

## Article

# The Chemical Signatures of Water Extract of *Zingiber officinale* Rosc

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**Abstract:** Background: Ginger (*Z. officinale* Rosc.) is a common herb and is widely used as a diet-based or home therapy in traditional medicine worldwide. However, fresh ginger turns into dried ginger after kiln drying and shows a different treatment effect in clinical practice. Objective: To characterize the changes of major bioactive constituents in dried ginger after the processing of fresh ginger. Methods: A novel, ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UHPLC–QTOF/MS) method was established to characterize the changes in the bioactive constituents of dried ginger. The novel strategy was split into two steps: firstly, the MS selected the most intense precursor ions of tandem MS; then, target MS/MS acquisition with different collision energies (10, 20, and 40 eV) was used to characterize the compound's accurate MS/MS spectra and compare the MS/MS spectrum with the building MS reference library and reference standards. Result: Fifty-three compounds, including diarylheptanoids, gingerols, gingerodiols, gingerdiones, and shogaol-related compounds, were identified based on summarized fragmentation patterns. Fifteen out of fifty-three compounds were diarylheptanoids, which was different from fresh ginger. Conclusion: These identified compounds could be used to characterize the quality of dried ginger, pharmacologic studies should focus on diarylheptanoids explaining the different treatment effects between fresh ginger and dried ginger.

**Keywords:** chromatography; ginger; mass spectrometry; plant extracts



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## 1. Introduction

Ginger (*Z. Rosc.*) is a common spice that is widely used as a diet-based or home therapy in various traditional systems of medicine around the world. It has been used in traditional medicine practice for the treatment of arthritis [1–3]; rheumatological diseases; gastrointestinal disorders such as distress symptoms, digestive disorders, and pain [4–6]. Clinical pharmacology research has shown that the active compounds in ginger are responsible for antioxidant and cancer-preventive properties [7–9], the prevention of chemotherapy-induced toxicity [10], and the improvement of inflammatory bowel disease and colitis [11,12]. However, the chemicals responsible for ginger's pharmacology vary considerably.

Gingerols, shogaols, and their homologues are the major volatile components of *Zingiber officinale* Rosc. The percentage of volatile components varies based on the plantation region and harvesting season. Non-volatile components include paradols and their derivatives; zingerone; monoterpenoids; organic acids; and flavonoids [13–17]. In clinical practice,

the decoction of dried ginger is a common practice in traditional medicine and shows different health effects from fresh ginger against chronic diseases [18]. One reason is that the major compounds in fresh ginger are liable to dehydrate and convert when exposed to heat and/or acidic conditions. The other is that during the decoction process, the active components vary sharply with changes in temperature and boiling time [19,20]. Therefore, in the current study, we explored the bioactive constituents in the aqueous extract of dried ginger.

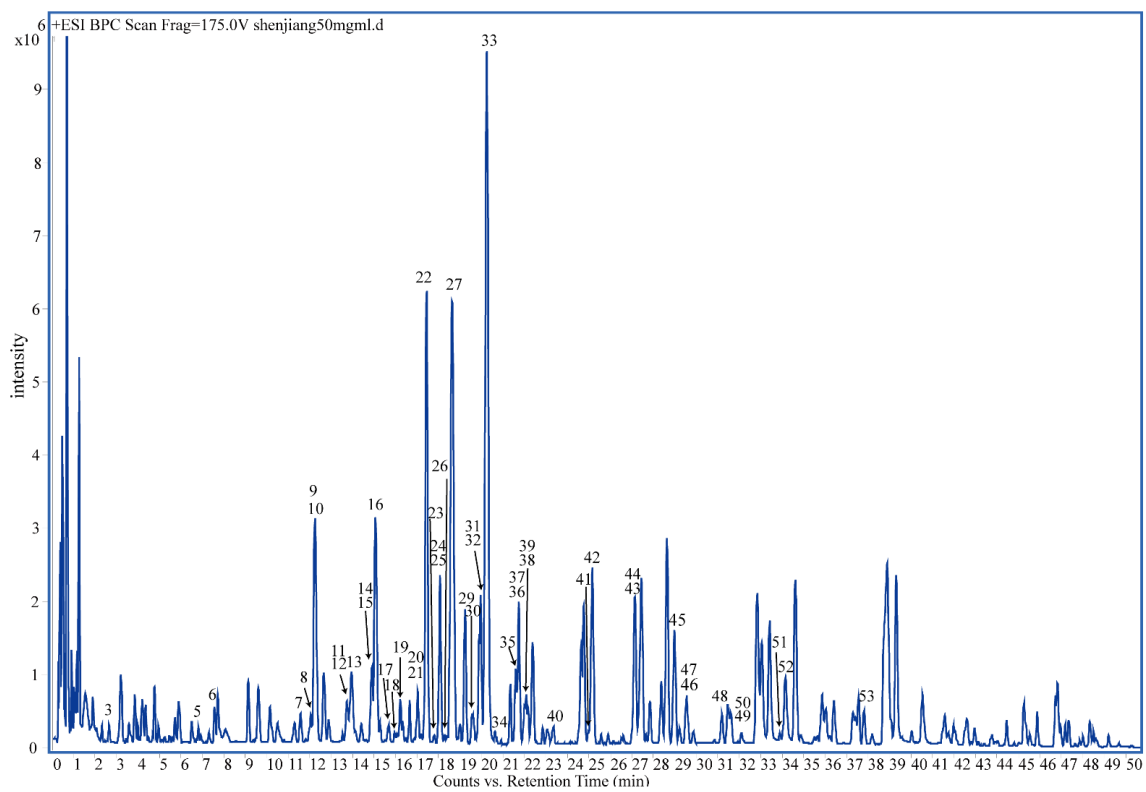
The components of fresh ginger has been chiefly studied by high-performance liquid chromatography or with a gas chromatography-mass spectrometer (GC-MS), because volatile oil is believed to be its main effective constituent [21]. These are also the usual ways to conduct quality assessments of ginger [22–24]. However, due to the characteristics of volatile oil, the quality control and bioactive components of ginger are still under investigation.

Mass spectrometry is currently one of the most robust and sensitive instrumental methods applied to the structural characterization of the secondary metabolite of ginger [25,26]. Therefore, we will use UHPLC-QTOF/MS to tentatively identify and characterize the chemical signature of the dried ginger. In addition, the gingerols and shogaols' homologues usually have the same skeletons as the parents. Therefore, the fragmentation of the parents molecules will provide specific structural information about the functionality of different compounds and is necessary and helpful for the characterization of the gingerols and shogaols' analogues in the tandem mass spectrometry (MS/MS) model.

