP wine. In previous studies, the chemical constituents of MSCP mainly include polysaccharides, flavonoids, alkaloids, terpenoids, and phenylpropanoids [2–4]. Pharmacological studies showed that the aqueous extracts of MSCP exhibited antifatigue, immunomodulatory, antioxidative, anti-hepatitis, and analgesic activities [3–7].

However, the root of MSCP consists of the radix and rhizome. Usually, the radix is swollen and powdery, commonly known as Niudali potato, whereas the rhizome is almost lignified and fibrous (Figure 1). Only the radix has been traditionally consumed [8]. Moreover, the price of the radix is higher than that of the rhizome in herbal markets. Therefore, the rhizome is often mixed into the radix before sale, which greatly affects the quality of the radix. Previous studies have mainly focused on the chemical composition and activity of MSCP [1,6,7,9]. A comparative analysis of alcohol soluble components
from the rhizome and radix of MSCP showed that the contents of flavonoids, saponins, and alkaloids in the rhizome were greater than those of the radix using ultraviolet–visible spectroscopy [10]. The antioxidant activities of the different parts of MSCP were also compared, and the activity of the root was higher than that of the stem, leaf, and flower [11]. The root of MSCP showed a good antioxidant activity [11]. The root is composed of the rhizome and the radix (Figure 1), but their texture and morphological characteristics are significantly different. There are no reports on the difference in chemical constituents and antioxidant activities between the rhizome and the radix of MSCP.

Ultra-high performance liquid chromatography coupled with the quadrupole time of flight mass spectrometry (UHPLC-QTOF-MS/MS) has been widely used in the separation and structural analysis of complex systems, such as traditional Chinese medicines, due to its rapid separation, high efficiency, high sensitivity, high resolution, and molecular weight accuracy [12]. It can be widely used for the chemical identification of different base sources and different medicinal parts of herbal medicines [13–15]. Recently, UHPLC-Q-Exactive Orbitrap MS, with a high selectivity and effectivity, was widely used in the identification of chemical constitutions.

In this study, the characteristic chemical components in twenty-eight batches of MSCP were qualitatively analyzed by UHPLC-Q-Exactive Orbitrap-MS. Moreover, principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to distinguish the differences between the rhizome and the radix of MSCP. In addition, the antioxidant activities were evaluated by the scavenging rate of ABTS and DPPH. Gray relational analysis (GRA) and partial least squares (PLS) were used to identify potential bioactive markers and to assess the correlation between the characteristic compounds and antioxidant activities.

2. Results and Discussion

2.1. Optimization of Chromatographic Conditions

In our previous study, we compared the effectiveness of the two columns in the analysis of MSCP samples and found that the ACQUITY HSS T 3 column (100 mm × 2.1 mm, 1.8 µm) was more suitable for the analysis of MSCP because of the greater number of compounds detected and separated. The chromatographic separation was optimized by investigating parameters, such as organic phases (acetonitrile and methanol), with mobile
phases (water, water-containing formic acid, and water-containing formic acid and ammonium), and temperatures (20, 25, 30, 35, and 40 °C). The optimal mobile phase consisted of 0.1% aqueous formic acid-acetonitrile with 35 °C, due to a superior peak pattern, better response value, and peak shapes. The base peak ions (BPIs) of rhizome and radix samples obtained in the negative ion mode are shown in Figure 2.

Figure 2. The BPI chromatograms of MSCP rhizome (A) and radix (B) based on UHPLC-Q-Exactive Orbitrap-MS in negative mode.

2.2. Identification of Components in the Rhizome and Radix Based on UHPLC-Q-Exactive Orbitrap MS

Under the optimal chromatographic and MS conditions, 91 compounds were unambiguously identified, including 71 flavones, 10 phenolic acids, 7 triterpenes, 3 alkaloids, and 10 fatty acid derivatives. Among them, 7 compounds were identified with corresponding reference standards (peaks 10, 28, 40, 55, 70, 72, and 81); however, the remaining 84 compounds were tentatively assigned in accordance with previously published MS data and literature. The retention times, MS data, and fragment ions of all detected compounds are shown in Table 1.
Table 1. The mass spectrometry data and identification of rhizome and radix in MSCP.

| NO. | Retention Time (min) | ESI-MS (m/z) | Error (ppm) | MS/MS Fragments Ions | Formula | Identification |
|-----|---------------------|--------------|-------------|----------------------|---------|----------------|
| 1   | 3.31                | 315.0715 [M-H]^- | -0.24       | 152.0101, 109.0202   | C13H16O5 | Protocatechuic acid-4-glucoside |
| 2   | 3.57                | 315.0714 [M-H]^- | -0.53       | 152.0101, 109.0201   | C12H15O5 | Protocatechuic acid-3-glucoside |
| 3   | 4.44                | 421.1330 [M+H]^+ | 0.16        | 289.0921, 128.0442, 127.0387, 97.0285, 85.0287 | C17H24O12 | NCGC00380493-01 |
| 4   | 4.54                | 461.1290 [M-H]^- | 0.39        | 329.0862, 167.0336, 152.0101, 108.0202 | C19H26O13 | Saccharoside C |
| 5   | 4.74                | 461.1292 [M-H]^- | 0.39        | 329.0858, 167.0336, 152.0101, 108.0202 | C19H26O13 | Saccharoside D |
| 6   | 4.86                | 315.0716 [M-H]^- | -0.05       | 153.0179, 109.0280   | C13H16O5 | Protocatechuic acid-2-glucoside |
| 7   | 5.13                | 447.1136 [M-H]^- | 0.56        | 315.0715, 152.0101, 108.0202 | C18H24O13 | 4-Hydroxy-5-(3',4',5'-Trihydroxyphenyl)-Valeric Acid-O-Methyl-O-Glucuronide |
| 8   | 5.17                | 447.1137 [M-H]^- | 0.54        | 315.0715, 152.0102, 108.0202 | C18H24O13 | 4-Hydroxy-5-(3',4',5'-Trihydroxyphenyl)-Valeric Acid-O-Methyl-O-Glucuronide |
| 9   | 5.23                | 477.1603 [M-H]^- | 0.09        | 345.1177, 183.0651, 168.0414, 153.0179 | C20H30O13 | Shamimino |
| 10  | 6.00                | 188.0703 [M+H]^+ | -1.40       | 170.0594, 143.0728, 118.0652, 115.0542, 91.0545 | C11H2O2N | trans-3-Indoleacrylic acid |
| 11  | 6.06                | 247.1434 [M+H]^+ | -3.8        | 188.0701, 146.0598, 118.0650, 60.0873 | C14H16O2N2 | Hypophorine |
| 12  | 6.07                | 188.0704 [M+H]^+ | -1.4        | 170.0595, 143.0720, 118.0651, 115.0541, 91.0544 | C11H2O2N | Indole-3-crylic acid |
| 13  | 6.69                | 577.1352 [M-H]^- | 1.96        | 407.0760, 289.0711, 245.0816, 161.0233, 125.0299 | C30H26O12 | Procyanidin B2 |
| 14  | 6.88                | 477.1602 [M-H]^- | 0.09        | 345.1178, 183.0650, 168.0415, 153.0179 | C20H30O13 | Kelampayoside A |
| 15  | 7.07                | 289.0708 [M-H]^- | 0.47        | 245.0809, 137.0229, 125.0229, 109.0260 | C13H14O6 | Epicatechin |
| 16  | 7.18                | 581.1500 [M-H]^- | -0.13       | 287.0554, 269.0449, 259.0605, 151.0023 | C26H30O15 | 5,7,3',4',5'-Tetrahydroxyflavanone-7-alpha-L-arabinofuranosyl(1->6)-glucoside |
| 17  | 7.32                | 449.1078 [M-H]^- | 0.29        | 287.0553, 269.0446, 259.0602, 174.0493, 151.0020 | C21H22O11 | Eriodictyol-7-O-glucoside |
| 18  | 7.58                | 505.1703 [M-H]^- | -0.35       | 343.1176, 325.1073, 310.0838 | C25H30O11 | Diosbulbinoside D |
| 19  | 7.70                | 563.1396 [M-H]^- | 0.10        | 431.0963, 269.0448 | C26H28O14 | Apiin |
| 20  | 7.93                | 583.1662 [M-H]^- | 0.77        | 167.0361, 152.0103, 123.0432 | C26H32O15 | Seguinidos K |
| 21  | 7.95                | 613.1751 [M-H]^- | 0.29        | 338.5223, 197.0443, 182.0204, 161.0439, 153.0543, 139.0382, 115.9812, | C27H34O16 | Alibbriassistos B |
| 22  | 8.02                | 431.1182 [M-H]^- | -0.49       | 299.0775, 137.0320, 93.0330 | C18H24O12 | Apiosylglucosyl-4-hydroxybenzoate |
| 23  | 9.09                | 271.0604 [M-H]^- | 1.81        | 243.0654, 227.0702, 225.0548, 163.0025, 135.0074, 109.0280, 91.0174 | C13H12O5 | 3',4',7-Trihydroxylavanne |
| 24  | 9.12                | 445.1127 [M-H]^- | -0.50       | 283.0665, 268.0370, 240.0420, 224.0464, 212.0472, 135.0073 | C22H22O10 | Calycosin-7-O-beta-D-glucoside |
Table 1. Cont.

