

Supplementary File

# Chemical Characterization and Evaluation of Antimicrobial Properties of the Wild Medicinal Mushroom *Ganoderma lucidum* Growing in Northern Moroccan Forests.

El Hadi Erbiai <sup>1,2</sup>, Benoutman Amina <sup>1</sup>, Abbassi Kaoutar <sup>1</sup>, Rabah Saidi <sup>1</sup>, Zouhaire Lamrani <sup>1</sup>, Eugénia Pinto <sup>3,4</sup>, Joaquim C.G. Esteves da Silva <sup>2</sup>, Abdelfettah Maouni <sup>1</sup>, and Luís Pinto da Silva <sup>2\*</sup>

<sup>1</sup> Biology, Environment, and Sustainable Development Laboratory, ENS, Abdelmalek Essaadi University, 93000 Tetouan, Morocco; elhadi.erbiai@etu.uae.ac.ma (E.H.E.); amina.benoutman@etu.uae.ac.ma; kaoutar.abbassi@etu.uae.ac.ma; r.saidi@uae.ac.ma (R.S.); zh.amrani@yahoo.fr (Z.L.); amaouni@uae.ac.ma (A.M.)

<sup>2</sup> Chemistry Research Unit (CIQUP), Institute of Molecular Sciences (IMS), Department of Sciences, Environment and Territorial Planning, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal; el.erbiai@fc.up.pt (E.H.E); jcsilva@fc.up.pt (J.C.G.E.d.S.)

<sup>3</sup> Laboratory of Microbiology, Biological Sciences Department, Faculty of Pharmacy, University of Porto (FFUP), 4050-313 Porto, Portugal; epinto@ff.up.pt (E.P.)

<sup>4</sup> Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, 4450-208 Matosinhos, Portugal

\* Correspondence: luis.silva@fc.up.pt (L.P.d.S.)

**Citation:** Erbiai, E.H.; Amina, B.; Kaoutar, A.; Saidi, R.; Lamrani, Z.; Pinto, E.; Esteves da Silva, J.C.G.; Maouni, A.; Pinto da Silva, L. Chemical Characterization and Evaluation of Antimicrobial Properties of the Wild Medicinal Mushroom *Ganoderma lucidum* Growing in Northern Moroccan Forests. *Life* **2023**, *13*, 1217. <https://doi.org/10.3390/life13051217>

Academic Editor: Stefania Lamponi

Received: 29 March 2023

Revised: 15 May 2023

Accepted: 17 May 2023

Published: 19 May 2023



**Copyright:** © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Table S1:** Sugar compositions of the derivatized methanolic extract of *Ganoderma lucidum* by GC–MS analysis.