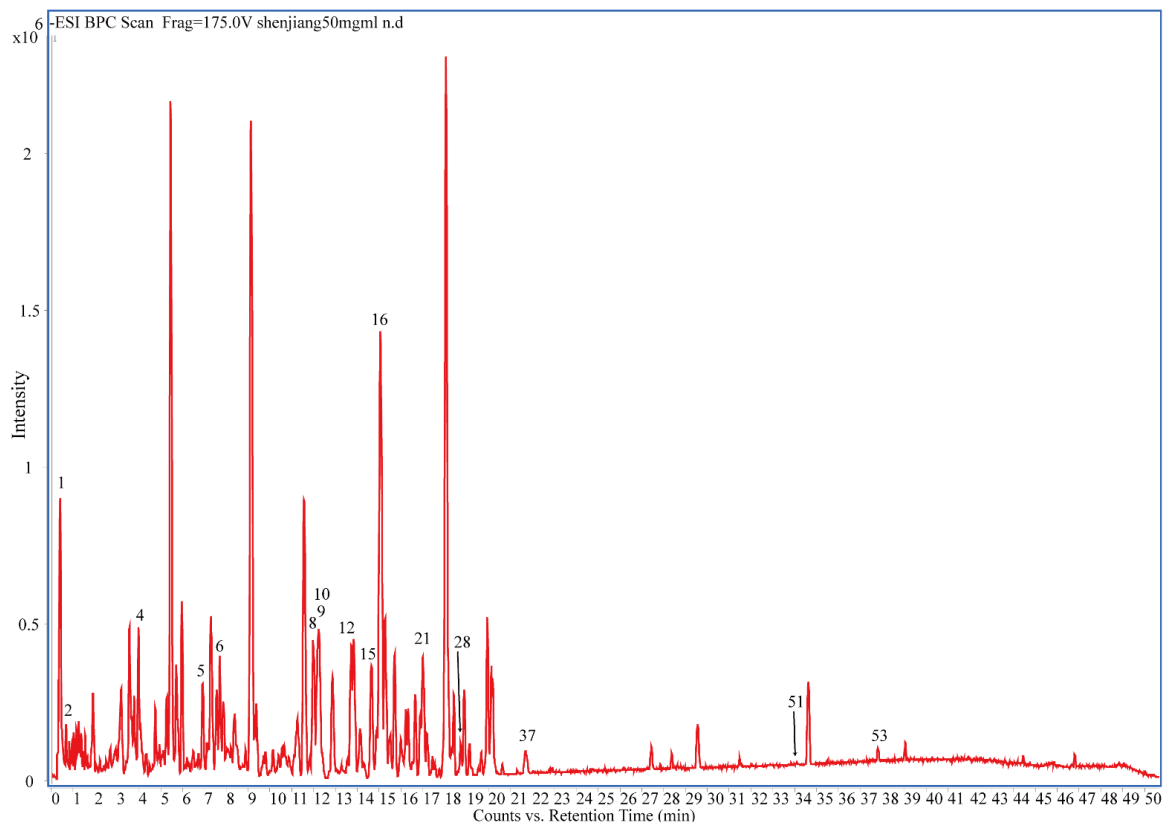
## 2. Results and Discussion

### 2.1. LC/MS Conditions

In this study, tandem mass spectrometry (MS/MS) was used to identify the molecular ions of different components. The results were shown in Figures 1 and 2 and Table 1. Most of the ingredients in dried ginger were identified within 40 min. Both positive and negative ionization modes were used to detect the compounds (Figures 1 and 2). It was found that the signal in the QTOF/MS positive mode was much more sensitive than that in the negative mode. Different polarity compounds are detected in the two ionization modes. It looks like the gingerol and shogaol derivatives ionize better in the negative mode. In the tandem MS negative mode, the intensity of the compound fragments was too weak to analyze. About 90 different ions were presented in the positive MS mode. In total, we characterized 53 compounds.



**Figure 1.** (+)-ESI base peak chromatogram (BPC) of the dried ginger water extract by UHPLC-ESI-QTOF-MS.



**Figure 2.** (-)-ESI base peak chromatogram (BPC) of the dried ginger water extract by UHPLC-ESI-QTOF-MS.

**Table 1.** UHPLC–ESI–QTOF–MS/MS results of the analysis of the dried ginger extract in the positive and negative models.

No.	Compound Name	Formula	Rt/ min	Detected Mass	Expected Tgt Mass	Diff/ ppm	Positive MS/MS	Negative MS/MS
1	Isoleucine	C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub>	0.50	131.0947	131.0946	0.14	N	130.0870[M–H] <sup>−</sup> , 115.0035[M–NH <sub>2</sub> ] <sup>−</sup> , 71.0140[M–NH <sub>2</sub> –COO] <sup>−</sup>
2	Phenylalanine	C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>	0.69	165.0785	165.0790	−2.70	N	164.0713[M–H] <sup>−</sup> , 147.8934[M–H <sub>2</sub> O] <sup>−</sup> , 103.0557[M–H <sub>2</sub> O–COO] <sup>−</sup> , and 72.0098
3	(Z)-citral	C <sub>10</sub> H <sub>16</sub> O	2.66	152.1197	152.1201	−2.88	153.1282[M+H] <sup>+</sup> , 93.0332[M+H–CH <sub>2</sub> COO] <sup>+</sup> , and 65.0383[M+H–CH <sub>2</sub> COO–CO] <sup>+</sup>	N
4	Galanganol C	C <sub>27</sub> H <sub>28</sub> O <sub>5</sub>	4.07	432.1937	432.1937	12.83	N	431.1914[M–H] <sup>−</sup> , 389.1797, 179.0545, 89.0243
5	Zingerone *	C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>	6.71	194.0942	194.0943	1.86	N	193.0863[M–H] <sup>−</sup> , 178.0573 [M–CH <sub>3</sub> –H] <sup>−</sup> , and 135.0450
6	Dihydrocurcumin	C <sub>21</sub> H <sub>22</sub> O <sub>6</sub>	7.75	370.1402	370.1416	−3.80	371.1481[M+H] <sup>+</sup> , 235.0943, 177.0535, 137.0589	369.1251[M–H] <sup>−</sup>
7	Tetrahydrocurcumin	C <sub>21</sub> H <sub>24</sub> O <sub>6</sub>	11.58	372.1557	372.1573	−4.18	373.1578[M+H] <sup>+</sup> , 179.0963, 153.0537, and 137.0589	
8	(E)-7-(3,4-dihydroxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)hept-2-en-1-one	C <sub>20</sub> H <sub>22</sub> O <sub>5</sub>	12.00	342.1451	342.1467	−4.64	343.1525[M+H] <sup>+</sup> , 258.2470, 147.0438, 137.0586, 123.0431, 107.0481, and 86.0960	327.1564[M–H] <sup>−</sup> , 135.0441
9	1,5-epoxy-3-hydroxy-1-(3,4-dihydroxy-5-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)heptane	C <sub>21</sub> H <sub>26</sub> O <sub>7</sub>	12.18	390.1659	390.1679	−5.09	391.1731[M+H] <sup>+</sup> , 179.0689, and 137.0586	389.1616[M–H] <sup>−</sup> , 165.0554
10	5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-3-heptanone	C <sub>20</sub> H <sub>24</sub> O <sub>5</sub>	12.23	344.1599	344.1624	−7.13	345.1631[M+H] <sup>+</sup> , 258.1470, and 123.0431	343.1552[M–H] <sup>−</sup>
11	Methyl diacetoxyl-[4]-gingerdiol	C <sub>20</sub> H <sub>30</sub> O <sub>6</sub>	13.73	366.2048	366.2042	1.39	389.1952[M+Na] <sup>+</sup> , 355.1524, 297.1104, 193.0484, and 137.0588	N
12	3,5-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)heptane	C <sub>22</sub> H <sub>30</sub> O <sub>7</sub>	13.73	406.1973	406.1992	−4.50	407.1992[M+H] <sup>+</sup> , 215.1382, 137.0584, 86.0959, and 70.0650	405.1530[M–H] <sup>−</sup> , 165.0558
13	[4]-Shogaol	C <sub>15</sub> H <sub>20</sub> O <sub>3</sub>	14.00	248.1403	248.1412	−3.84	249.1481[M+H] <sup>+</sup> , 163.0745, 137.0590, and 131.0480	N
14	1,5-epoxy-3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-4-methoxyphenyl)heptane	C <sub>22</sub> H <sub>28</sub> O <sub>7</sub>	14.86	404.1819	404.1835	−3.90	405.1885[M+H] <sup>+</sup> , 217.1210, 167.0693, and 139.0854	N
15	Gingerenone B	C <sub>22</sub> H <sub>26</sub> O <sub>6</sub>	14.87	386.1714	386.1729	−4.01	387.1729[M+H] <sup>+</sup> , 247.1316, 193.0848, 167.0692, and 137.0592	385.1603[M–H] <sup>−</sup> , 341.1071, 223.0609, and 101.0248,
16	3-acetoxy-5-hydroxy-1-(4-hydroxyphenyl)-7-(3,4-dihydroxyphenyl)heptane	C <sub>21</sub> H <sub>26</sub> O <sub>6</sub>	15.04	374.1709	374.1729	−5.43	375.1780[M+H] <sup>+</sup> , 341.1720, 217.1207, 163.0744, and 137.0588	373.1661[M–H] <sup>−</sup> , 331.1540, and 175.0753

Table 1. Cont.