| NO. | Retention Time (min) | ESI-MS (m/z) | Error (ppm) | MS/MS Fragments | Formula | Identification               |
|-----|----------------------|--------------|-------------|-----------------|---------|-----------------------------|
| 25  | 9.49                 | 271.0611 [M-H]⁻   | 0.97        | 243.0654, 227.0731, 225.0540, 163.0028, 135.0075, 109.0280, 91.0173 | C₁₅H₁₂O₅ | 3’,4’,7-trihydroxyisoflavanone |
| 26  | 9.97                 | 627.1902 [M-H]⁻   | −1.7        | 556.2740, 459.1497, 183.0654, 167.0336, 152.0102, 123.0437 | C₂₈H₉₀O₁₆ | Khaephuoside B               |
| 27  | 10.26                | 271.0597 [M+H]⁻   | −3.6        | 253.0482, 225.0542, 215.0690, 197.0593, 137.0230 | C₁₅H₁₀O₃ | 5’,3’,4’-Trihydroxyflavone   |
| 28  | 10.37                | 137.0229 [M-H]⁻   | −2.31       | 93.0329         | C₇H₆O₃ | Salicylic acid               |
| 29  | 10.49                | 271.0606 [M-H]⁻   | 0.97        | 253.0505, 135.0437, 134.0388, 91.0173 | C₁₅H₁₂O₅ | butein                      |
| 30  | 10.64                | 187.0963 [M-H]⁻   | −1.2        | 169.0850, 143.1064, 125.0956, 97.0643 | C₉H₁₆O₄ | Azelaic acid                |
| 31  | 10.71                | 577.1554 [M-H]⁻   | 0.37        | 445.1126, 283.0606 | C₂₇H₃₀O₁₄ | Yuankanin                   |
| 32  | 10.74                | 271.0601 [M-H]⁻   | −0.31       | 243.0654, 227.0715, 163.0028, 135.0073, 109.0280, 91.0173 | C₁₅H₁₂O₃ | 2’,4’,7-trihydroxyisoflavanone |
| 33  | 10.91                | 269.0448 [M-H]⁻   | 0.32        | 241.0495, 227.0430, 225.0524, 224.0544, 213.0547, 195.0441, 185.0593, 135.0073, 133.0280, 91.0173 | C₁₅H₁₀O₅ | Sulfuretin                  |
| 34  | 10.95                | 273.0758 [M-H]⁻   | 0.22        | 255.0658, 227.0699, 167.0336, 137.0299, 109.0280 | C₁₅H₁₄O₅ | 2’,4’,4’,a-tetrahydroxy-dihydrochalcone |
| 35  | 10.98                | 445.1131 [M-H]⁻   | 0.33        | 283.0605, 268.0369, 239.0343, 224.0472, 212.0464, 151.0032, 132.0202 | C₂₂H₂₂O₁₀ | Sissotrin                   |
| 36  | 11.68                | 285.0760 [M-H]⁻   | 0.27        | 270.0511, 269.0411, 253.0500, 241.0487, 270.0511, 253.0500, 241.0487, 180.0051, 161.0230, 148.0148, 135.0444, 123.0436, 91.0173 | C₁₆H₁₄O₅ | Vestiton                    |
| 37  | 11.82                | 285.0748 [M+H]⁻   | 0.92        | 270.0515, 242.0564, 225.0535, 197.0583, 137.0230 | C₁₆H₁₂O₅ | 5’,4’-dihydroxy-3’-methoxy-isoflavone |
| 38  | 11.91                | 253.0497 [M-H]⁻   | −3.5        | 223.0395, 208.0521, 195.0440, 180.0568, 135.0072, 91.0173 | C₁₅H₁₁O₄ | Daidzein                    |
| 39  | 12.02                | 255.0659 [M-H]⁻   | 2.72        | 135.0075, 119.0487, 91.0174 | C₁₅H₁₂O₄ | dihydrodaidzein             |
| 40  | 12.20                | 255.0652 [M-H]⁻   | 0.25        | 135.0074, 119.0487, 91.0173 | C₁₅H₁₀O₄ | Liquiritigenin              |
| 41  | 12.31                | 283.0602 [M-H]⁻   | 0.38        | 268.0371, 251.0337, 239.0342, 224.0495, 211.0388, 195.0441, 183.0441, 167.0484, 156.0567, 148.0153, 135.0073, 91.0173 | C₁₆H₁₃O₅ | Isoprunetin                 |
| 42  | 12.40                | 285.0758 [M-H]⁻   | −0.36       | 270.0520, 228.1277, 194.5045, 149.0230, 135.0073, 121.0283, 91.0173 | C₁₆H₁₄O₅ | 3’,4’-Dihydroxy-7’-methoxyflavanone |
| 43  | 12.62                | 315.0850 [M-H]⁻   | 2.12        | 176.0103, 163.0020, 135.0071 | C₁₇H₁₄O₆ | Violanone                   |
| NO. | Retention Time (min) | ESI-MS (m/z) | Error (ppm) | MS/MS Fragments | Formula | Identification |
|-----|----------------------|--------------|-------------|----------------|---------|----------------|
| 44  | 12.65                | 285.0758 [M-H]^- | -0.38       | 270.0520, 269.0444, 228.1277, 194.5045, 149.0230, 135.0073, 121.0283, 91.0173 | C_{16}H_{14}O_5 | 3’,7-Dihydroxy-4’-methoxyisoflavanone |
| 45  | 12.72                | 283.0603 [M-H]^- | 1.2         | 268.0370, 251.0348, 224.0465, 211.0387, 195.0440, 183.0445, 167.0490, 156.0565, 148.0152, 135.0073, 91.0173 | C_{16}H_{14}O_5 | 2’-Hydroxyformononetin |
| 46  | 12.79                | 297.0398 [M-H]^- | 0.33        | 269.0488, 253.0501, 225.0548, 211.0396, 197.0596, 181.0643, 161.0228, 147.0069, 135.0075, 91.0173 | C_{16}H_{10}O_5 | Griffonianone H |
| 47  | 12.88                | 285.0759 [M-H]^- | 0.39        | 270.0520, 228.1277, 194.5045, 167.0490, 156.0565, 148.0152, 135.0073, 91.0173 | C_{16}H_{14}O_5 | 3,8-Dihydroxy-9-methoxypterocarpan |
| 48  | 13.34                | 297.0762 [M-H]^- | 1.41        | 282.0527, 267.0291, 254.0577, 239.0340, 223.0391, 211.0392, 195.0439, 183.0437, 167.0488, 132.0199 | C_{17}H_{14}O_5 | 8-O-Methylretusin |
| 49  | 13.38                | 269.0799 [M-H]^- | 1.2         | 254.0555, 226.0610, 151.0413, 118.0411 | C_{16}H_{12}O_4 | 4’-hydroxy-7-methoxyisoflavone |
| 50  | 13.51                | 269.0812 [M-H]^- | 0.38        | 253.0499, 237.0547, 225.0549, 211.0394, 161.0225, 145.0278, 133.0188, 117.0331, 108.0201 | C_{16}H_{14}O_4 | medicarpin |
| 51  | 13.58                | 255.0655 [M-H]^- | 1.2         | 135.0073, 119.0487 | C_{16}H_{12}O_4 | 5,4’-dihydroxy-flavanone |
| 52  | 13.83                | 301.0699 [M-H]^- | -2.40       | 286.0463, 269.0443, 241.0485, 229.0488, 153.0177, 134.0351 | C_{16}H_{12}O_6 | 2’-Hydroxybiochanin A |
| 53  | 14.15                | 271.0598 [M-H]^- | -1.81       | 253.0488, 135.0436, 134.0359, 91.0173 | C_{15}H_{12}O_5 | Garbanzol |
| 54  | 14.18                | 297.0759 [M-H]^- | 0.66        | 282.0526, 267.0292, 254.0577, 239.0341, 223.0390, 211.0389, 195.0438, 183.0439, 167.0488, 132.0202 | C_{17}H_{14}O_5 | Afrormosin |
| 55  | 14.35                | 271.0606 [M-H]^- | 0.36        | 187.0385, 165.0176, 151.0022, 119.0487, 107.0123 | C_{15}H_{12}O_5 | Naringenin |
| 56  | 14.40                | 287.0912 [M+H]^+ | -0.52       | 269.0803, 254.0559, 226.0622, 198.0659, 153.0544, 135.0437, 107.0492 | C_{16}H_{14}O_5 | 6,7-Dihydroxy-4’-methoxyisoflavanone |
| 57  | 14.40                | 271.0957 [M+H]^+ | -2.54       | 151.0386, 119.0489 | C_{16}H_{14}O_4 | 2’4-dihydroxy-4’-methoxychalcone |
| 58  | 14.55                | 297.0761 [M-H]^- | 0.65        | 282.0526, 267.0293, 254.0575, 239.0340, 223.0392, 221.0390, 195.0437, 183.0439, 167.0489, 132.0202 | C_{17}H_{14}O_5 | Alfalone |
Table 1. Cont.