Compound names	Chemical formula	Molecular weight	Kovats' RI*	Area %
1,5-Anhydrohexitol	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>	164.16	1989.85	1.52
1-Deoxy-d-ribitol	C <sub>5</sub> H <sub>12</sub> O <sub>4</sub>	136.15	1533.83	0.35
D-Allofuranose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.16	1996.64	1.19
D-Arabinitol	C <sub>5</sub> H <sub>12</sub> O <sub>5</sub>	152.15	1710.45	0.28
D-Erythofuranose (isomer 2)	C <sub>4</sub> H <sub>8</sub> O <sub>4</sub>	120.10	1833.33	0.27
D-Fructofuranose (isomer 1)	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.16	2155.17	0.69
D-Gluconic acid	C <sub>6</sub> H <sub>12</sub> O <sub>7</sub>	196.16	2017.01	0.79
D-Glucopyranosiduronic acid	-	-	3090.24	0.33
DL-Arabinopyranose	C <sub>5</sub> H <sub>10</sub> O <sub>5</sub>	150.13	1993.62	0.22
D-Ribofuranose (isomer 1)	C <sub>5</sub> H <sub>10</sub> O <sub>5</sub>	150.13	2665.19	0.52
D-Ribono-1,4-lactone	C <sub>5</sub> H <sub>8</sub> O <sub>5</sub>	148.11	1634.80	0.13
D-Talofuranose (isomer 2)	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.16	1788.03	0.24
<b>Galactitol</b>	<b>C<sub>6</sub>H<sub>14</sub>O<sub>6</sub></b>	<b>182.17</b>	<b>1991.19</b>	<b>5.56</b>
Gluconolactone	C <sub>6</sub> H <sub>10</sub> O <sub>6</sub>	178.14	1777.31	0.32
Glyceric acid	C <sub>3</sub> H <sub>6</sub> O <sub>4</sub>	106.08	1318.28	0.27
Glycerol	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	92.09	1260.23	2.31
Lactulose (isomer 2)	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	342.30	2777.84	2.69
L-Arabinose	C <sub>5</sub> H <sub>10</sub> O <sub>5</sub>	150.13	1781.42	0.34
L-Sorbose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.16	2010.31	0.35
Methyl alpha-D-glucofuranoside	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194.18	2736.36	0.93
Myo-Inositol	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.16	1952.72	2.26
N-Acetyl-D-galactosamine (isomer 2)	C <sub>8</sub> H <sub>15</sub> NO <sub>6</sub>	221.21	2109.77	0.82
Threonic acid	C <sub>4</sub> H <sub>8</sub> O <sub>5</sub>	136.10	1538.81	0.11
Trehalose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	342.30	2634.18	2.49
<b>Turanose</b>	<b>C<sub>12</sub>H<sub>22</sub>O<sub>11</sub></b>	<b>342.30</b>	<b>2627.85</b>	<b>5.25</b>
<b>Xylitol</b>	<b>C<sub>5</sub>H<sub>12</sub>O<sub>5</sub></b>	<b>152.15</b>	<b>1726.87</b>	<b>7.69</b>
<b>α-D-Allopyranose</b>	<b>C<sub>6</sub>H<sub>12</sub>O<sub>6</sub></b>	<b>180.16</b>	<b>1998.07</b>	<b>5.28</b>
α-D-Glucopyranose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.16	1986.33	0.76
α-D-Mannopyranose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.16	1650.36	0.94
<b>α-D-Talopyranose</b>	<b>C<sub>6</sub>H<sub>12</sub>O<sub>6</sub></b>	<b>180.16</b>	<b>2080.54</b>	<b>4.59</b>

\*Kovats' RI: Kovats retention index of compound's derivative form.

**Table S2:** Organic acids of the derivatized methanolic extract of *G. lucidum* by GC–MS analysis.

Compound names	Chemical formula	Molecular weight	Kovats' RI*	Area %
3-Hydroxyisobutyric acid	C <sub>4</sub> H <sub>8</sub> O <sub>3</sub>	104.10	1146.99	0.06
Carbamic acid	CH <sub>3</sub> NO <sub>2</sub>	61.04	1024.84	0.27
<b>Citric acid</b>	<b>C<sub>6</sub>H<sub>8</sub>O<sub>7</sub></b>	<b>192.12</b>	<b>1820.71</b>	<b>1.31</b>
<b>Fumaric Acid</b>	<b>C<sub>4</sub>H<sub>4</sub>O<sub>4</sub></b>	<b>116.07</b>	<b>1329.57</b>	<b>1.89</b>
Glycolic acid	C <sub>2</sub> H <sub>4</sub> O <sub>3</sub>	76.05	1066.46	0.12
Lactic Acid	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	90.08	1053.42	0.2
<b>Malic acid</b>	<b>C<sub>4</sub>H<sub>6</sub>O<sub>5</sub></b>	<b>134.09</b>	<b>1479.08</b>	<b>3.64</b>
Phthalic acid	C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>	166.13	2541.45	0.42
Pyruvic acid	C <sub>3</sub> H <sub>4</sub> O <sub>3</sub>	88.06	1079.50	0.11
Succinic acid	C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>	118.09	1298.86	0.87

\*Kovats' RI: Kovats retention index of compound's derivative form.

**Table S3:** Fatty acids of the derivatized methanolic extract of *G. lucidum* by GC–MS analysis.

Compound names	Chemical formula	Molecular weight	Kovats' RI*	Area %
10,12-Docosadiynedioic acid	C <sub>22</sub> H <sub>34</sub> O <sub>4</sub>	362.50	2813.30	0.11
2-Bromosebacic acid	C <sub>10</sub> H <sub>17</sub> BrO <sub>4</sub>	281.14	2954.77	0.30
2-Hydroxyglutaric acid	C <sub>5</sub> H <sub>8</sub> O <sub>5</sub>	148.11	1563.68	0.12
<b>Linoleic acid</b>	<b>C<sub>18</sub>H<sub>32</sub>O<sub>2</sub></b>	<b>280.45</b>	<b>2197.13</b>	<b>5.13</b>
Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.46	2205.92	0.46
Palmitic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.42	2027.84	0.92
Pentadecanoic acid	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242.40	1988.93	0.36
Stearic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.48	2223.08	0.35

\*Kovats' RI: Kovats retention index of compound's derivative form.