No.	Compound Name	Formula	Rt/ min	Detected Mass	Expected Tgt Mass	Diff/ ppm	Positive MS/MS	Negative MS/MS
17	Dihydro-[6]-paradol	C <sub>17</sub> H <sub>28</sub> O <sub>3</sub>	15.64	280.2050	280.2038	4.13	303.1941[M+Na] <sup>+</sup> , 287.1989, 163.0742, and 103.0383	N
18	3,5-diacetoxy-1,7-bis(3,4-dihydroxy-5-methoxyphenyl)heptane	C <sub>25</sub> H <sub>32</sub> O <sub>10</sub>	15.96	492.1971	492.1996	−5.00	510.2883, 235.1176, 137.0589, 110.0708	N
19	Isomer of number 18	C <sub>25</sub> H <sub>32</sub> O <sub>10</sub>	16.10	492.1973	492.1996	−4.41	510.2883, 235.1176, 137.0589, and 110.0708	N
20	3-acetoxy-5-hydroxy-1-(3,4-dihydroxy-5-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)heptane	C <sub>23</sub> H <sub>30</sub> O <sub>8</sub>	17.01	434.1924	434.1941	−3.89	435.1989[M+H] <sup>+</sup> , 385.1987, 357.1692, 207.1003, 193.0846, 181.0847, 167.0694, 163.0745, 153.0539, and 137.0590	N
21	3,5-diacetoxy-1-(3,4-dihydroxyphenyl)-7(4-hydroxyphenyl)heptane	C <sub>23</sub> H <sub>28</sub> O <sub>7</sub>	17.01	416.1817	416.1835	−4.37	417.1890[M+H] <sup>+</sup> , 324.1414, 217.1211, 207.1009, 153.0539, 137.0590, and 81.0694	415.1761[M-H] <sup>−</sup> , 371.2049, and 359.1597
22	Isomer of number 45	C <sub>22</sub> H <sub>34</sub> O <sub>6</sub>	17.45	394.2334	394.2355	N	N	N
23	3-acetoxy-5-hydroxy-1-(3,4-dihydroxy-5-methoxyphenyl)heptane	C <sub>24</sub> H <sub>32</sub> O <sub>8</sub>	17.97	448.2075	448.2097	−4.93	449.2141[M+H] <sup>+</sup> , 373.1634, 313.1423, 123.0435,	N
24	3,5-diacetoxy-1-(3,4-dihydroxyphenyl)-7-(3,4-dihydroxy-5-methoxyphenyl)heptane	C <sub>24</sub> H <sub>30</sub> O <sub>9</sub>	18.06	462.1860	462.1890	−6.41	480.2202, 179.0685, and 137.0593	N
25	Dehydro-[6]-gingerdione	C <sub>17</sub> H <sub>22</sub> O <sub>4</sub>	18.07	290.1526	290.1518	2.86	177.0538, 145.0277	N
26	Isomer of number 18	C <sub>25</sub> H <sub>32</sub> O <sub>10</sub>	18.38	492.1969	492.1996	−4.38	510.2883, 235.1176, 137.0589, and 110.0708	N
27	unknown	unknown	18.71	466.2413	466.2413	2.34	Unknown	N
28	Trihydroxy octadecenoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>5</sub>	18.72	330.2405	330.2406	−0.40	N	329.233[M-H] <sup>−</sup> , 283.2613
29	Curcumadiol	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	19.24	238.1946	238.1933	7.94	261.1839[M+Na] <sup>+</sup> , 229.1566, 177.0886, 163.0743, 145.0637, 137.0590, 131.0488, 117.0692, and 103.0536	N
30	3,5-diacetoxy-1-(3,4-dihydroxy-5-methoxyphenyl)-7-(4-hydroxy-3,5-dimethoxyphenyl)heptane	C <sub>26</sub> H <sub>34</sub> O <sub>10</sub>	19.64	506.2129	506.2152	−4.61	507.2168[M+H] <sup>+</sup> , 355.1523, 215.1054, 179.0695, and 137.0587	N
31	[6]-Paradol	C <sub>17</sub> H <sub>26</sub> O <sub>3</sub>	19.91	278.1869	278.1882	−4.74	279.0941[M+H] <sup>+</sup> , 261.1840[M+H−H <sub>2</sub> O] <sup>+</sup> , 233.0954[M+H−H <sub>2</sub> O−CO] <sup>+</sup> , and 137.0595	N
32	3,5-diacetoxy-1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxy-5-methoxyphenyl)heptane	C <sub>25</sub> H <sub>32</sub> O <sub>9</sub>	19.93	476.2032	476.2046	−2.94	477.2855[M+H] <sup>+</sup> , 285.2163, 179.0695, 137.0591, and 69.0693	N
33	[6]-Gingerol*	C <sub>17</sub> H <sub>26</sub> O <sub>4</sub>	20.24	294.1819	294.1831	−4.10	317.1712[M+Na] <sup>+</sup> , 177.0906, 137.0600, 99.0797, 69.0697	N
34	6-hydroxy-[6]-shogaol	C <sub>17</sub> H <sub>24</sub> O <sub>4</sub>	20.63	292.1664	292.1675	−3.79	293.1742[M+H] <sup>+</sup> , 179.0690, and 137.0588	N

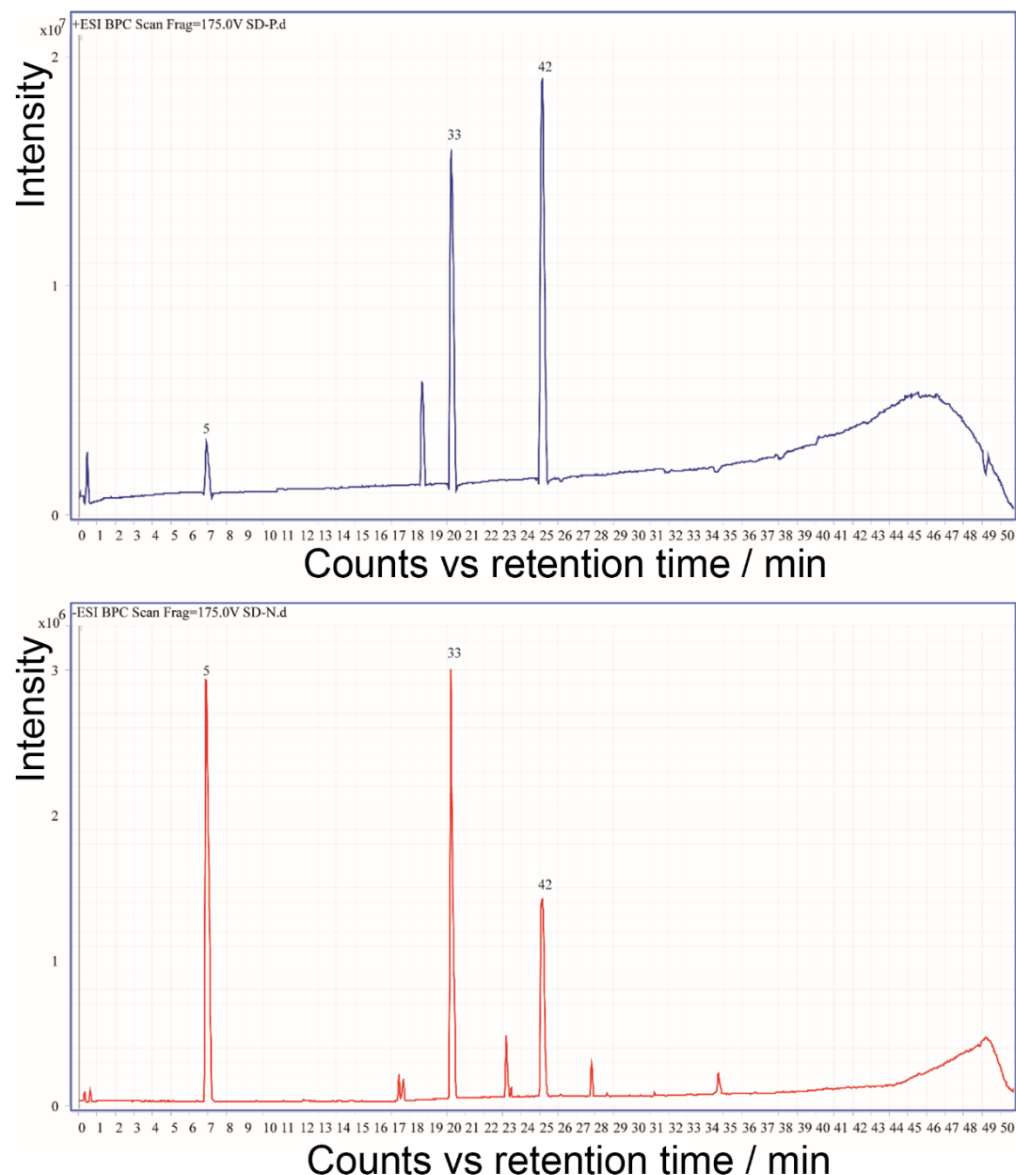
Table 1. Cont.