| NO. | Retention Time (min) | ESI-MS (m/z) | Error (ppm) | MS/MS Fragments Ions | Formula | Identification          |
|-----|----------------------|--------------|-------------|----------------------|---------|-------------------------|
| 59  | 14.66                | 301.0701 [M+H]^+ | −0.59       | 286.0467, 285.0336, 269.0467, 241.0486, 229.0486, 213.0538, 184.0517, 139.0541, 93.0377 | C_{16}H_{12}O_{6} | Tectorigenin       |
| 60  | 14.67                | 283.0604 [M-H]^− | 1.02        | 268.0370, 239.0334, 224.0468, 211.0387, 195.0443, 183.0436, 167.0491, 135.0073, 91.0174  | C_{16}H_{12}O_{5} | Calycosin        |
| 61  | 14.85                | 285.0747 [M+H]^+ | −3.75       | 267.0634, 239.0691, 211.0700, 196.0515, 151.0386 | C_{16}H_{12}O_{5} | 3',4'-Dihydroxy-7-methoxyisoflavone |
| 62  | 14.88                | 301.0697 [M+H]^+ | 1.35        | 286.0456, 285.0329, 269.0433, 241.0488, 229.0487, 213.0532, 187.0383, 153.0177, 134.0359 | C_{16}H_{12}O_{6} | Pratensein        |
| 63  | 15.01                | 315.0850 [M+H]^+ | −4.08       | 300.0615, 272.0644, 257.0429, 255.0637, 244.0714, 240.0440, 227.0696, 216.0788, 212.0449, 201.0551, 175.0388, 167.0355, 152.0096, 148.0514, 133.0228 | C_{17}H_{14}O_{6} | 5',4'-Dihydroxy-7,3'-dimethoxyisoflavone |
| 64  | 15.12                | 315.0504 [M-H]^− | 2.4         | 300.0644, 284.0270, 256.0344, 148.0128, 125.0229 | C_{16}H_{12}O_{7} | 3',5,6,7-Tetrahydroxy-4'-methoxyisoflavone |
| 65  | 15.15                | 283.0604 [M-H]^− | 1.13        | 268.0369, 256.0370, 239.0344, 224.0467, 211.0388, 195.0444, 183.0441, 167.0489, 132.0201 | C_{16}H_{12}O_{5} | Glycitein         |
| 66  | 15.25                | 285.0761 [M-H]^− | 1.36        | 270.0526, 267.0656, 255.0288, 241.0493, 224.0466, 211.0395, 183.0438, 153.0169, 149.0230 | C_{16}H_{12}O_{5} | 3',4'-Dihydroxy-7-methoxyisoflavonone |
| 67  | 15.32                | 313.0346 [M-H]^− | 0.33        | 285.0393, 269.1279, 257.0460, 245.0443, 227.0341, 217.0496, 203.0340, 175.0387, 161.0231, 151.0022, 149.0231, 133.0282, 109.0277, 107.0123 | C_{16}H_{10}O_{7} | Luteolal          |
| 68  | 15.51                | 301.0712 [M-H]^− | 0.43        | 286.0425, 151.0028, 125.0229 | C_{16}H_{11}O_{6} | Ferreirin         |
| 69  | 15.55                | 281.0446 [M-H]^− | 0.92        | 253.0496, 223.0398, 208.0524, 195.0441, 180.0564, 167.0487, 155.0476, 135.0070, 132.0200, 91.0173 | C_{16}H_{10}O_{5} | Pseudobaptigenin |
| 70  | 15.60                | 257.0802 [M+H]^+ | −3.5        | 137.0229, 119.0491, 93.0368 | C_{15}H_{12}O_{4} | Isoliquiritigenin |
| 71  | 15.68                | 329.2324 [M-H]^− | 0.78        | 229.1436, 211.1329, 183.1377, 171.1014 | C_{13}H_{34}O_{5} | 9,12,13-Trihydroxy-10-octadecenoic acid |
| 72  | 15.75                | 267.0656 [M-H]^− | 1.2         | 252.0418, 223.0390, 208.0522, 195.0439, 180.0563, 167.0487, 135.0071, 132.0202, 91.0174 | C_{16}H_{13}O_{4} | Formononetin       |
Table 1. Cont.

| NO. | Retention Time (min) | ESI-MS (m/z) | Error (ppm) | MS/MS Fragments Ions | Formula | Identification |
|-----|----------------------|-------------|-------------|----------------------|---------|----------------|
| 73  | 15.88                | 297.0758    | -2.5        | 284.0671, 269.0441,  | C_{17}H_{14}O_{5} | 7-O-Methylbiochanin A |
|     |                      |             |             | 256.0725, 241.0491,  |         |                 |
|     |                      |             |             | 282.0527, 267.0291,  |         |                 |
|     |                      |             |             | 254.0576, 239.0342,  |         |                 |
|     |                      |             |             | 223.0391, 221.0389,  |         |                 |
|     |                      |             |             | 195.0438, 183.0440,  |         |                 |
|     |                      |             |             | 167.0496, 132.0202   |         |                 |
| 74  | 15.93                | 297.0757    | -0.34       | 282.0525, 267.0291,  | C_{17}H_{14}O_{5} | Cladrin |
|     |                      |             |             | 254.0577, 239.0340,  |         |                 |
|     |                      |             |             | 223.0402, 221.0386,  |         |                 |
|     |                      |             |             | 195.0451, 183.0445,  |         |                 |
|     |                      |             |             | 167.0497, 135.0202   |         |                 |
| 75  | 15.96                | 297.0396    | 2.3         | 269.0446, 241.0494,  | C_{16}H_{10}O_{6} | 5,7-dihydroxy-3',4'-methylenediosysoflavone |
|     |                      |             |             | 225.0544, 213.0548,  |         |                 |
|     |                      |             |             | 197.0594, 183.0440,  |         |                 |
|     |                      |             |             | 161.0225, 149.0277,  |         |                 |
|     |                      |             |             | 133.0327             |         |                 |
| 76  | 16.15                | 313.0710    | 1.00        | 298.046, 297.0396,  | C_{17}H_{14}O_{5} | Khrinone E |
|     |                      |             |             | 283.0215, 269.0449,  |         |                 |
|     |                      |             |             | 254.0580, 225.0544,  |         |                 |
|     |                      |             |             | 161.0299, 149.0277,  |         |                 |
|     |                      |             |             | 135.0071, 121.0208,  |         |                 |
| 77  | 16.28                | 285.0745    | -1.92       | 270.0910, 255.0642,  | C_{16}H_{12}O_{5} | Homopterocarpin |
|     |                      |             |             | 163.0388, 151.0385,  |         |                 |
|     |                      |             |             | 147.0432, 123.0438,  |         |                 |
|     |                      |             |             | 93.0336             |         |                 |
| 78  | 16.69                | 271.0968    | 0.34        | 147.0433, 137.0593,  | C_{16}H_{14}O_{4} | Echinatin |
|     |                      |             |             | 123.0438             |         |                 |
| 79  | 16.78                | 941.5099    | 1.02        | 923.4996, 879.5104,  | C_{48}H_{78}O_{18} | Soyasaponin I |
|     |                      |             |             | 795.4318, 633.4003,  |         |                 |
|     |                      |             |             | 615.3887, 597.3769,  |         |                 |
|     |                      |             |             | 533.3872, 457.3671,  |         |                 |
|     |                      |             |             | 437.3403, 409.3475,  |         |                 |
|     |                      |             |             | 247.0819, 205.0713,  |         |                 |
|     |                      |             |             | 163.0610, 157.0147,  |         |                 |
|     |                      |             |             | 143.0339, 139.0029   |         |                 |
| 80  | 16.95                | 329.0657    | 0.46        | 314.0406, 313.0349,  | C_{17}H_{14}O_{7} | Tricin |
|     |                      |             |             | 286.0451, 285.0392,  |         |                 |
|     |                      |             |             | 271.0222, 245.0480,  |         |                 |
|     |                      |             |             | 177.0179, 152.0100,  |         |                 |
|     |                      |             |             | 151.0025, 136.0102,  |         |                 |
|     |                      |             |             | 107.0122             |         |                 |
| 81  | 17.19                | 283.0604    | 0.81        | 269.0403, 268.0367,  | C_{16}H_{14}O_{3} | maackiain |
|     |                      |             |             | 239.0341, 223.0390,  |         |                 |
|     |                      |             |             | 195.0451, 183.0441,  |         |                 |
|     |                      |             |             | 167.0491, 135.0074,  |         |                 |
|     |                      |             |             | 132.0202, 91.0174    |         |                 |
| 82  | 17.22                | 315.0867    | 1.32        | 300.0629, 285.0403,  | C_{17}H_{16}O_{6} | Homofereirin |
|     |                      |             |             | 241.0492, 214.9861,  |         |                 |
|     |                      |             |             | 196.0044, 164.0105,  |         |                 |
|     |                      |             |             | 151.0023, 107.0122   |         |                 |
| 83  | 19.72                | 295.2271    | 1.0         | 277.2159, 171.1024   | C_{18}H_{20}O_{3} | 9-hydroxyoctadeca-10,12-dienoic acid |
| 84  | 21.58                | 271.2273    | 1.19        | 226.2241, 225.2216   | C_{15}H_{10}O | 2-Pentadecanone |
| 85  | 21.71                | 457.3676    | 0.36        | 439.3570, 381.3133,  | C_{30}H_{48}O_{3} | betulinic acid |
|     |                      |             |             | 248.1692, 191.1788   |         |                 |
| 86  | 22.04                | 617.3844    | 1.02        | 453.3359, 163.0392,  | C_{29}H_{54}O_{6} | 27-p-Coumaroyloxyursolic acid |
|     |                      |             |             | 145.0273, 119.0495   |         |                 |
Table 1. Cont.

| NO. | Retention Time (min) | ESI-MS (m/z) | Error (ppm) | MS/MS Fragments Ions | Formula | Identification |
|-----|----------------------|--------------|-------------|----------------------|---------|----------------|
| 87  | 22.06                | 617.3851 [M-H]− | 1.6         | 437.3422, 179.0399, 161.0243, 134.0365 | C_{60}H_{52}O_{13} | 3-β-O-trans-cafeoylbutenolic acid |
| 88  | 22.08                | 617.3853 [M-H]− | 2.3         | 437.3417, 179.0345, 161.02441, 134.0363 | C_{60}H_{52}O_{13} | 3-O-Caffeoyloleanolonic acid |
| 89  | 22.38                | 339.2321 [M-H]− | 0.3         | 163.1115             | C_{21}H_{22}O_{2} | 2,2′-Methylenebis |
| 90  | 22.47                | 603.4050 [M-H]− | 1.5         | 179.0341, 161.0240, 134.0367 | C_{30}H_{29}O_{5} | Betulin-3-cafeate |
| 91  | 22.51                | 603.4051 [M-H]− | 0.6         | 179.0333, 161.0240, 134.0364 | C_{30}H_{29}O_{5} | uvaol-3-cafeate |

2.2.1. Identification of Flavones, Isoflavones, Flavone Glycosides, and Isoflavone Glycosides

The flavonoid compounds reported in *M. speciosa* were mainly divided into several types, including flavones, isoflavones, flavanones, and chalcones. The fragmentation pathways of flavonoids were followed by Retro-Diels-Alder (RDA) [16]. In this study, a total of 30 flavones, isoflavones, and glycosides were identified. Compounds 37, 41, 45, 60, 61, and 65 were isomers that all showed ion peaks at m/z 283 [M-H]− and 285 [M+H]+ and had a similar molecular formula of C_{16}H_{12}O_{5}. Compound 37 produced fragment ions at m/z 270.0515 [M+H-CH_{3}]+, 242.0564 [M+H-CH_{3}-CO]+, 225.0535 [M+H-CH_{3}-CO-H_{2}O]+, and 197.0583 [M+H-CH_{3}-2CO-H_{2}O]−, and it yielded RDA fragment ions at m/z 137 (1,2-A') in positive mode, indicating that one hydroxy group was attached to ring A and one hydroxy group and one methoxy group were attached to ring B. Compound 37 was tentatively identified as 5,4′-dihydroxy-3′-methoxy-isoflavone [1]. Compounds 41, 45, 60, and 65 were tentatively identified as isoprenetin, 2′-hydroxyformononoinetin, calycosin, and glycictein, respectively, based on data reported in the literature [9] and the RDA fragmentation pathway. Similarly, compound 61 was deduced to be 3′,4′-dihydroxy-7-methoxyisoflavone [9].