**Table S4:** Amino acids of the derivatized methanolic extract of *G. lucidum* by GC–MS analysis.

Compound names	Chemical formula	Molecular weight	Kovats' RI*	Area %
Alanine	C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>	89.09	1093.79	0.44
Aspartic acid	C <sub>4</sub> H <sub>7</sub> NO <sub>4</sub>	133.10	1512.94	0.50
Glutamic acid	C <sub>5</sub> H <sub>9</sub> NO <sub>4</sub>	147.13	1610.29	0.23
Leucine	C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub>	131.17	1282.95	0.43
<b>Pidolic acid</b>	<b>C<sub>5</sub>H<sub>7</sub>NO<sub>3</sub></b>	<b>129.11</b>	<b>1524.38</b>	<b>4.72</b>
Serine	C <sub>3</sub> H <sub>7</sub> NO <sub>3</sub>	105.09	1347.85	0.38
Threonine	C <sub>4</sub> H <sub>9</sub> NO <sub>3</sub>	119.12	1376.34	0.32
Valine	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>	117.15	1205.11	0.42

\*Kovats' RI: Kovats retention index of compound's derivative form.

**Table S5:** Steroids of the derivatized methanolic extract of *G. lucidum* by GC–MS analysis.

Compound names	Chemical formula	Molecular weight	Kovats' RI*	Area %
16 $\alpha$ -Hydroxypregnенолон	C <sub>21</sub> H <sub>32</sub> O <sub>3</sub>	332.50	3027.53	0.23
<b>Ergosta-7,22-dien-3<math>\beta</math>-ol</b>	<b>C<sub>28</sub>H<sub>46</sub>O</b>	<b>398.70</b>	<b>3260.87</b>	<b>3.02</b>
Ergosta-7-en-3 $\beta$ -ol	C <sub>28</sub> H <sub>48</sub> O	400.70	3342.53	0.52
<b>Ergosterol</b>	<b>C<sub>28</sub>H<sub>44</sub>O</b>	<b>396.65</b>	<b>3242.78</b>	<b>3.55</b>

\*Kovats' RI: Kovats retention index of compound's derivative form.