No.	Compound Name	Formula	Rt/min	Detected Mass	Expected Tgt Mass	Diff/ppm	Positive MS/MS	Negative MS/MS
35	3,5-diacetoxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)heptane	C <sub>26</sub> H <sub>34</sub> O <sub>9</sub>	21.60	490.2176	490.2203	−5.53	491.2525[M+H] <sup>+</sup> , 431.2055[M+H−CH <sub>3</sub> COO] <sup>+</sup> , 371.1837[M+H−2CH <sub>3</sub> COO] <sup>+</sup> , 339.1577, 247.1314, and 193.0852	N
36	3,5-diacetoxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)heptane	C <sub>24</sub> H <sub>30</sub> O <sub>7</sub>	21.74	430.1969	430.1992	−5.31	431.2926[M+H] <sup>+</sup> , 193.0848, and 167.0691	N
37	1,7bis-(4-hydroxy-3-methoxyphenyl)-5-methoxy-3-heptanone	C <sub>22</sub> H <sub>28</sub> O <sub>6</sub>	21.83	388.1887	388.1886	0.15	N	387.1814[M−H] <sup>−</sup> , 329.1384, 207.1025, 165.0552, and 122.0372
38	3,5-diacetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptane	C <sub>25</sub> H <sub>32</sub> O <sub>8</sub>	22.01	460.2078	460.2097	−4.10	478.2418[M+NH <sub>4</sub> ] <sup>+</sup> , 341.1732, 217.1212, and 137.0593	N
39	Palmitic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	22.16	256.2392	256.2402	−4.03	257.2620[M+H] <sup>+</sup> , 191.1054, and 106.0856	N
40	Diacetoxy-[4]-gingerdiol	C <sub>19</sub> H <sub>28</sub> O <sub>6</sub>	23.35	352.1863	352.1886	−6.48	370.2203[M+H <sub>2</sub> O] <sup>+</sup> , 137.0591 337.1985[M+H] <sup>+</sup> , 279.0995,	N
41	Acetoxy-[6]-gingerol	C <sub>19</sub> H <sub>28</sub> O <sub>5</sub>	25.12	336.1921	336.1937	−4.82	261.0898, 163.0739, 137.0590, 131.0484, 122.0359, 103.0537, and 94.0409	N
42	[6]-shogaol*	C <sub>17</sub> H <sub>24</sub> O <sub>3</sub>	25.24	276.1719	276.1725	−4.19	277.1787[M+H] <sup>+</sup> , 137.0591, 122.0356, and 94.0408	N
43	Diacetoxy-[6]-gingerdiol	C <sub>21</sub> H <sub>32</sub> O <sub>6</sub>	27.14	380.2178	380.2199	−5.60	403.2071[M+Na] <sup>+</sup> , 321.2052 [M+H−CH <sub>3</sub> COO] <sup>+</sup> , 137.0592	N
44	Dehydro-[8]-gingerol	C <sub>19</sub> H <sub>28</sub> O <sub>4</sub>	27.14	320.1975	320.1988	−3.93	321.2049[M+H] <sup>+</sup> , 261.1840, 177.0904,163.0742, and 137.0590	N
45	Methyl,diacetoxy-[6]-gingerdiol	C <sub>22</sub> H <sub>34</sub> O <sub>6</sub>	28.96	394.2334	394.2355	−5.39	417.2227[M+Na] <sup>+</sup> , 412.2678[M+H <sub>2</sub> O] <sup>+</sup> , 335.2210[M+H−CH <sub>3</sub> COO] <sup>+</sup> , 275.1911[M+H−2CH <sub>3</sub> COO] <sup>+</sup> , 177.0900, 151.0747, 137.0588,	N
46	Isomer of number 21	C <sub>23</sub> H <sub>28</sub> O <sub>7</sub>	29.40	416.1817	416.1835	N	417.1890[M+H] <sup>+</sup> , 324.1414, 217.1211, 207.1009, 153.0539, and 137.0590	N
47	Dehydro-10-gingerdione	C <sub>21</sub> H <sub>30</sub> O <sub>4</sub>	29.55	346.2141	346.2144	−0.82	347.2206[M+H] <sup>+</sup> , 177.0902, and 137.0592	N
48	[6]-gingerdiol	C <sub>17</sub> H <sub>28</sub> O <sub>4</sub>	31.20	296.1997	296.1988	3.27	319.1890[M+Na] <sup>+</sup> , 177.0899, 137.0589, and 94.0399	N
49	[10]-shogaol	C <sub>21</sub> H <sub>32</sub> O <sub>3</sub>	32.11	332.2334	332.2351	−5.14	333.2410[M+H] <sup>+</sup> , 137.0590, 94.0403	N
50	Acetoxy-[10]-gingerol	C <sub>23</sub> H <sub>36</sub> O <sub>5</sub>	32.11	392.2537	392.2563	−6.59	393.2606[M+H] <sup>+</sup> , 195.1220, 163.0737, and 137.0592	N
51	6-hydroxy-[10]-shogaol	C <sub>21</sub> H <sub>32</sub> O <sub>4</sub>	34.06	348.2284	348.2301	−4.88	349.2357[M+H] <sup>+</sup> , 179.0696, 161.0949, 137.0588, 121.0587, 95.0847	347.2227[M−H] <sup>−</sup>
52	Oleamide	C <sub>18</sub> H <sub>35</sub> NO	34.16	281.2708	281.2719	−3.82	282.2781[M+H] <sup>+</sup> , and 187.0727	N
53	Dehydro-[12]-gingerdione	C <sub>23</sub> H <sub>34</sub> O <sub>4</sub>	37.81	374.2440	374.2457	−4.52	375.2514[M+H] <sup>+</sup> , 177.0901, and 137.0590	373.1646[M−H] <sup>−</sup> , 313.1437, 191.1078, and 122.0374

\* means identified by Standard reference. N means not detected, Rt means retention time.

## 2.2. Establishment of Fragmentation Patterns

Reference standards (zingerone, 6-gingerol, and 6-shogaol) were used in this study to demonstrate identity (Figure 3). These compounds were dissolved in 50% methanol at a final concentration of 1  $\mu$ M for the UHPLC–ESI QTOF MS/MS analyses. Figure 3 shows the reference standards' base peak chromatograms (BPC) in both positive and negative ionization modes. All constituents were identified by comparing the UHPLC retention time, accurate mass, and mass spectrum with those standards (Table 1). The abnormal peak in Figure 3 was the background contamination ion in the MS, and it did not impact our analysis.



**Figure 3.** The base peak chromatograms (BPC) in positive negative and positive modes of the 3 standards of *Zingiber officinale* Rosc.: 5 zingerone, 33 6-gingerol, and 42 6-shogaol.

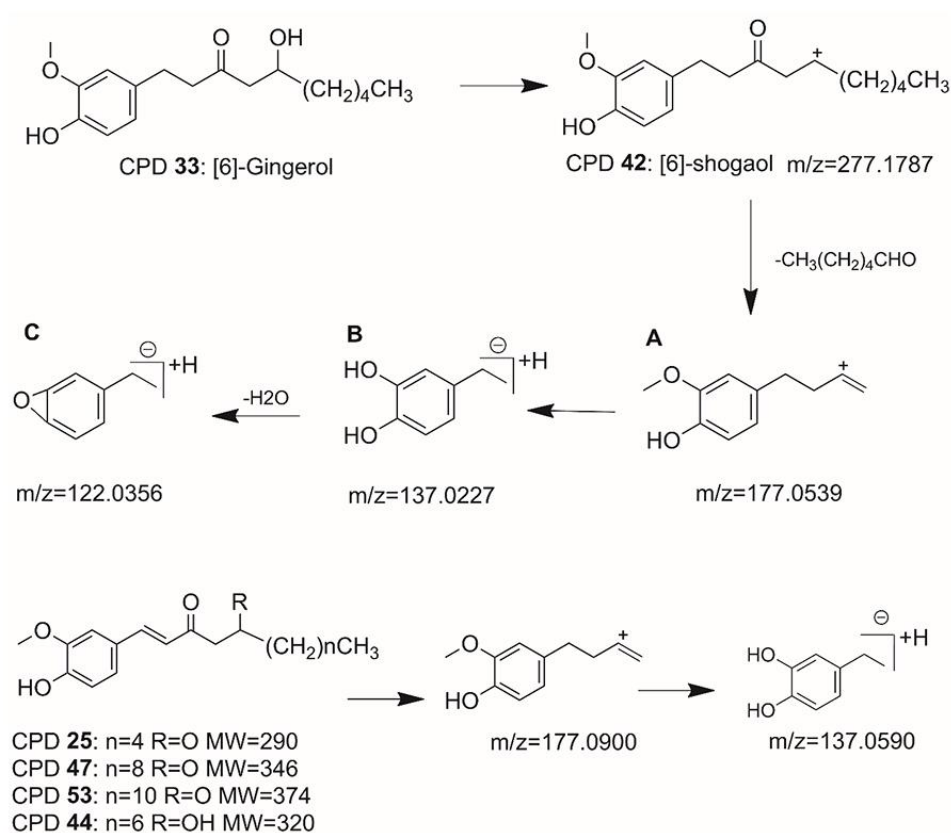
## 2.3. Gingerol-Related Compounds

Gingerol-related compounds are the most important components in fresh ginger. Gingerol, gingerdione, gingerdiol, and shogaol contribute the predominant peppery taste in *Z. officinale* Rosc. Additionally, they all have a 4-hydroxy-3-methoxyphenyl moiety with different hydrocarbon chains. In our study, only a few gingerol-related compounds were identified in the extraction of dried ginger, including compound 33 ([6]-gingerol),

41(acetoxy-[6]-gingerol), 44(acetoxy-[10]-gingerol), and 50 (dehydro-[8]-gingerol). These results are consistent with Tao and Li's study [27].