Compound 19 showed an [M-H]− ion at m/z 563.1396 with the molecular formula of C_{26}H_{29}O_{14}, which further produced fragment ions at m/z 431.0963 [M-H-132]− and 269.0448 [M-H-132-162]−. According to the literature [17,18], compound 19 was tentatively identified as apiuin. Compounds 24 and 35 produced an [M-H]− ion at m/z 445.1127 and 445.1131, respectively, with the same molecular formula of C_{22}H_{22}O_{10}. Their MS² spectra produced a fragment ion at m/z 283, indicating the loss of a hexose moiety. Compound 24 showed fragment ions at m/z 268.0370, 240.0420, 224.0464, 212.0472, and 135 (1,3-A−); therefore, it was tentatively identified as calycosin-7-O-beta-D-glucoside [19]. However, compound 35 gave characteristic fragment ions at m/z 151 (1,3-A−) and 132 (1,3-B−) by the C-ring RDA fragment, which showed that two hydroxy groups were attached to the A-ring and one methoxo group was attached to the B-ring. Compound 35 was tentatively identified as sissotrin. Similarly, according to the reported literature and MS data in Table 1, compound 31 was tentatively identified as yuankanin [20].

Compound 27 showed an [M+H]⁺ ion at m/z 271.0597 (C_{15}H_{10}O_{5}). It produced fragment ions at m/z 253.0482 and 137.0230 (1,3-A⁺) in positive mode. Therefore, compound 27 was tentatively assigned as 5,3′,4′-trihydroxyflavone [1]. Using a similar method, compound 38 was tentatively assigned as daidzein [9]. Compounds 46 and 75 showed an [M-H]− ion peak at m/z 297.0398 and 297.0396, respectively, with the same formula of C_{16}H_{12}O_{5}. Compound 46 produced an RDA fragment ion at m/z 135 (1,3-A−); thus, compound 46 was tentatively identified as griffonione H [9]. Compound 75 showed characteristic fragment ions at m/z 133 (1,3-A−-H_{2}O) and 161 (0,3-B−), indicating that two hydroxy groups were attached to ring A. Thus, compound 75 was tentatively identified as 5,7-dihydroxy-3′,4′-methyleneedioxyisoflavone [9].

Compounds 48, 54, 58, 73, and 74 produced an [M-H]− ion at m/z 297.07 with the same formula of C_{17}H_{14}O_{5}. Their MS² spectra produced fragment ions at m/z 282,269, indicating...
that there were two methoxy groups. Compounds 48, 54, 58, and 73 yielded fragment ions at m/z 167 \((1,3\text{A}^-+\text{H})\) and 132 \((1,3\text{B}^-)\) by RDA reactions, indicating that one methoxy group and hydroxy group were assumed to attach to ring A and one methoxy group was assumed to attach to ring B. Compared with MS data and literature data [1,9], compounds 48, 54, 58, and 73 were tentatively determined to be 8-O-methylretusin, afrormosin, alfalfa, and 7-O-methylbiochanin A, respectively. Compound 74 generated the RDA fragment ion at m/z 135 \((1,3\text{A}^-)\), indicating that one hydroxy group was attached to ring A. Compound 74 was tentatively identified as cladactin [9].

Compounds 49 and 72 exhibited a molecular ion at m/z 269.0799 \([\text{M+H}]^+\) with the same formula of \(\text{C}_{16}\text{H}_{12}\text{O}_4\). Compound 49 was observed at m/z 254.0555, with the loss of a \(\text{CH}_3\) \((-15\text{Da})\). The fragment ions at m/z 151.0413 \((1,3\text{A}^-)\) and 118.0411 \((1,3\text{B}^-)\) were generated by the RDA reaction, which indicated that one methoxy group was on ring A and one hydroxy group was on ring B. Compound 49 was tentatively identified as 4'-hydroxy-7-methoxyisoflavone [1]. Compared with the reference standard, compound 72 was confirmed as formononetin [1,9].

Compounds 52, 59, and 62 displayed an \([\text{M+H}]^+\) ion at m/z 301 and a product ion at m/z 286, indicating the loss of \(\text{CH}_3\) \((-15\text{Da})\). Compounds 52 and 62 produced RDA fragment ions at m/z 153 \((1,3\text{A}^-)\) and 134 \((1,3\text{B}^--\text{CH}_3)\) in positive mode, indicating that two hydroxy groups were attached to ring A and hydroxy and methoxy groups were attached to ring B. Compounds 59 and 62 produced a fragment ion at m/z 286 \([\text{M-H-CH}_4]\) \((-\text{CH}_4)\), which demonstrated that the position of methoxy is ortho to hydroxy. Therefore, compounds 52 and 62 were tentatively assigned as 2'-hydroxybiochanin A [1] and pratensein [9], respectively. Compound 59 exhibited a fragmentation ion at m/z 93, with the loss of ring B by the breakage of the C3-C9 bond. This indicated that ring B was substituted by one hydroxy group. Compound 59 was tentatively identified as tectorigenin [9,21].

Compound 63 generated an \([\text{M+H}]^+\) ion at m/z 315.0850, with the formula \(\text{C}_{17}\text{H}_{14}\text{O}_6\). It showed characteristic fragments at m/z 167.0335 \((1,3\text{A}^-)\), 152.0096 \((1,3\text{A}^-+\text{CH}_3)\), 148.0514 \((1,3\text{B}^-)\), and 133.0228 \((1,3\text{B}^--\text{CH}_3)\), which were generated by the RDA reaction. This suggests that one methoxy and hydroxy group was attached to ring A and ring B, respectively. Compound 63 was tentatively identified as 5,4'-dihydroxy-7,3'-dimethoxyisoflavone [1]. Using a similar method, compounds 64, 69, 76, and 80 were tentatively assigned as 3',5,6,7-tetrahydroxy-4'-methoxyisoflavone [9], pseudobaptigenin [9], krinone E [22], and tricin [23], respectively. Compound 67 exhibited an \([\text{M+H}]^+\) ion at m/z 313.0346 with a molecular formula of \(\text{C}_{16}\text{H}_{10}\text{O}_7\). The characteristic fragment ions were observed at m/z 285.0393, 269.1279, and 257.0460, corresponding to the losses of \(\text{CO} (-28 \text{Da})\), \(\text{CO}_2 (-44 \text{Da})\), and \(2\text{CO} (-56 \text{Da})\), respectively. It displayed RDA fragment ions at m/z 151 \((1,3\text{A}^-)\), 161 \((1,3\text{B}^-)\), and 109 with the breakage of the C3-C9 bond, indicating that ring A and ring B were simultaneously substituted by two hydroxy groups. Compound 67 was tentatively assigned as luteolal.

2.2.2. Identification of Flavonone, Isoflavonone, and Flavonone Glycosides

As shown in Table 1, a total of 18 compounds were assigned to flavonone, isoflavonone, and flavonone glycosides. Compound 16 showed an \([\text{M-H}]^-\) ion at m/z 581.1500 with the molecular formula of \(\text{C}_{26}\text{H}_{30}\text{O}_{15}\), which produced a fragment \([\text{M-H}]^-\) ion at m/z 287.0554 by the loss of the glucosyl moiety \((294 \text{Da})\). The continuous loss of \(\text{H}_2\text{O} (-18 \text{Da})\) yielded fragment ions at m/z 269.0446. It also generated an RDA fragment ion at m/z 151 \((1,3\text{A}^-)\) in negative mode, meaning that ring A was substituted by two hydroxy groups. Compound 16 was tentatively identified as 5,7,3',4'-tetrahydroxyflavanone-7-alpha-L-arabinofuranosyl-(1->6)-glucoside. Using a similar method, compound 17 was tentatively assigned as erydocitoyl-7-O-glucoside [24].

Compounds 23, 25, 32, 53, and 55 produced an \([\text{M-H}]^-\) ion at m/z 271 with the same formula, \(\text{C}_{15}\text{H}_{12}\text{O}_5\). Compounds 23, 25, and 32 generated fragment ions at m/z 243 [\(\text{M-H-CO}^-\)] and 227 [\(\text{M-H-CO}_2^-\)]. Compounds 23, 25, and 32 yielded characteristic fragmentation ions at m/z 135 \((1,3\text{A}^-)\) and 109 with the breakage of the C3-C9 bond by RDA reactions, indicating that ring A had one hydroxy group and that ring B had two hydroxy groups.
Compounds 23, 25, and 32 were tentatively identified as 3′,4′,7-trihydroxyflavanone [25], 3′,4′,7-trihydroxy-isoflavanone, and 2′,4′,7-trihydroxyisoflavanone through a comparison with MS data and literature data. Compound 53 generated fragment ions at m/z 253 [M-H-H2O]− and 135 (1,3-A−) and was identified as garbanzol. Compound 55 showed fragment ions at m/z 151 (1,3-A−) and 119 (1,3-B−), which were definitively identified as naringenin [9] via a comparison with the reference standard.