**Table S6:** Polyphenols of the derivatized methanolic extract of *G. lucidum* by GC–MS analysis.

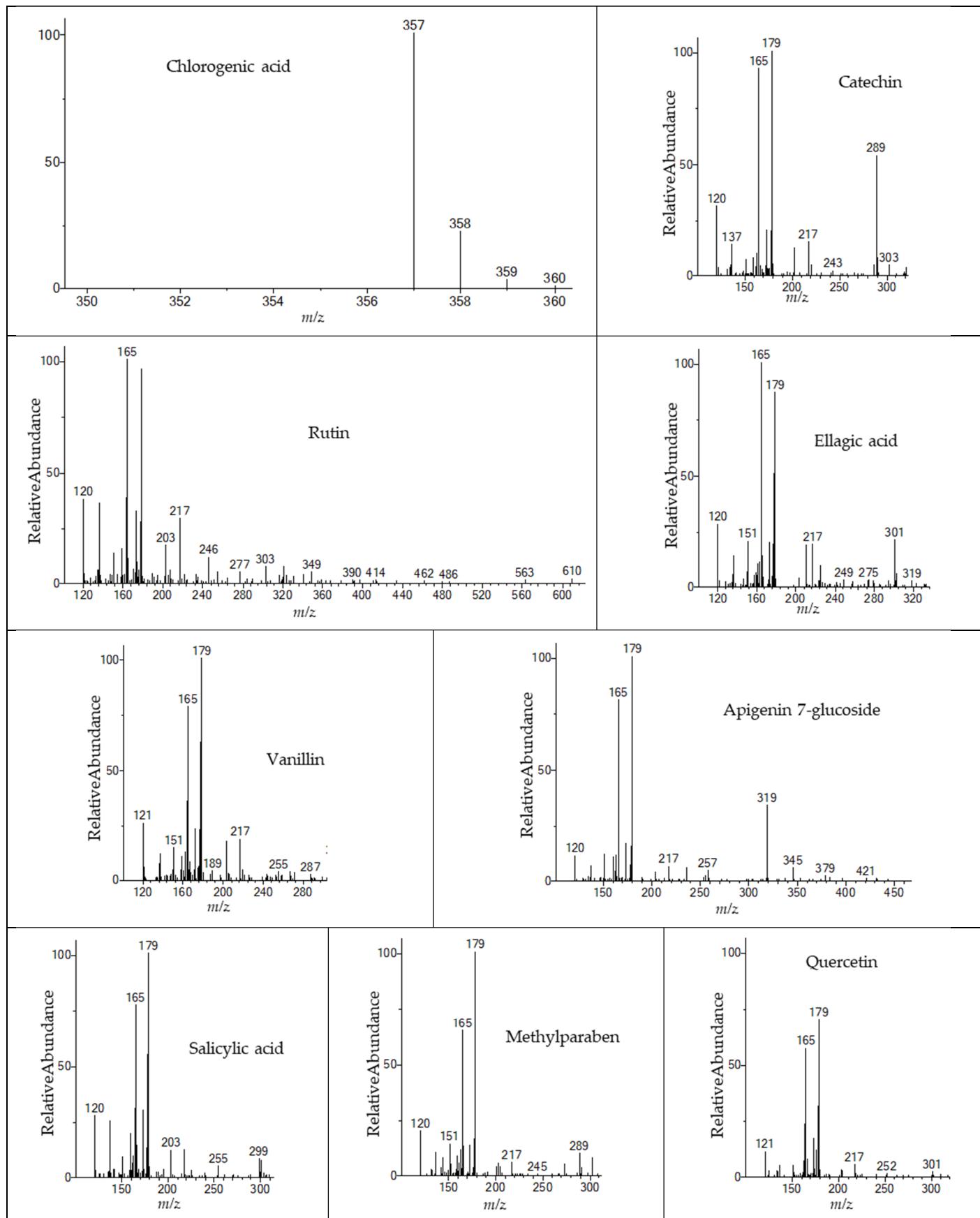
Compound names	Chemical formula	Molecular weight	Kovats' RI*	Area %
1,1-Dichloro-2,2-bis(4-methoxyphenyl)ethane	C <sub>16</sub> H <sub>16</sub> Cl <sub>2</sub> O <sub>2</sub>	311.2	1217	0.22
<b>Benzene, (3-chloro-1-propenyl)-</b>	<b>C<sub>9</sub>H<sub>9</sub>Cl</b>	<b>152.621</b>	<b>934.18</b>	<b>3.79</b>
Flavone, 5,7-dihydroxy-6c-glucoside	C <sub>27</sub> H <sub>30</sub> O <sub>14</sub>	578.5	3128.57	0.46
Gentisic acid	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	154.1201	1772.14	0.65
Phenol	C <sub>6</sub> H <sub>6</sub> O	94.11	919.62	0.31
Pyrogallol	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11	1597.01	0.49

\*Kovats' RI: Kovats retention index of compound's derivative form.