#### 2.4. Gingerdione-Related Compounds

Gingerdione, a gingerol derivative, is one of the major constituents of dried ginger. Compared to the standard compound, 6-gingerol, compound 25 showed a decrease of 4 Da for its corresponding  $[M+H]^+$  291.1580 in (+) ESI-MS. Protonated ions of compound 25 were further fragmented by losing a neutral alkyl moiety and a rearrangement (Figure 4), leading to the formation of predominance A at  $m/z$  177.0539  $m/z$  145.0227. Compounds 47 and 53 had similar fragmentation behaviors to compound 25. Compared to compound 25, the protonated ions of compounds 47 and 53 showed increases of 48 and 72 Da, respectively. Therefore, compounds 47 and 53 were tentatively identified as 1-dehydro-[10]-gingerdione and 1-dehydro-[12]-gingerdione.



**Figure 4.** The typical fragmentation mechanisms of 6-gingerol and dehydro-gingerdione in positive mode.  $m/z$  177.0900 (A),  $m/z$  137.0590 (B), and  $m/z$  122.0356 (C) were the base peaks of the derivative compounds in MS/MS.

#### 2.5. Gingerdiol-Related Compounds

The precursor ion of compound 48 was sodium adduct ions in (+) ESI-MS, which is different from the standard compound, [6]-gingerol (Table 1). However, the fragmentation pattern of [6]-gingerdiol was similar to that of [6]-gingerol, and they both broke into  $m/z$  177.0899 and 137.0589. According to the (+) ESI-MS/MS spectra for compound 43, we know that the parent ion fragments into  $321.2052[M+H-CH_3COO]^+$  due to the loss of 60 Da (AcOH). This information suggests that the acetoxy group, and not the hydroxy group, is present on the aliphatic side chain of compound 48. Compound 43 was identified as diacetoxy-[6]-gingerdiol. Compound 45 was identified as methyl-diacetoxy-[6]-gingerdiol. Sodium adduct ions (417.2227) fragmented into  $m/z$   $335.2210[M+H-CH_3COO]^+$ ,  $275.1911[M+H-2CH_3COO]^+$ , 177.0900, 151.0747, and 137.0588. Compounds 11 and 40 were identified as methyl-diacetoxy-[4]-gingerdiol and diacetoxy-[4]-gingerdiol, which had the



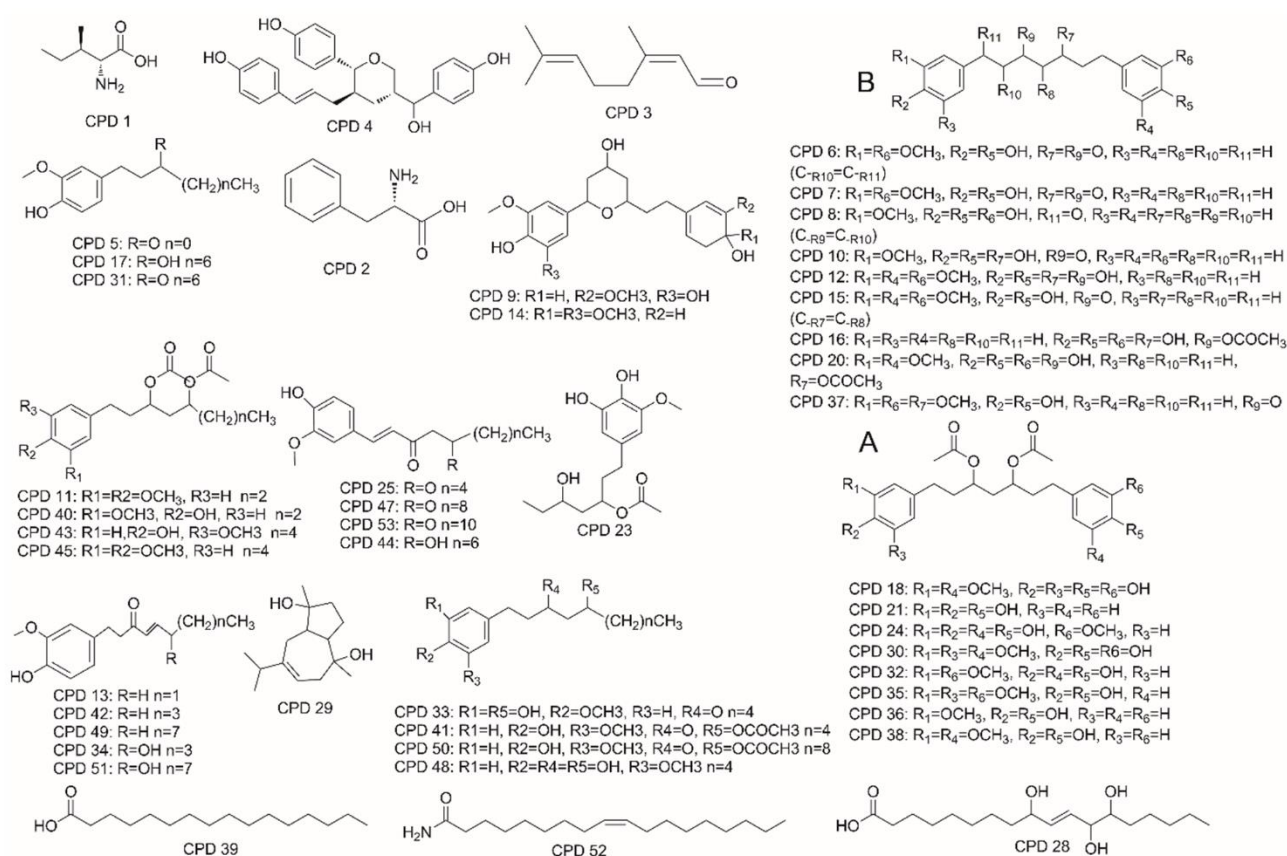
same basic skeleton and fragmentation pathway as compound 45 and have been reported previously in the literature.

## 2.6. Shogaol and Paradol-Related Compounds

Compounds 13, 42, 34, 49, and 51 were identified as [4]-shogaol, [6]-shogaol, 6-hydroxy-[6]-shogaol, [10]-shogaol, and 6-hydroxy-[10]-shogaol. These are all shogaol derivatives and have similar fragmentation patterns according to the mass spectrum(B). This fragmentation pattern was consistent with Hongliang Jiang's research results [28]. 6-hydroxy-[6]-shogaol and 6-hydroxy-[10]-shogaol can fragment into  $m/z$  177.0535(A). Compounds 31 and 17 were identified as [6]-paradol and dihydro-[6]-paradol in positive ionization mode. They also fragmented into ions at  $m/z$  137.0595—the same as the shogaol. [6]-paradol was detected as a protonated ion ( $m/z$  279.0941) and fragmented into ions at  $m/z$  261.1840[M+H-H<sub>2</sub>O]<sup>+</sup>, 233.0954[M+H-H<sub>2</sub>O-CO]<sup>+</sup>, and 137.0595(B).