Compounds 36, 42, 44, and 66 produced an [M-H]− ion at m/z 285 with the same formula of C18H14O5, which produced a fragment [M-H]− ion at m/z 270 by the loss of CH2 (15 Da). Compounds 36 and 44 generated an RDA fragment ion at m/z 135 (1,3-A−) in negative ion mode, indicating that ring A had one hydroxy group. Compound 44 exhibited a fragment [M-H]− ion at m/z 269 by the loss of CH4 (16 Da), meaning that the position of methoxy is ortho to hydroxyl in ring B. Therefore, compounds 36 and 44 were assigned as vestitone [9] and 3′,7-dihydroxy-4′-methoxyisoflavanone, respectively. Compounds 42 and 66 generated fragment ions at m/z 149 (1,3,A−) and 135 (1,3-A-CH3) by the RDA reaction, meaning that ring A had one methoxy group. Compounds 42 and 66 were assigned as 3′,4′-dihydroxy-7-methoxyflavanone and 3′,4′-dihydroxy-7-methoxyisoflavanone [9]. Compound 56 produced an [M+H]+ ion at m/z 287.0912 with the formula of C18H14O5. In addition, MS2 fragmentation at m/z 269.0803 [M+H-H2O]+ and 153.0544 (1,3-A+) were generated, meaning that two hydroxy groups were in the ortho-position of ring A, and the structure was tentatively assigned as 6,7-dihydroxy-4′-methoxyisoflavanone.

Compounds 39, 40, and 51 yielded an [M-H]− ion at m/z 255 with the same formula of C15H12O4. They produced characteristic fragment ions at m/z 135 (1,3-A−) and 119 (1,3-B−), indicating that one hydroxy group was attached to ring A and ring B, respectively. Compound 40 was unambiguously identified as liquiritigenin [9] through a comparison with the reference standard. Compounds 39 and 51 were tentatively assigned as dihydrodaidzein [9] and 5,4′-dihydroxyisoflavanone, respectively. Compounds 43 and 82 produced an [M-H]− ion at m/z 315 with the formula of C17H16O5. Compound 43 further yielded a fragment [M-H]− ion at m/z 135.0071 (1,3-A−), and it was tentatively assigned as violanone [9]. Compound 82 generated fragment ions [M-H]− ions at m/z 300.0629, 285.0401, and 151.0023 (1,3-A−). Thus, it was tentatively assigned as homoferreirin. Similarly, compound 68 was tentatively assigned as ferreirin [1].

2.2.3. Identification of Chalcone, Dihydrochalcone, and Flavanones

Chalcone, dihydrochalcone, and flavanones give weak RDA fragments and commonly lose ring B by breaking at different positions [16]. Compound 29 exhibited an [M-H]− ion at m/z 271.0606 with the formula C15H12O5. It gave characteristic fragment ions at m/z 253.050 and 135.043. Compound 29 was tentatively assigned as butein. Compound 34 produced an [M-H]− ion at m/z 273.0758, which was 2 Da more than that of compound 29, indicating a dihydrochalcone. Further fragmentation led to ions at m/z 255.0658 [M-H-H2O], 167.0336, and 137.0029, indicating that ring B was substituted by one hydroxy group and ring A was substituted by two hydroxy groups. Ring C was also substituted by one hydroxy group. Compound 34 was tentatively identified as 2′,4,4′,α-tetrahydroxydihydrochalcone [25]. Compounds 57 and 78 showed an [M+H]+ ion at m/z 271 with the same formula of C16H14O4. Compound 57 yielded characteristic fragment [M+H]+ ions at m/z 151.0386 and 119.0489, indicating that ring B had one hydroxy group. Compound 57 was tentatively assigned as 2′,4-dihydroxy-4′-methoxychalcone [7]. Compound 78 generated a characteristic fragment [M+H]+ ion at m/z 123.0438, and it was tentatively assigned as echinatin [1]. Compound 70 yielded an [M+H]+ ion at m/z 257.0802 with product ions at 137.0229 and 119.049. Compound 70 was confirmed as isoliquiritigenin [9] with the corresponding reference standard. Compound 13 exhibited an [M-H]− ion at m/z 577.1352 (C30H26O12) and produced ions at m/z 289.0711 and 125.0299. Compound 13 was tentatively identified as procyanidin B2 [26]. Compound 15 produced an [M-H]− ion at m/z 289.0708 with the formula C15H14O8. The characteristic fragment ions at m/z 245.0809, 137.0229, 125.0229, and 109.0280 were generated from (-)-epicatechin [26].
2.2.4. Identification of Pterocarpans and Other Flavonoids

Usually, pterocarpan cannot produce RDA reactions because of its tight structure. The proposed fragmentation pathways of pterocarpan compounds are summarized in Figure 3A. The cleavage of the C-C bonds of pterocarpan compounds mainly occurred on the side of C_{6a} and C_{11a} to generate ion fragments a, b, and c. For example, compound 50 displayed [M-H]⁻ ions at m/z 269.0812 with the formula C_{16}H_{14}O_{4}. The characteristic fragment in negative mode included 253.0499 [M-H-O]⁻, 237.0547 [M-H-CH_{3}-O]⁻, 225.0549 [M-H-CH_{3}-CO]⁻, 161.0225 (C_{9}H_{5}O_{3}, c-2H), 145.0278 (C_{9}H_{5}O_{2}, b-2H), 133.0188 (C_{8}H_{5}O_{2}, a-CH_{3}), and 117.0331 (C_{8}H_{5}O, c-2H-CO_{2}) in its MS² spectrum (Figure 3B), indicating that ring I was substituted by one hydroxy group and ring IV was substituted by one methoxy group. The hypothesized fragmentation pattern of compound 50 is demonstrated in Figure 3C. Thus, compound 50 was tentatively identified as medicarpin.

Figure 3. (A) Fragmentation pathway of Pterocarpans compounds in MSCP; (B) The MS/MS spectrum of compound 50; (C) The hypothesized fragmentation pathway of compound 50.
Compound 47 produced an \([M-H]^-\) ion at \(m/z\) 285.0759 with the formula C16H14O5, and produced characteristic ions at \(m/z\) 270.0520 \([M-H-CH_3]^-\), 269.0431 \([M-H-CH_4]^-\), 163.0365 (C9H7O3, a), 148.0190 (C8H4O3, a-CH3), and 147.0433 (C9H7O2, b). This indicates that ring I was substituted by one hydroxy group and ring IV was substituted by hydroxy ortho with a methoxy group. Thus, compound 47 was tentatively confirmed as 3,8-dihydroxy-9-methoxypterocarpan. Compounds 77 and 81 showed ions at \(m/z\) 285 \([M+H]^+\) and 283 \([M-H]^-\) with the same formula, C16H14O4. Compound 77 exhibited characteristic ions \(m/z\) 270.0910 \([M+H-CH_3]^+\), 255.0642 \([M+H-2CH_3]^+\), 163.0388 (C9H7O3, b), and 147.0432 (C9H7O3, b-CH3) in positive mode, and it was tentatively assigned as homopterocarpin. Compound 81 yielded an \([M-H]^-\) ion at \(m/z\) 268.0367 \([M-H-CH_3]^-\), 239.0341 \([M-H-CO_2]^-\), 223.0393 \([M-H-CO_2-O]^-\), 211.0390 \([M-H-CO_2-CO]^-\), and 195.0451 \([M-H-2CO_2]^-\), but it also produced major product ions at \(m/z\) 163.0348 (C9H7O3, b) and 151.0385 (C8H7O3, b-C) in positive mode. Compound 81 was confirmed as a mixture using the corresponding reference standards.

Compound 33 produced an \([M-H]^-\) ion at \(m/z\) 269.0448 with the formula C16H10O5. In the MS2 spectra, it gave ions at \(m/z\) 241.0495 \([M-H-CO]^-\), 225.0524 \([M-H-CO_2]^+\), 213.0547 \([M-H-2CO]^+\), 185.0593 \([M-H-3CO]^+\), 135.0073 \(1,2A^-\), 133.0280 \(1,2B^-\), and 91.0173 \(1,2A^-\) \(CO_2\)). The hypothesized fragmentation pattern of compound 33 is presented in Figure 4. Thus, compound 33 was tentatively identified as sulfuretin, belonging to aurone.

![The hypothesized fragmentation pathway of compound 33.](image-url)

**Figure 4.** The hypothesized fragmentation pathway of compound 33.
2.2.5. Identification of Steroid Saponins

Steroid saponins are major biological compounds present in MSCP. The ions of saponins tended to be present in the negative mode, with successive losses of several sugar moieties. In this work, seven steroid saponins were rapidly identified using UHPLC-Q-Exactive Orbitrap MS by matching the structural data combined with the literature data of published compounds. For example, compound 79 yielded an [M-H]$^-$ ion at m/z 941.5099 with the formula C$_{46}$H$_{76}$O$_{18}$. It gave fragment ions at m/z 795, 633, and 457 by the successive losses of deoxyhexose, hexose, and glucuronic acid moieties, respectively. It was tentatively confirmed as soyasaponin I. Compound 85 generated an [M+H]$^+$ ion at m/z 457.3676, and it produced fragment ions at m/z 439.3570 [M+H-H$_2$O]$^+$, 381.3133 [M+H-2H$_2$O]$^+$, 248.1692, and 191.1788. It was tentatively identified as betulinic acid. Compounds 86, 87, and 88 showed an [M-H]$^-$ ion at m/z 617 with the same formula, C$_{39}$H$_{54}$O$_{6}$. Compound 86 exhibited fragment ions at m/z 453, 163, 145, and 119 and was tentatively identified as 27-p-coumaroyloxyursolic acid. Compounds 87 and 88 gave fragment ions at m/z 437, 179, 161, and 134, respectively. They were tentatively assigned as 3-β-O-trans-caffeoyl-betulinic acid and 3-O-caffeoyl-oleanolic acid, respectively. Compounds 90 and 91 gave an [M-H]$^-$ ion at m/z 603 with the same formula, C$_{39}$H$_{54}$O$_{6}$. They gave fragment ions at m/z 179, 161, and 134. Compounds 90 and 91 were tentatively assigned as betulin-3-cafeate and uvaol-3-cafeate, respectively.