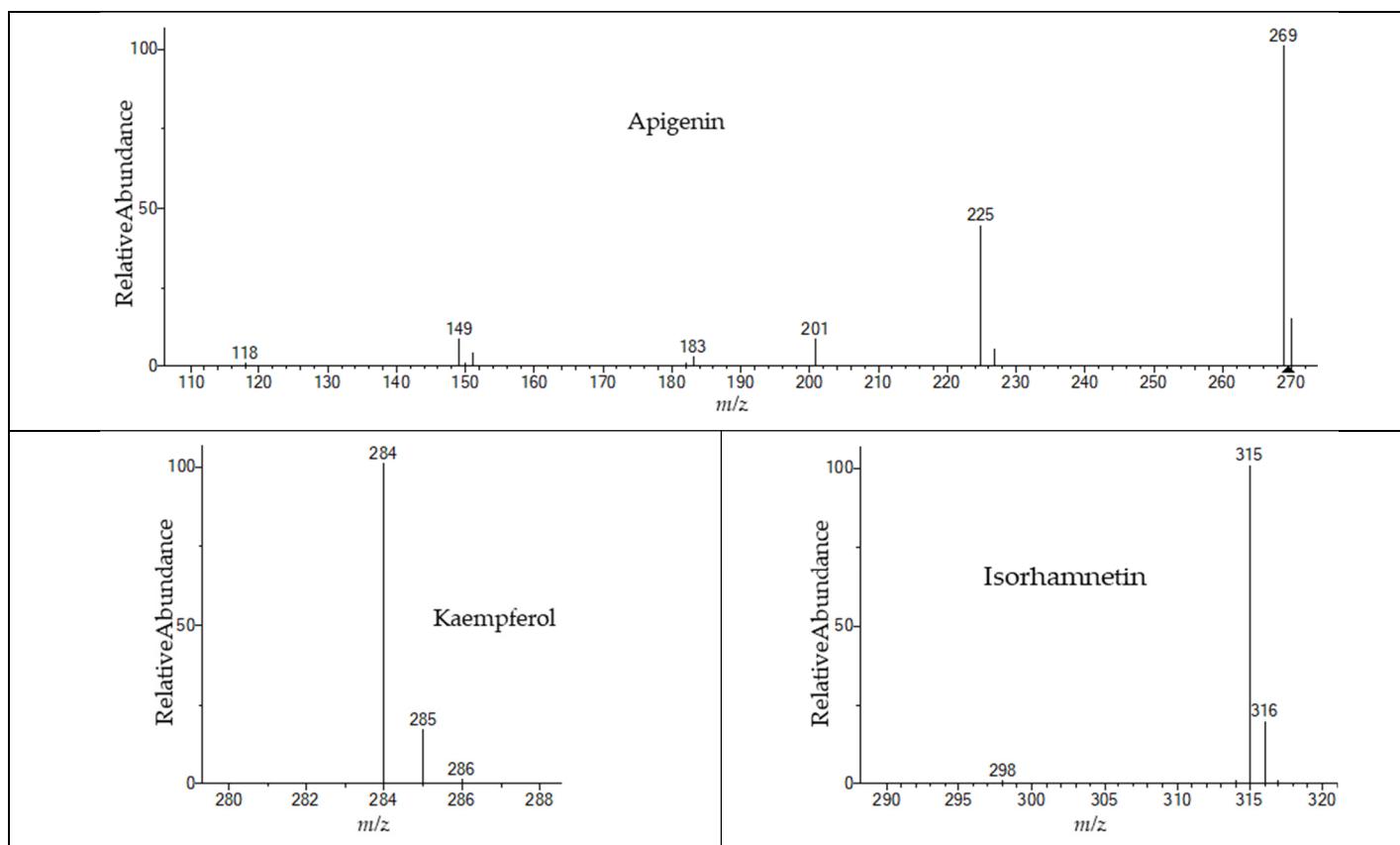
**Table S7:** Other biomolecules group of the derivatized methanolic extract of *G. lucidum* by GC–MS analysis.

Compound names	Chemical formula	Molecular weight	Kovats' RI*	Area %
1,5-Pentanediol	C <sub>5</sub> H <sub>12</sub> O <sub>2</sub>	104.15	1225.57	0.21
2,4-Dimethylthiazole	C <sub>5</sub> H <sub>7</sub> N <sub>s</sub>	113.18	897.42	0.03
2-Monoolein	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	356.50	2934.44	0.09
3-Methoxypentane	C <sub>6</sub> H <sub>14</sub> O	102.17	1730.35	0.26
3-Methyl-5-phenyl-1H-pyrazole	C <sub>10</sub> H <sub>10</sub> N <sub>2</sub>	158.20	2210.06	0.22
4-Pyridinol	C <sub>5</sub> H <sub>5</sub> No	95.10	1037.27	0.11
6-Undecanol	C <sub>11</sub> H <sub>24</sub> O	172.31	2616.46	0.44
Adenosine	C <sub>10</sub> H <sub>13</sub> N <sub>5</sub> O <sub>4</sub>	267.24	2644.30	0.31
Deanol	C <sub>4</sub> H <sub>11</sub> NO	89.14	911.39	0.67
Glycerol monostearate	C <sub>21</sub> H <sub>42</sub> O <sub>4</sub>	358.60	2764.77	0.15
<b>Glycerol-3-phosphate</b>	<b>C<sub>3</sub>H<sub>9</sub>O<sub>6</sub>P</b>	<b>172.07</b>	<b>1764.68</b>	<b>2.63</b>
Guanosine	C <sub>10</sub> H <sub>13</sub> N <sub>5</sub> O <sub>5</sub>	283.24	2785.80	0.15
<b>Phosphoric acid</b>	<b>H<sub>3</sub>PO<sub>4</sub></b>	<b>97.99</b>	<b>1267.05</b>	<b>5.7</b>
<b>Prostaglandin D<sub>2</sub></b>	<b>C<sub>20</sub>H<sub>32</sub>O<sub>5</sub></b>	<b>352.47</b>	<b>3078.05</b>	<b>2.19</b>