## 2.7. Diarylheptanoids

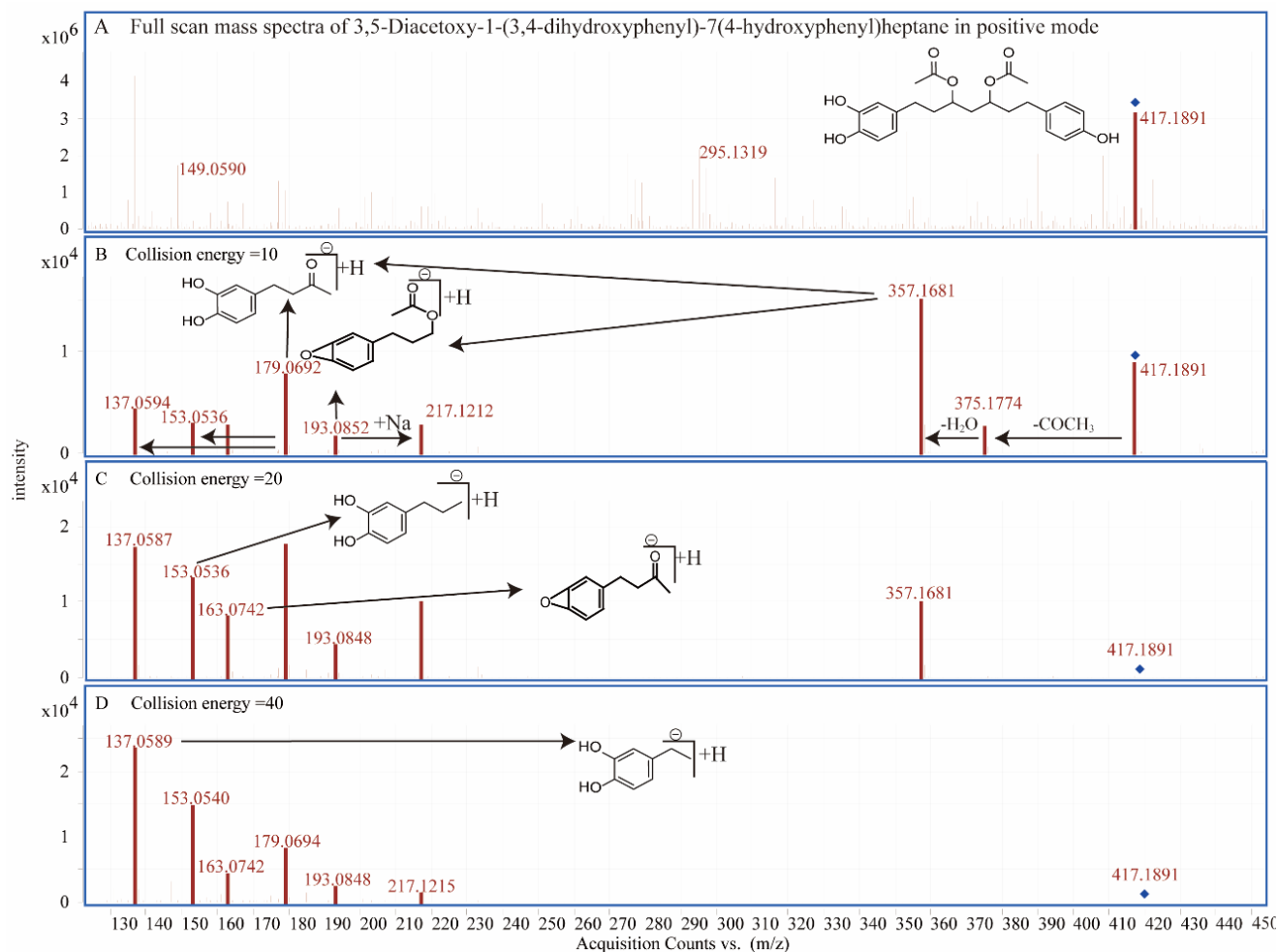
More than 15 compounds were identified as diarylheptanoids, which are primarily responsible for cytotoxicity and apoptosis in ginger [9,29,30]. These diarylheptanoids were characterized by the presence of 5-hydroxy and 3-oxo groups on the heptane skeleton (Figure 5A,B).



**Figure 5.** Chemical structure of the compounds identified by UHPLC–ESI–QTOF–MS/MS from dried ginger's water extract. **A**, diarylheptanoids with two CH<sub>3</sub>CO atomic groups; **B** diarylheptanoids with one CH<sub>3</sub>CO atomic group.

Compounds 8, 10, 12, 16, 20, and 37 are diarylheptanoids with -OH, -H, =O, or CH<sub>3</sub>CO atomic groups at the 1, 3, or 5 positions of the carbon chain. Compounds 18, 21, 24, 30, 32, 35, 36, and 38 have CH<sub>3</sub>CO- at both the 3 and 5 positions of the carbon chain. Most of them can fragment into  $m/z$  177.0690 (Figure 5A) or 137.0588 (Figure 5B) at different collision energies (Figure 6). We identified these compounds based on the characteristics of

the compounds shown in research conducted by Riethmüller [31,32] and Svarc-Gajic [33]. The typical scheme of diarylheptanoid fragmentation was shown in Figure 6.



**Figure 6.** The mass spectra and the fragmentation pathway of compound 21, which was identified as 3,5-diacetoxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl) heptane. The mass spectra of compound 21 was shown in (A) with different collision energies 10 V (B), 20 V (C), and 40 V (D).

The precursor ions of compound 6 were observed at  $m/z$  371.1481  $[M+H]^+$  in (+) ESI-MS and  $m/z$  369.1251  $[M-H]^-$  in (−) ESI-MS, indicating a molecular weight of 370. When compared to compound 7, compound 6 showed an increase of 2 Da on the precursor ion, and its product ions are shown in Table 1, indicating that it may be a homolog of compound 6 with a difference in the carbon–carbon double bond. The protonated ion compound 15 (387.1729) is 14 Da larger than compound 7 (373.1578), which indicates that compound 15 has a methoxy group. Compounds 9 and 14 displayed the same fragmentation behavior; however, protonated ion compound 14 (405.1885) is 14 Da larger than compound 9 (391.1731), differing in a methoxy group. The fragmentation pattern of compound 23 is unique and was identified as 3-acetoxy-5-hydroxy-1-(3,4-dihydroxy-5-methoxyphenyl)heptane by referring to the literature [34].

## 2.8. Two Amino Acids, Four Fatty Acids, and Others

The compound 1 deprotonated ion is 130.0870  $[M-H]^-$ , and it fragments into  $m/z$  115.0035  $[M-NH_2]^-$  and 71.0140  $[M-NH_2-COO]^-$  at the negative ionization mode with different collision energy. Therefore, compound 1 was identified as isoleucine. Compound 2 showed  $[M-H]^-$  ions with  $m/z$  164.0713. The precursor ion and daughter ion matched with what we obtained from phenylalanine [34].

In the precursor ion scan spectrum,  $m/z$  of compound 3  $[M+H]^+$  was 153.1282. It can fragment into  $m/z$  93.0332 $[M+H-CH_2COO]^+$  and 65.0383 $[M+H-CH_2COO-CO]^+$  in the positive ionization mode. Therefore, compound 3 was identified as citral [35]. Compounds 28, 39, and 52 are fatty acids and were tentatively identified by comparison with the literature [36].

Compounds 4 and 29 were also identified in the extractions of *Z. mioga* and *Z. officinale*, and their daughter ions matched galanganol C and curcumadiol's daughter ions [37].

### 3. Materials and Methods

#### 3.1. Chemicals and Reagents

HPLC-grade acetonitrile, formic acid, and methanol were purchased from Merck KGaA (Darmstadt, Germany). Deionized water was re-distilled. Three standard materials—zingiberone (Lot J0108AS), 6-gingerol (Lot O1014AS), and 6-shogaol (Lot O1020AS)—were purchased from Meilunbio company (Liaoning, Dalian, China, purity > 99.0%)

#### 3.2. Plant-Material and Sample Preparation

Dried ginger materials appeared as yellow primrose pieces of wood and were purchased from Kangmei Chinese Traditional Medicine. Co. Ltd. (Guangdong, China). Original herbs were produced from Sichuan in 2017 (MAN: 2017.04.01)—10 g in each package (LOP: 170400541). The dried ginger was immersed in eight-fold volumes of water (1:8, w/v) for 30 min and boiled for 1 h. The decoction was filtered through 8 layers of gauze and was lyophilized to dried powder. We evaluated the amount of solvent (1:2, 1:4, and 1:8) and the extraction time (0.25, 0.5, 1, and 2 h) impact on the extraction efficiency, and we found that the ginger was boiled for a long time (2 h) or short time (0.5 h), removing valuable components from the extractions. Therefore, we closed 8-fold water and only boiled 1 h to acquire more peaks in the mass spectrum.

The LC-MS sample preparation: 1 mL of deionized water was added to 50 mg of the freeze-dried powder, vortexed for 10 min, and then sonicated for 15 min at 40 °C. The mixture was then vortexed vigorously for 3 min, after centrifugation at 15,000 rpm for 30 min. Five microliters of supernatant was injected into the UPLC-MS/MS system for qualitative analysis.

#### 3.3. Accurate-Mass QTOF LC/MS System

The analysis of dried ginger was performed on an Agilent 1290UHPLC system coupled to an in-line diode array detector (DAD) and an Agilent 6540 Accurate-Mass QTOF LC/MS system with Agilent Jet Stream technology for electrospray ionization (Agilent Technologies, Santa Clara, CA, USA). The LC conditions were as follows: separation column, Acquity HPLC BEH C18 column (50 × 2.1 mm, i.d. 1.7 μM; Waters); the mobile phase consisted of 0.1% aqueous formic acid (*v:v*) (A) and acetonitrile (B), using a gradient elution of 5% B for 0–2 min, 5–10% B for 2–5 min, 10–14% B for 5–8 min, 14% B for 8–14 min, 14–17% B for 14–20 min, 17–20% B for 20–25 min, 20–25% B for 25–30 min, 25–50% B for 30–40 min, 50–100% B for 40–50 min, 100% B for 50–55 min, and 100–5% B for 55–60 min. The pastime was 3 min for the re-equilibrated systems. The flow rate was 0.5 mL/min, the temperature was 40 °C, and the injection volumes were 2 μL in MS mode and target MS/MS mode.