2.2.6. Identification of Alkaloids

Alkaloid compounds tend to produce signals in negative ion mode. In this work, three alkaloid compounds were rapidly identified. Compounds 10 and 12 showed an [M+H]$^+$ ion at m/z 188 with the same formula, C$_{11}$H$_{16}$O$_{2}$N. The fragment ions at m/z 170 [M+H-H$_2$O]$^+$, 143 [M+H-CO$_2$H]$^+$, 118 [M+H-C$_2$H$_2$COOH]$^+$, and 91 [M+H-C$_4$H$_3$COOH]$^+$ were produced in the MS$^2$ spectrum. They were tentatively identified as trans-3-indoleacrylic acid and indole-3-acrylic acid, respectively. Compound 11 generated an [M+H]$^+$ ion at m/z 247.1434, and it further produced fragment ions at m/z 188.0701 [M+H-NCH$_3$]$^+$ and 146.0598 [M+H-NCH$_3$-CO$_2$]$^+$. It was unambiguously identified as hypaphorine using the reference standard.

2.2.7. Identification of Phenolic Acids and Their Derivatives

Phenolic acids and derivatives extensively exist in the plant. Deprotonated ions were detected in negative ion mode. Compounds 1, 2, and 6 produced an [M-H]$^-$ ion at m/z 315 with the same formula, C$_{13}$H$_{14}$O$_{9}$. As shown in Table 1, they produced characteristic fragment ions at m/z 152 [M-H-glu]$^-$ and 108 [M-H-glu-CO$_2$]$^-$ by the successive elimination of Glu and CO$_2$ groups, respectively. They were tentatively identified as protocatechuic acid-4-glucoside, protocatechuic acid-3-glucoside, and protocatechuic acid-2-glucosid. Compounds 4 and 5 showed an [M-H]$^-$ ion at m/z 461 with the same formula, C$_{19}$H$_{26}$O$_{13}$. In the MS$^2$ spectrum, the successive elimination of pentose, hexose, CH$_3$, and CO$_2$ groups generated fragment ions at m/z 329, 167, 152, and 108, respectively. Compounds 4 and 5 were tentatively assigned as saccharosamide C and saccharosamide D, respectively. Compounds 7 and 8 generated an [M-H]$^-$ ion at m/z 447 with the same formula, C$_{18}$H$_{25}$O$_{13}$. In the MS$^2$ spectrum, the successive elimination of pentose, hexose, and CO$_2$ groups generated fragment ions at m/z 315, 152, and 108, respectively. Compounds 7 and 8 were tentatively assigned as 5-[2-O-(beta-d-apiofuranosyl)-beta-d-glucopyranosyl]oxy]-2-hydroxybenzoic acid and 4-hydroxy-5-(3′,4′,5′-trihydroxyphenyl)-valeric acid-O-methyl-O-glucuronide, respectively. By using a similar approach, compounds 20, 21, 22, and 26 were tentatively assigned as seguinoside K [27], alibrisinoside B [27], apiosylglucosyl-4-hydroxybenzoate, and khaephuside B [27] through a comparison of the structural data and literature data. Compound 28 produced an [M-H]$^-$ ion at m/z 137.0229, which further yielded a fragment ion at m/z 93.0329 by the loss of the CO$_2$ group. It was identified as salicylic acid [9] using the reference standard.
2.2.8. Identification of Phenols, Fatty Acid and Other Compounds

Compounds 9 and 14 exhibited an [M-H]$^-$ ion at m/z 477 with the same formula of C$_{20}$H$_{30}$O$_{13}$. Their MS$^2$ spectrum showed fragment ions at m/z 345, 183, 168, and 153, corresponding to the loss of pentose, hexose, and two CH$_3$ molecules. Compounds 9 and 14 were tentatively assigned as shamiminol and kelampayoside A, respectively. Using a similar method, compound 18 was tentatively assigned as diosbulbinside D.

Compound 30 exhibited an [M-H]$^-$ ion at m/z 187.0963 with the formula C$_9$H$_{16}$O$_4$. The fragment ions at 169.0850 [M-H-H$_2$O]$^-$, 143.1064 [M-H-CO$_2$]$^-$, and 125.0956 [M-H-CO$_2$-H$_2$O]$^-$ were observed in the MS$^2$ spectrum. It was identified as a fatty acid in M. speciosa and tentatively assigned as azelaic acid [9]. Through a similar method, compounds 71, 83, and 84 were tentatively assigned as 9,12,13-trihydroxy-10-octadecenoic acid [1], 9-hydroxyoctadeca-10,12-dienoic acid [1], and 2-pentadecanone [1], respectively. Compound 3 showed an [M+H]$^+$ ion at m/z 289 and 127, corresponding to the loss of pentose and hexose, respectively. It was tentatively identified as NCGC00380493-01. Compound 89 produced an [M-H]$^-$ ion at m/z 339.2321 with the formula C$_{23}$H$_{32}$O$_2$. The fragment ion at m/z 163 was obtained, and compound 89 was tentatively assigned as 2,2′-methylenebis.

2.3. Discrimination of Chemical Profiles of Rhizome and Radix

As shown in Figure 2, a total of 91 chemical constituents were identified in the rhizome and radix samples. It is worth noting that all of the intensities of the chemical constituent peaks of the rhizome were stronger than those of the radix under the same analytical conditions. In total, the intensities of the peaks were comparatively low, within 6.2–8, 8.5–10, and 13.5–14 min in the radix, and the intensities of peaks 5, 9, 13–15, 17, 18, 20, 21, 23, 24, 28, 33–35, 43, 44, 48, and 49 were almost undetectable in the radix samples. To establish representative chromatographic fingerprints, the established method was applied to analyze 16 batches of rhizome and 12 batches of radix. There were 91 common compounds identified in the MS spectra fingerprints from the rhizome and radix. However, considering many common peaks and a large amount of peak area data, it was difficult to distinguish rhizome and radix samples using this information. Multivariate analysis, including PCA and OPLS-DA, was performed on mass spectral data sets using SIMCA-P14.1 software. The loading plot from OPLS-DA together with the variable importance in the projection (VIP) were applied to reveal potential markers [14–16]. Thus, it is necessary to use multivariate analysis to reduce the dimensionality of the primal data.

2.4. Principal Components Analysis (PCA)

To efficiently visualize the differences between 16 batches of rhizomes and 12 batches of radix from MSCP, PCA was applied to analyze the MS spectral data using SIMCA-P14.1 software. The parameters R$^2$(cum) and Q$^2$(cum) are generally used to explain the quality and reliability of the models. The PCA results are presented in two score plots (Figure 5A), and 28 batches of MSCP samples were divided into two types containing rhizome or radix (R$^2$(cum) = 0.235 and Q$^2$(cum) = 0.377). The samples from different parts were clearly distinguished into two groups according to the PCA model. As shown in Figure 5A, the radix (A1–A16) of MSCP is displayed on the left side of the score plot, whereas rhizome (B1–A12) is displayed on the right side of the plot, indicating a difference between the rhizome and radix in terms of chemical composition. Therefore, it is possible to distinguish the rhizome and radix samples based on UHPLC-Q-Exactive Orbitrap MS fingerprint analysis with PCA. These results revealed the distinctive differences in chemical composition between the rhizome and radix samples of MSCP.
2.5. Chemical Markers to Distinguish the Rhizome from Radix with OPLS-DA

To identify chemical markers unique to the rhizome and radix samples, OPLS-DA was utilized to further process the MS spectral data by SIMCA-P14.1 software. The loading S-plot was used to characterize the chemical difference between the rhizome and the radix. In Figure 5B, the spots located at the end of the plot indicate that the contribution of the variable to the differentiation is higher. In this S-plot, every spot represents an ion \( t_R \)-m/z pair. The x-axis presents the alterable contributions of the variables, whereas the y-axis presents the alterable confidence levels of the variables. Therefore, when the distance between the ion \( t_R \)-m/z pair spots and zero increases, the confidence level of the difference between the rhizome and radix also increases. Thus, those spots located at the end of the plot were tentatively regarded as potential chemical markers, leading to differences between the rhizome and radix samples.

In addition, the VIP value was employed to confirm the potential markers, which represent the differentiation between the rhizome and radix. When the VIP is higher, the variables are more important to the model. The S-plot, together with the variables of VIP \( \geq 1 \), suggests the influence of the differentiation of samples using OPLS-DA.
From Figure 5B, one ion (a) in the bottom left corner and eight ions (b–j) in the top right corner of the S-plot contributed the most to the differentiation of the rhizome and radix. Combining this analysis with the base peak ion (BPI) chromatogram, ten compounds generated from peak 79 (soyasaponin I, VIP 2.66), peak 89 (2,2′-methylenebi, VIP 3.29), peak 90 (betulin-3-caffeate, VIP 4.42), peak 91 (uvaol-3-caffeate, VIP 3.91), peak 88 (3-O-caffeoyloleanolic acid, VIP 3.90), peak 76 (khrinone E, VIP 3.83), peak 87 (3-β-O-trans-cafeoyl betulinic acid, VIP 4.25), peak 82 (homopterocarpin, VIP 2.78), peak 85 (betulinic acid, VIP 3.20), and peak 86 (27-p-coumaroyloxyursolic acid, VIP 3.95) were confirmed to be the most characteristic compounds. Therefore, these 10 chemical constituents were considered as potential markers to distinguish the rhizome and radix of MSCP samples, and this method could be applied for the differentiation of the rhizome and radix in other samples.