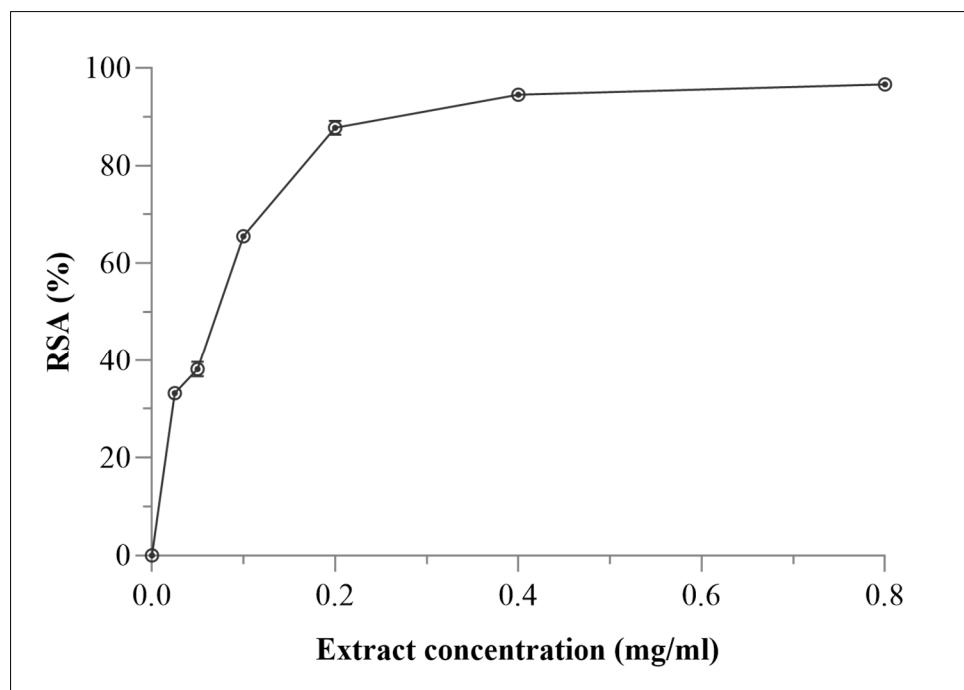
\*Kovats' RI: Kovats retention index of compound's derivative form.



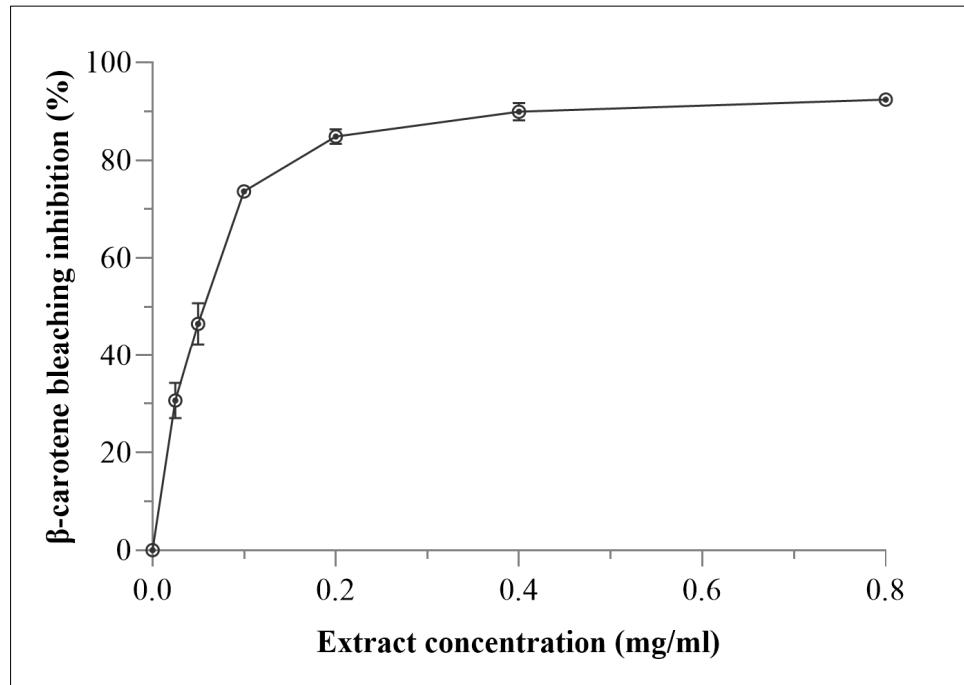
**Figure S1:** ESI-MS<sup>n</sup> spectrum of the most important phenolic compounds identified in *G. lucidum*: Chlorogenic acid at  $m/z$  353.14, catechin at  $m/z$  289.11, rutin at  $m/z$  609.98, ellagic acid at  $m/z$  301.22, vanillin at  $m/z$  151.27, apigenin 7-glucoside at  $m/z$  432.02, salicylic acid at  $m/z$  137.36, methylparaben at  $m/z$  151.20 and quercetin at  $m/z$  301.49.