#### 3.4. Mass Spectrometry

Full acquisition MS, auto MS/MS, and targeted MS/MS were performed with a 6540 QTOF Mass Spectrometer in both positive and negative ionization modes. Full acquisition MS spectra were collected over a mass range of  $m/z$  100–1700, and the acquisition rate was 1 Hz at 1000 ms/scan. In the auto MS/MS and targeted MS/MS modes, the precursor MS spectrum was from  $m/z$  100 to 1500, and the acquisition rate was 2 Hz with 500 ms/scan; the MS/MS spectrum was from  $m/z$  50 to 1000, and the acquisition rate was 3 Hz with 333 ms/scan. The conditions of the ESI source were as follows: a drying gas (N<sub>2</sub>) flow rate of 11 (L/min); a drying gas temperature of 300 °C; a sheath gas temperature of 350 °C; a

nebulizer at 40 psi; a capillary voltage of 3.5 kV (negative mode) or 4 kV (positive mode); a fragmentor at 175 V; a skimmer voltage of 60 V; and an octopole RF of 250 V. Every day, prior to the analyses of the samples, the mass axis was calibrated. All of the operations, acquisitions, and analyses of data were controlled by Mass Hunter software version B.06.00 (Agilent Technologies, Santa Clara, CA, USA).

### 3.5. Building the Chemical Database of Ginger

The ginger database was created using the Agilent software, Personal Compound Database Library (PCDL). The database contained the formulas, accurate masses, compound names, and original plants. The ginger database is available in Supplementary Table S1. The records of 236 compounds were input into the database by comprehensively searching databases such as Sci Finder, PubMed, TCM Database@Taiwan, Chinese National Knowledge Infrastructure of Tsinghua University, and KNApSack for all of the compounds reported in the literature for ginger (Supplementary Table S1).

### 3.6. Preparation of the Reference Standard

The stock solutions of the reference standard (gingerol, zingerone, and 6-shogaol) were prepared in ethanol/DMSO (4:1, *v:v*) at final concentrations of 10 mM. Then, each stock solution was diluted by 50% methanol to 1  $\mu$ M for analysis.

## 4. Conclusions

A novel UHPLC–ESI–QTOF/MS approach was developed to identify chemical profiles of *Z. officinale* Rosc.. Many studies have demonstrated that the major function of the drying process is to reduce the gingerol concentration, increase the terpene hydrocarbon level, and convert some monoterpene alcohols into their corresponding acetates [13,24,38,39]. Our study results showed that the major components of dried ginger are diarylheptanoids. However, the main compounds in fresh ginger, gingerols, and shogaols, which are responsible for the bioactivity and spicy taste, were present in very low concentrations. This result may support the fact that dried ginger has different chemical constituents and pharmacological activities when compared with fresh herbs in clinical practice. Therefore, future pharmacologic studies should focus on these diarylheptanoids.

Most of the gingerol-related compounds have -OH and -OCH<sub>3</sub> groups on their benzene ring. All of them fragment into the basement product ion  $m/z$  137 (2-hydroxy-3-methoxyphenyl-CH<sub>2</sub><sup>+</sup> and 2,3-methoxyphenyl<sup>+</sup>). This fragmentation pattern was useful for diagnosing fragmentation behavior in positive and negative ESI–QTOF/MS, and analyzing the structures of homologs and allowed us to classify compounds by group and identify them based on key structural features. Overall, our novel strategy only requires 1–2 h to complete each peak, with each compound feature guided by the in-house database.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/molecules27227818/s1>, Table S1: Data of chemical substances database of *Zingiber officinale* Rosc.

**Author Contributions:** H.C. and F.L. wrote the initial manuscript with major input from W.X.; R.S. H.C., F.L. and W.X. performed the experiments and analyzed the results; S.L. carried out the data interpretation; and S.L., W.X. and R.S. designed the study and obtained funding for the studies. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** Ethical standards were complied with.

**Informed Consent Statement:** Ethical approval and informed consent are not applicable in this study.



**Data Availability Statement:** All data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest:** The authors declare that they have no conflict of interest.

**Sample Availability:** Dried ginger is available from the authors.

## References

1. Funk, J.L.; Frye, J.B.; Oyarzo, J.N.; Chen, J.; Zhang, H.; Timmermann, B.N. Anti-Inflammatory Effects of the Essential Oils of Ginger (*Zingiber officinale* Roscoe) in Experimental Rheumatoid Arthritis. *PharmaNutrition* **2016**, *4*, 123–131. [[CrossRef](#)] [[PubMed](#)]
2. Ramadan, G.; Al-Kahtani, M.A.; El-Sayed, W.M. Anti-inflammatory and anti-oxidant properties of *Curcuma longa* (turmeric) versus *Zingiber officinale* (ginger) rhizomes in rat adjuvant-induced arthritis. *Inflammation* **2011**, *34*, 291–301. [[CrossRef](#)] [[PubMed](#)]
3. Ramadan, G.; El-Menshawly, O. Protective effects of ginger-turmeric rhizomes mixture on joint inflammation, atherogenesis, kidney dysfunction and other complications in a rat model of human rheumatoid arthritis. *Int. J. Rheum. Dis.* **2013**, *16*, 219–229. [[CrossRef](#)] [[PubMed](#)]
4. Ghayur, M.N.; Gilani, A.H. Pharmacological basis for the medicinal use of ginger in gastrointestinal disorders. *Dig. Dis. Sci.* **2005**, *50*, 1889–1897. [[CrossRef](#)] [[PubMed](#)]
5. Walstab, J.; Kruger, D.; Stark, T.; Hofmann, T.; Demir, I.E.; Ceyhan, G.O.; Feistel, B.; Schemann, M.; Niesler, B. Ginger and its pungent constituents non-competitively inhibit activation of human recombinant and native 5-HT<sub>3</sub> receptors of enteric neurons. *Neurogastroenterol Motil.* **2013**, *25*, 439–447-e302. [[CrossRef](#)]
6. Nikkhah Bodagh, M.; Maleki, I.; Hekmatdoost, A. Ginger in gastrointestinal disorders: A systematic review of clinical trials. *Food Sci. Nutr.* **2019**, *7*, 96–108. [[CrossRef](#)]
7. Wang, C.Z.; Qi, L.W.; Yuan, C.S. Cancer Chemoprevention Effects of Ginger and its Active Constituents: Potential for New Drug Discovery. *Am. J. Chin. Med.* **2015**, *43*, 1351–1363. [[CrossRef](#)]
8. Zick, S.M.; Turgeon, D.K.; Ren, J.; Ruffin, M.T.; Wright, B.D.; Sen, A.; Djuric, Z.; Brenner, D.E. Pilot clinical study of the effects of ginger root extract on eicosanoids in colonic mucosa of subjects at increased risk for colorectal cancer. *Mol. Carcinog.* **2015**, *54*, 908–915. [[CrossRef](#)]
9. Mao, Q.Q.; Xu, X.Y.; Cao, S.Y.; Gan, R.Y.; Corke, H.; Beta, T.; Li, H.B. Bioactive Compounds and Bioactivities of Ginger (*Zingiber officinale* Roscoe). *Foods* **2019**, *8*, 185. [[CrossRef](#)]
10. Marx, W.; Ried, K.; McCarthy, A.L.; Vitetta, L.; Sali, A.; McKavanagh, D.; Isenring, L. Ginger-Mechanism of action in chemotherapy-induced nausea and vomiting: A review. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 141–146. [[CrossRef](#)]
11. Murakami, A.; Hayashi, R.; Tanaka, T.; Kwon, K.H.; Ohigashi, H.; Safitri, R. Suppression of dextran sodium sulfate-induced colitis in mice by zerumbone, a subtropical ginger sesquiterpene, and nimesulide: Separately and in combination. *Biochem. Pharmacol.* **2003**, *66*, 1253–1261. [[CrossRef](#)]
12. Zhang, M.; Xu, C.; Liu, D.; Han, M.K.; Wang, L.; Merlin, D. Oral Delivery of Nanoparticles Loaded With Ginger Active Compound, 6-Shogaol, Attenuates Ulcerative Colitis and Promotes Wound Healing in a Murine Model of Ulcerative Colitis. *J. Crohns Colitis.* **2018**, *12*, 217–229. [[CrossRef](#)] [[PubMed](#)]
13. Alinkina, E.S.; Misharina, T.A.; Fatkullina, L.D.; Burlakova, E.B. Comparison of the antiradical activity of ionol, components of fresh ginger, and its extracts. *Prikl. Biokhim. Mikrobiol.* **2012**, *48*, 564–569. [[CrossRef](#)] [[PubMed](#)]
14. Azam, F.; Amer, A.M.; Abulifa, A.R.; Elzwawi, M.M. Ginger components as new leads for the design and development of novel multi-targeted anti-Alzheimer's drugs: A computational investigation. *Drug Des. Devel. Ther.* **2014**, *8*, 2045–2059. [[CrossRef](#)] [[PubMed](#)]
15. Ghasemzadeh, A.; Jaafar, H.Z.; Rahmat, A. Identification and concentration of some flavonoid components in Malaysian young ginger (*Zingiber officinale* Roscoe) varieties by a high performance liquid chromatography method. *Molecules* **2010**, *15*, 6231–6243. [[CrossRef](#)]
16. Gong, F.; Fung, Y.S.; Liang, Y.Z. Determination of volatile components in ginger using gas chromatography-mass spectrometry with resolution improved by data processing techniques. *J. Agric. Food Chem.* **2004**, *52*, 6378–6383. [[CrossRef](#)]
17. Shao, X.; Lv, L.; Parks, T.; Wu, H.; Ho, C.T.; Sang, S. Quantitative analysis of ginger components in commercial products using liquid chromatography with electrochemical array detection. *J. Agric. Food Chem.* **2010**, *58*, 12608–12614. [[CrossRef](#)]
18. Sang, S.; Snook, H.D.; Tareq, F.S.; Fasina, Y. Precision Research on Ginger: The Type of Ginger Matters. *J. Agric. Food Chem.* **2020**, *68*, 8517–8523. [[CrossRef](#)]
19. Wu, H.; Tang, Z.; Qiu, L.; Ye, D. The comparison of component contents and pharmacological actions between two kinds of processed products of *Pinellia rhizoma* prepared by ginger juice and alum. *Zhongguo Zhong Yao Za Zhi* **1999**, *24*, 25–28, 63.
20. Zhong, L.Y.; Su, D.; Zhu, J.; Deng, Y.F. Comparison of *Coptidis Rhizoma* processed with different ginger juice based on metabolomics. *Zhongguo Zhong Yao Za Zhi* **2016**, *41*, 2712–2719.
21. El-Ghorab, A.H.; Nauman, M.; Anjum, F.M.; Hussain, S.; Nadeem, M. A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). *J. Agric. Food Chem.* **2010**, *58*, 8231–8237. [[CrossRef](#)] [[PubMed](#)]