2.6. Antioxidant Activity Test Results

The UHPLC-Q-Exactive Orbitrap MS results show that MSCP extracts mainly contained flavonoids, phenolic acids, and steroid saponins. Previous studies have confirmed that plants with antioxidant activity are closely related to their chemical compounds, such as flavonoids, phenolic acids, and steroid saponins [28]. Antioxidants are closely associated with human health. The damaging effects of free radicals can directly or indirectly lead to diseases and cancers [29]. The antioxidant activities of different extracts between the rhizome and radix of MSCP were determined by ABTS and DPPH antioxidant assays. As shown in Table 2, different batches of MSCP showed different antioxidant abilities, compared with the IC_{50} values. In addition, a significant difference in the IC_{50} values between rhizomes and radices was observed using ABTS and DPPH antioxidant tests. The IC_{50} values of the rhizome and radix extracts were 5.05–10.13 µg/mL and 2.09–4.56 µg/mL in the ABTS test, respectively. The IC_{50} values of the rhizome and radix extracts were 5.86–11.86 µg/mL and 2.76–5.77 µg/mL in the DPPH test, respectively. The lower IC_{50} values indicate a higher antioxidant activity [30]. Therefore, in both the ABTS and DPPH antioxidant assays, the antioxidant activity of radix samples was obviously higher than that of rhizome samples. The results indicate that both rhizome and radix extracts of MSCP presented remarkable antioxidant activities in vitro. However, it is still unknown whether the variability in the chemical components is related to differences in antioxidant activity and whether the variability influences the ability to discriminate between the rhizomes and radix. Therefore, further study is necessary to reveal the relationship between chemical components and antioxidant efficacy via statistical analysis.

Table 2. Sample collection information and antioxidant activity of MSCP (n = 3).

| No. | Collecting Location | GPS Data | Collection Year | Classification | ABTS IC_{50} (µg/mL) | DPPH IC_{50} (µg/mL) |
|-----|---------------------|----------|-----------------|----------------|---------------------|---------------------|
| A1  | Pubei, Guangxi      | N22°15'12" E 109°34'25" | June. 2019      | radix          | 3.10 ± 0.13         | 5.16 ± 0.49         |
| A2  | Pubei, Guangxi      | N22°09'53"E 109°28'47" | June. 2019      | radix          | 3.94 ± 0.18         | 5.41 ± 0.32         |
| A3  | Hexian, Guangxi     | N22°41'57" E 109°19'38" | June. 2019      | radix          | 4.56 ± 0.28         | 5.25 ± 0.11         |
| A4  | Shangxi, Guangxi    | N22°09'43.45" E 108°08'35" | Oct. 2019      | radix          | 3.57 ± 0.16         | 4.83 ± 0.79         |
| A5  | Shangxi, Guangxi    | N22°07'30" E 108°06'54" | Oct. 2019      | radix          | 2.09 ± 0.13         | 2.76 ± 0.21         |
| A6  | Jiangmen, Guangdong | N22°34'30" E 113°02'45" | May. 2020      | radix          | 3.64 ± 0.35         | 4.84 ± 0.73         |
| A7  | Jiangmen, Guangdong | N22°34'43" E 113°02'41" | May. 2020      | radix          | 4.34 ± 0.32         | 5.77 ± 0.15         |
| A8  | Heshan, Guangdong   | N22°42'35" E 113°1'16" | May. 2020      | radix          | 3.73 ± 0.18         | 4.89 ± 0.98         |
Table 2. Cont.

| No. | Collecting Location | GPS Data | Collection Year | Classification | ABTS IC50 (µg/mL) | DPPH IC50 (µg/mL) |
|-----|---------------------|----------|-----------------|----------------|-------------------|-------------------|
| A9  | Heshan, Guangdong   | N22°42'35" E 113°0'16" | May. 2020 | radix          | 3.59 ± 0.26       | 4.11 ± 0.42       |
| A10 | Taizhou, Zhejiang   | N28°38'17" E 121°16'25" | June. 2020 | radix          | 3.51 ± 0.14       | 4.21 ± 0.88       |
| A11 | Taizhou, Zhejiang   | N28°38'28" E 121°16'20" | June. 2020 | radix          | 3.99 ± 0.20       | 4.48 ± 0.76       |
| A12 | Nanning, Guangxi    | N22°30'7" E 108°0'35" | June. 2020 | radix          | 4.15 ± 0.65       | 4.85 ± 0.48       |
| A13 | Nanning, Guangxi    | N22°24'38" E 108°29'23" | Aug. 2020 | radix          | 3.10 ± 0.18       | 5.11 ± 0.67       |
| A14 | Nanning, Guangxi    | N22°24'38" E 108°29'23" | Aug. 2020 | radix          | 3.45 ± 0.51       | 4.20 ± 0.36       |
| A15 | Qinzhou, Guangxi    | N22°25'4.50" E 109°35'18" | Aug. 2020 | radix          | 3.26 ± 0.14       | 5.47 ± 0.22       |
| A16 | Qinzhou, Guangxi    | N22°26'35" E 109°41'19" | Aug. 2020 | radix          | 3.61 ± 0.22       | 5.66 ± 0.55       |
| B1  | Pubei, Guangxi      | N22°15'12" E 109°34'25" | June. 2019 | rhizome        | 7.39 ± 0.54       | 7.81 ± 0.65       |
| B2  | Pubei, Guangxi      | N22°0'33" E 109°28'47" | June. 2019 | rhizome        | 6.15 ± 0.16       | 6.46 ± 0.74       |
| B3  | Hexian, Guangxi     | N22°41'57" E 109°19'38" | June. 2019 | rhizome        | 6.45 ± 0.29       | 7.72 ± 1.93       |
| B4  | Shangxi, Guangxi    | N22°0'43" E 108°08'35" | Oct. 2019 | rhizome        | 5.19 ± 0.34       | 6.3 ± 0.63        |
| B5  | Shangxi, Guangxi    | N22°0'30" E 108°06'54" | Oct. 2019 | rhizome        | 9.01 ± 1.24       | 10.14 ± 2.2       |
| B6  | Jiangmen, Guangdong | N22°34'30" E 113°02'45" | May. 2020 | rhizome        | 5.78 ± 0.33       | 7.40 ± 0.79       |
| B7  | Jiangmen, Guangdong | N22°34'43" E 113°02'41" | May. 2020 | rhizome        | 6.00 ± 0.21       | 8.12 ± 0.99       |
| B8  | Heshan, Guangdong   | N22°42'35" E 113°1'16" | June. 2020 | rhizome        | 7.91 ± 0.22       | 9.69 ± 0.44       |
| B9  | Heshan, Guangdong   | N22°42'35" E 113°0'16" | June. 2020 | rhizome        | 5.64 ± 0.33       | 7.06 ± 0.79       |
| B10 | Taizhou, Zhejiang   | N28°38'17" E 121°16'25" | Aug. 2020 | rhizome        | 5.05 ± 0.26       | 5.86 ± 0.48       |
| B11 | Taizhou, Zhejiang   | N28°38'28" E 121°16'20" | Aug. 2020 | rhizome        | 10.13 ± 1.20      | 11.92 ± 0.75      |
| B12 | Nanning, Guangxi    | N22°30'7" E 108°08'35" | Aug. 2020 | rhizome        | 5.33 ± 0.24       | 7.21 ± 0.18       |

2.7. Correlations between the Characteristic Chemical Compounds and Antioxidant Activities

2.7.1. GRA

As shown in Table 3, the GRA results indicate that the relational grade between the 10 peaks and the antioxidant activity was in the range of 0.5661–0.7846. In ABTS assays, the relational degrees of peaks a, c, d, e, and f exceeded 0.7000, which indicates that these compounds play important roles in the correlation. In DPPH assays, the relational grade of peaks b, c, d, e, and f were more than 0.7000, and these peaks may be closely related to DPPH antioxidant activity. Therefore, betulin-3-ceaffeate (peak 90), uvaol-3-ceaffeate (peak 91), 3-O-cafeoyloleanolic acid (peak 88), and khrinone E (peak 76) were assigned as essential markers for antioxidant activity, and the contents of these components may be related to antioxidant activity. Notably, the common peak c had the highest relational grade in both ABTS and DPPH assays. Thus, it is likely to be the most relevant compound to reflect antioxidant activity. To determine whether the gray correlation degree was positively or negatively correlated with biological activity, the PLS model was used for further statistical analysis, as described below [31].
Table 3. Correlations and grade of GRA.

| Peaks | ABTS | | DPPH | |
|-------|------|------|------|------|
|       | Correlations | Rank | Correlations | Rank |
|       | 0.7070 | 6    | 0.6778 | 7    |
| a     | 0.6412 | 9    | 0.7087 | 4    |
| b     | 0.7846 | 1    | 0.7811 | 1    |
| c     | 0.7407 | 3    | 0.7087 | 4    |
| d     | 0.7750 | 2    | 0.7770 | 2    |
| e     | 0.7114 | 4    | 0.7433 | 3    |
| f     | 0.6893 | 8    | 0.6344 | 8    |
| g     | 0.6901 | 7    | 0.6841 | 6    |
| h     | 0.7073 | 5    | 0.5975 | 10   |
| i     | 0.5651 | 10   | 0.6098 | 9    |
| j     | 0.5651 | 10   | 0.6098 | 9    |

2.7.2. PLS Model Analysis

PLS analysis was further used to predict the correlation between the characteristic chemical components and antioxidant activity. Figure 6 was drawn to reflect the correlation between 10 peaks and antioxidant activity. The regression equations obtained by the PLS model are as follows:

\[
Y_1(\text{ABTS}) = -0.202X_a + 0.056X_b - 0.056X_c + 0.066X_d + 0.003X_e + 0.157X_f + 0.048X_g - 0.110X_h + 0.033X_i + 0.086X_j \quad (1)
\]

\[
Y_2(\text{DPPH}) = -0.039X_a + 0.065X_b + 0.056X_c + 0.066X_d + 0.056X_e + 0.067X_f + 0.058X_g + 0.037X_h - 0.014X_i + 0.053X_j \quad (2)
\]