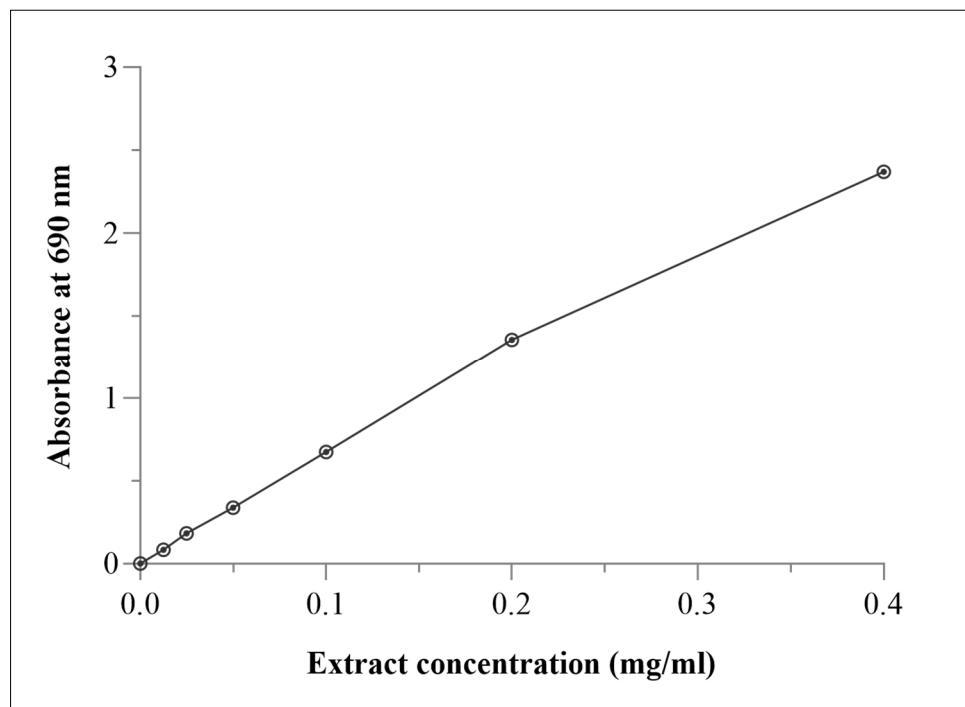
**Figure S1** (continued): ESI-MS<sup>n</sup> spectrum of the most important phenolic compounds identified in *G. lucidum*: apigenin at  $m/z$  269.22, kaempferol at  $m/z$  285.79, and Isorhamnetin at  $m/z$  316.14.



**Figure S2:** Radical-scavenging activity on DPPH radicals. Each value is expressed as mean  $\pm$  SD ( $n = 3$ ).



**Figure S3:** Lipid peroxidation inhibition measured by the  $\beta$ -carotene bleaching inhibition. Each value is expressed as mean  $\pm$  SD ( $n = 3$ ).



**Figure S4:** Reducing power. Each value is expressed as mean  $\pm$  SD ( $n = 3$ ).