22. Koch, W.; Kukula-Koch, W.; Marzec, Z.; Kasperek, E.; Wyszogrodzka-Koma, L.; Szwerc, W.; Asakawa, Y. Application of Chromatographic and Spectroscopic Methods towards the Quality Assessment of Ginger (*Zingiber officinale*) Rhizomes from Ecological Plantations. *Int. J. Mol. Sci.* **2017**, *18*, 452. [[CrossRef](#)] [[PubMed](#)]
23. Zhao, H.B.; Wang, Z.H.; He, F.; Meng, H.; Peng, J.H.; Shi, J.L. Analysis of Volatile Oils from Different Processed Products of *Zingiber officinale* Rhizome by GC-MS. *Zhong Yao Cai* **2015**, *38*, 723–726. [[PubMed](#)]
24. Li, Y.; Hong, Y.; Han, Y.; Wang, Y.; Xia, L. Chemical characterization and antioxidant activities comparison in fresh, dried, stir-frying and carbonized ginger. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2016**, *1011*, 223–232. [[CrossRef](#)]
25. Wu, C.Y.; Kong, M.; Zhang WLong, F.; Zhou, J.; Zhou, S.S.; Xu, J.D.; Xu, J.; Li, S.L. Impact of sulphur fumigation on the chemistry of ginger. *Food Chem.* **2018**, *239*, 953–963. [[CrossRef](#)]
26. Li, M.Q.; Hu, X.Y.; Wang, Y.Z.; Zhang, X.J.; Li, J.P.; Song, Z.M.; Liu, Y.F.; Feng, W.S. Qualitative analysis on chemical constituents from different polarity extracted fractions of the pulp and peel of ginger rhizomes by ultra-high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2021**, *35*, e9029.
27. Tao, Y.; Li, W.; Liang, W.; Van Breemen, R.B. Identification and quantification of gingerols and related compounds in ginger dietary supplements using high-performance liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* **2009**, *57*, 10014–10021. [[CrossRef](#)]
28. Jiang, H.; Solyom, A.M.; Timmermann, B.N.; Gang, D.R. Characterization of gingerol-related compounds in ginger rhizome (*Zingiber officinale* Rosc.) by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2957–2964. [[CrossRef](#)]
29. Jiang, H.; Timmermann, B.N.; Gang, D.R. Characterization and identification of diarylheptanoids in ginger (*Zingiber officinale* Rosc.) using high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 509–518. [[CrossRef](#)]
30. Li, T.; Pan, D.B.; Pang, Q.Q.; Zhou, M.; Yao, X.J.; Yao, X.S.; Li, H.B.; Yu, Y. Diarylheptanoid analogues from the rhizomes of *Zingiber officinale* and their anti-tumour activity. *RSC Adv.* **2021**, *11*, 29376–29384. [[CrossRef](#)]
31. Riethmuller, E.; Alberti, A.; Toth, G.; Beni, S.; Ortolano, F.; Kery, A. Characterisation of diarylheptanoid- and flavonoid-type phenolics in *Corylus avellana* L. leaves and bark by HPLC/DAD-ESI/MS. *Phytochem. Anal.* **2013**, *24*, 493–503. [[CrossRef](#)] [[PubMed](#)]
32. Riethmuller, E.; Toth, G.; Alberti, A.; Vegh, K.; Burlini, I.; Konczol, A.; Balogh, G.T.; Kéry, Á. First characterisation of flavonoid- and diarylheptanoid-type antioxidant phenolics in *Corylus maxima* by HPLC-DAD-ESI-MS. *J. Pharm. Biomed. Anal.* **2015**, *107*, 159–167. [[CrossRef](#)] [[PubMed](#)]
33. Švarc-Gajić, J.; Cvetanović, A.; Segura-Carretero, A.; Linares, I.B.; Mašković, P. Characterisation of ginger extracts obtained by subcritical water. *J. Supercrit. Fluids.* **2017**, *123*, 92–100. [[CrossRef](#)]
34. Ni, J.; Xu, L.; Li, W.; Wu, L. Simultaneous determination of thirteen kinds of amino acid and eight kinds of acylcarnitine in human serum by LC-MS/MS and its application to measure the serum concentration of lung cancer patients. *Biomed. Chromatogr.* **2016**, *30*, 1796–1806. [[CrossRef](#)]
35. Tian, H.; Yang, X.; Ho, C.T.; Huang, Q.; Song, S. Development of a solid phase microextraction protocol for the GC-MS determination of volatile off-flavour compounds from citral degradation in oil-in-water emulsions. *Food Chem.* **2013**, *141*, 131–138. [[CrossRef](#)] [[PubMed](#)]
36. Ferrer, I.; Ruiz-Sala, P.; Vicente, Y.; Merinero, B.; Perez-Cerda, C.; Ugarte, M. Separation and identification of plasma short-chain acylcarnitine isomers by HPLC/MS/MS for the differential diagnosis of fatty acid oxidation defects and organic acidemias. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2007**, *860*, 121–126. [[CrossRef](#)]
37. Han, J.S.; Lee, S.; Kim, H.Y.; Lee, C.H. MS-Based Metabolite Profiling of Aboveground and Root Components of *Zingiber mioga* and *officinale*. *Molecules* **2015**, *20*, 16170–16185. [[CrossRef](#)]
38. Fang, W.T.; Zhan, Z.L.; Peng, H.S.; Huang, L.Q. Historical evolution and change of differentiation on dried ginger, fresh ginger and baked ginger. *Zhongguo Zhong Yao Za Zhi* **2017**, *42*, 1641–1645.
39. Wang, L.; Fang, L.; Zhao, H.; Wang, S.; Du, J.; Wang, X. Analysis of gingerol-related compounds in fresh ginger by HPLC-ESI-Q-TOF-MS/MS. *Zhongguo Zhong Yao Za Zhi* **2011**, *36*, 3467–3471.