Figure 6. Regression coefficients of antioxidant activity obtained with PLS (A) is the regression coefficient figure of ABTS antioxidant activity; (B) is the regression coefficient figure of DPPH antioxidant activity.
As shown in Figure 6A and Equation (1), the areas of peaks b, d, e, f, g, i, and j showed a clear positive correlation with ABTS antioxidant activity, whereas peaks a, c, and h were negatively correlated with antioxidant activity. Figure 6B and Equation (2) show that the areas of peaks b, c, d, e, f, g, h, and j are positively correlated with DPPH antioxidant activity, whereas peaks a and i are negatively correlated with antioxidant activity. Therefore, 2,2′-methylenebis (peak 89), uvaol-3-caffeate (peak 91), 3-O-caffeoyloleanolic acid (peak 88), khrinone E (peak 76), 3-β-O-trans-caffeoyl betulinic acid (peak 87), and 27-p-coumaroyloxyursolic acid (peak 86) represent compounds that significantly contribute to the pharmacological effects of MSCP. Khrinone E (peak 76) showed the highest regression coefficient and was considered to have the highest contribution to antioxidant activity.

Therefore, correlation analysis shows that uvaol-3-caffeate (peak 91), 3-O-caffeoyloleanolic acid (peak 88), and khrinone E (peak 76) are the main active markers for the antioxidant activity of MSCP.

3. Materials and Methods

3.1. Reagents and Chemicals

HPLC-grade acetonitrile and methanol were obtained from Fisher Scientific Co. (Loughborough, UK). Deionized water was used. High-purity (>98.0%) hypaphorine, salicylic acid, liquiritigenin, naringin, isoliquiritigenin, formononetin, and maackiain were purchased from the National Institutes for Food and Drug Control (Beijing, China). All other chemicals were of analytical grade. The ABTS and DPPH were purchased from Sigma–Aldrich (St. Louis, MO, USA).

3.2. Sample Collection

The MSCP was collected from different locations in Guangxi Province and authenticated by Professor Zhifeng Zhang (Institute of Qinghai-Tibetan Plateau, Southwest Minzu University). The sample information is shown in Table 2. The fresh sample was sectioned and dried in the sun. The samples were kept in the herbarium of Qin Zhou Provincial Health School (Qinzhou, China).

3.3. Preparation of Standard and Sample Solutions

Standard stock solutions of hypaphorine, salicylic acid, liquiritigenin, naringin, isoliquiritigenin, formononetin, and maackiain were prepared in methanol/water (50% v/v), and the final concentration was 0.1 mg/mL. The stock solutions were further diluted and stored in a refrigerator at −20 °C until UHPLC-Q-Exactive Orbitrap MS analysis.

The dried sample was ground to a powder and saved in desiccators at normal temperature for future use. Subsequently, the sample powder (0.3 g) was extracted with 10 mL of 70% methanol in an ultrasonic water bath for 30 min at room temperature. The solution was filtered through a 0.22 µm microfiltration membrane.

3.4. Instrumentation and Chromatographic Conditions

The solutions were measured using a Thermo Scientific™ Vanquish™ Flex UHPLC (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a binary solvent system, autosampler, and full UV wavelength spectrophotometer. The chromatographic conditions were set and modified according to our previous work [32]. The chromatographic separation was performed with an ACQUITY HSS T 3 column (100 mm × 2.1 mm, 1.8 µm). The solvent system consisted of 0.1% aqueous formic acid (A) and acetonitrile (B), with the following gradient elution program: 3–3% B (0–1.5 min), 3–15% B (1.5–5 min), 15–30% B (5–12 min), 30–95% B (12–22 min), 95–95% B (22–25 min), 95–3% B (25–26 min), and 3–3% B (26–30 min). The flow rate was 0.3 mL/min. The column was kept at 35 °C, and the injection volume was 3 µL.

Mass spectrometry was performed on a Thermo UHPLC-Q-Exactive Orbitrap mass spectrometer equipped with an electrospray ionization (ESI) source. Both positive and negative ionization modes were applied to acquire a scanning range from 100 to 1000 Da.
with a scanning time of 0.2 s and a 30 min detection period. The MS parameters were set as follows: the capillary voltage was set to 3.5 kV (positive mode) and 3.2 kV (negative mode); the source and desolvation temperatures were 100 and 350 °C, respectively; the drying gas flow rate was 600 L/h; and the cone flow rate was 50 L/h. Finally, processing and analysis of the data were carried out using Xcalibur 2.1 software (Thermo Fisher Scientific, Bremen, Germany).

3.5. Determination of Antioxidant Activity

3.5.1. ABTS Activity Assay

The ABTS assay was performed according to a previously described study by Wang et al. [11] with a few modifications. The ABTS aqueous solution (7 mM) was mixed with K$_2$S$_2$O$_8$ (2.45 mM) and protected from light at room temperature for 12 h. The configured ABTS$^+$ solution was diluted with anhydrous ethanol, and an absorbance of 0.70 ± 0.02 was measured at 734 nm for ABTS$^+$ analysis. Five different concentrations of sample solutions were prepared by diluting with anhydrous ethanol. Then, 0.4 mL of the sample extract was mixed with 4 mL of the diluted ABTS$^+$ solution at 25 °C for exactly 5 min. The absorbance was determined at 734 nm. The ABTS activity was calculated using the following Equation.

\[
\text{ABTS activity (\%) = } (1 - \frac{A_{\text{sample}}}{A_{\text{blank}}}) \times 100\% \tag{3}
\]

where $A_{\text{sample}}$ is the absorbance of 0.4 mL of sample extract combined with 4 mL of the diluted ABTS$^+$ solution, and $A_{\text{blank}}$ is 0.4 mL of anhydrous ethanol combined with 4 mL of the diluted ABTS$^+$ solution. The half inhibition concentration (IC$_{50}$) value was evaluated using a regression equation from serial concentrations of the sample extract.

3.5.2. DPPH Activity Assay

The DPPH scavenging capacity was determined as previously described by Wang et al. [11] with a small modification. The extracts were concentrated to five different concentrations by distillation to prepare the sample solutions. A 4 mL DPPH (0.04 mg/mL) solution was mixed with 1 mL of the sample and kept in the dark at 25 °C for 30 min. The absorbance was recorded at 517 nm, and DPPH activity was calculated via the following Equation.

\[
\text{DPPH activity (\%) = } (1 - \frac{A_{\text{sample}}}{A_{\text{blank}}}) \times 100\% \tag{4}
\]

where $A_{\text{sample}}$ is the absorbance of 1 mL of sample extract combined with 4 mL of the diluted DPPH solution, and $A_{\text{blank}}$ is 1 mL of anhydrous ethanol combined with 4 mL of the diluted DPPH solution. Regression analysis of the data was used to estimate the IC$_{50}$ values.

3.6. Statistical Analysis

The processed data with accurate mass were exported from Xcalibur 2.1 software. The match factor and retention time window of the peaks were set to 0.3 ppm and 0.05 min, respectively.

The spectral information was imported into SIMCA software (SIMCA-P 14.1, Umetrics Inc., Umeå, Sweden) for multivariate statistical analysis. Principal component analysis (PCA) and orthogonal partial least-squares discriminate analysis (OPLS-DA) were used to distinguish between rhizome and radix samples. The S-plot and the importance in the projection (VIP) were used for predicting potentially characteristic chemical compounds between the rhizome and radix. Molecular formulas were determined using fragment information obtained from the workstation, as well as the established database and information from the literature. The cleavage process of each compound was predicted based on the MS and MS/MS information. Data were compared by one-way ANOVA followed by Turkey’s test for multiple comparisons. A value of $p < 0.05$ was considered to represent a significant difference.
3.7. Correlation Analysis

3.7.1. Gray Relational Analysis

GRA was performed using the DPS software (DPS 9.5, China) to calculate the correlation degree between the characteristic chemical compound peak area of MSCP and the antioxidant activities (ABST and DPPH radical scavenging activity). The gray relational grade was calculated with a distinguishing coefficient of 0.5.

3.7.2. Partial Least Squares Analysis

PLS analysis was performed using SIMCA software (SIMCA-P 14.1, Umetrics Inc., Umea, Sweden) for regression analysis between the peak area of characteristic components and the antioxidant activities. In the PLS model, the areas of characteristic component peaks were the independent variables (X), and the scavenging activities of ABTS and DPPH were the dependent variables (Y).

4. Conclusions

In this study, the UHPLC-Q-Exactive Orbitrap MS with multivariate analysis approach was established to identify and compare the chemical profiles of rhizome and radix MSCP. A total of 91 compounds were tentatively identified, and 10 compounds were selected as quality control markers to distinguish them. Additionally, the ABTS and DPPH assays were used to evaluate their antioxidant efficiency. GRA and PLS analysis indicated that uvaol-3-caffeate (peak 91), 3-O-caffeoyloleanolic acid (peak 88), and khrinone E (peak 76) are the primary bioactive markers for their antioxidant activity. The radix of MSCP is a rich source to be considered as a natural antioxidant reagent. The study could be helpful for future exploration of its material base and bioactive mechanism. In addition, it would be considered to be used as a new method for the quality control of *M. speciosa*.

Author Contributions: This study was designed by Z.Z., J.Z. conducted the UHPLC-Q-Exactive Orbitrap MS analysis and drafted the manuscript. J.W. and Y.W. performed the antioxidant experiments. M.C. and X.S. identified the chemical compounds. X.Z. conducted statistical analyses. All authors have read and agreed to the published version of the manuscript.

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