Phenylethanoid and Phenylmethanoid Glycosides from the Leaves of *Ligustrum robustum* and Their Bioactivities

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Abstract: The phytochemical study on the leaves of *Ligustrum robustum*, which have been used as Ku-Ding-Cha, led to the isolation and identification of three new phenylethanoid glycosides and three new phenylmethanoid glycosides, named ligurobustosides R₁ (1b), R₂–3 (2), R₄ (3), S₁ (4b), S₂ (5), and S₃ (6), and five reported phenylethanoid glycosides (7–11). In the bioactivity test, (Z)-osmanthuside B₆ (11) displayed strong fatty acid synthase (FAS) inhibitory activity (IC₅₀: 4.55 ± 0.35 µM) as the positive control orlistat (IC₅₀: 4.46 ± 0.13 µM), while ligurobustosides R₄ (3) and S₂ (5), ligupurpuroside B (7), cis-ligupurpuroside B (8), ligurobustoside N (9), osmanthuside D (10), and (Z)-osmanthuside B₆ (11) showed stronger ABTS radical scavenging activity (IC₅₀: 2.68 ± 0.05–4.86 ± 0.06 µM) than the positive control L-(+)-ascorbic acid (IC₅₀: 10.06 ± 0.19 µM). This research provided a theoretical basis for the leaves of *L. robustum* as a tea with function in treating obesity and diabetes.

Keywords: *Ligustrum robustum*; phenylethanoid glycoside; phenylmethanoid glycosides; FAS; α-glucosidase; antioxidant; anti-obesity; hypoglycemic

1. Introduction

Ku-Ding-Cha, a tea with functions in clearing heat, removing toxins, and treating obesity and diabetes, has been applied widely in Southwest China for nearly 2000 years [1,2]. It was derived from the leaves of more than 30 plants belonging to 13 genera in 12 families [3]. *Ligustrum robustum* (Roxb.) Blume (Oleaceae), classified as a food by the Chinese Ministry of Health since 2011, has been used as Ku-Ding-Cha in Southwest China [4,5]. In the previous investigations on *L. robustum* [1–16], more than 60 chemical constituents, including monoterpenoid glycosides, phenylethanoid glycosides, phenylmethanoid glycosides, iridoid glycosides, flavonoid glycosides, lignan glycosides, and triterpenoids, were discovered, and the antioxidative, anti-obesity, and anti-inflammatory effects of the aqueous extract, the inhibitory activities on FAS, α-glucosidase, and α-amylase, and the antioxidant effects of some constituents, were observed. To further elucidate the active components for preventing obesity and diabetes, the phytochemical and biological study on the leaves of *L. robustum*, which had been performed preliminarily [12,13], was carried out. As a result, three new phenylethanoid glycosides and three new phenylmethanoid glycosides, named ligurobustosides R₁ (1b), R₂–3 (2), R₄ (3), S₁ (4b), S₂ (5), and S₃ (6), and five reported phenylethanoid glycosides (7–11) (Figure 1) were isolated from the leaves of *L. robustum*. This article discusses the isolation and structure identification of compounds
1–11 and deals with their inhibitory effects on FAS, α-glucosidase, α-amylase, and their antioxidant activities.

Figure 1. Structures of compounds 1–11 from the leaves of *L. robustum*.

2. Material and Methods

2.1. General Experimental Procedure

Optical rotation value was determined with an AUTOPOL VI automatic polarimeter (Rudolph, Hackettstown, NJ, USA). The UV spectrum was measured on a UV2700 spectrophotometer (Shimadzu, Kyoto, Japan). IR absorption spectrum was carried out with a PerkinElmer Spectrum Two FT-IR spectrometer (PerkinElmer, Waltham, MA, USA). NMR spectra were recorded using an Agilent 600/54 Premium Compact NMR spectrometer (Agilent, Santa Clara, CA, USA) (\(^1\)H at 600 MHz, \(^{13}\)C at 150 MHz) or a Bruker Ascend\(^{TM}\) 400 NMR spectrometer (Bruker, Germany) (\(^1\)H at 400 MHz, \(^{13}\)C at 100 MHz) with CD\(_3\)OD (compound 3: CD\(_3\)OD + DMSO-d\(_6\)) as the solvent at 25 °C. Chemical shifts are reported in...
δ (ppm) with tetramethylsilane (TMS) as the internal standard, while coupling constants (J) are expressed in Hz. High-resolution electrospray ionization mass spectroscopy (HRESIMS) was measured on a Waters Q-TOF Premier mass spectrometer (Waters, Milford, MA, USA).

Column chromatography (CC) was carried out on silica gel (SiO$_2$: 200–300 mesh, Qingdao Ocean Chemical Industry Co., Pingdu, Qingdao, China), polyamide (60–90 mesh, Jiangsu Changfeng Chemical Industry Co., Gulou, Nanjing, China), and MCI-gel CHP-20P (75–150 µm, Mitsubishi Chemical Co., Tokyo, Japan). Preparative HPLC was carried out on a GL3000-300 mL system instrument (Chengdu Gelai Precision Instruments Co., Ltd., Dayi, Chengdu, China) with a UV-3292 detector (detection wavelength 215 nm) and a GL C-18 column (particle size 5 µm, 50 × 450 mm), eluting with MeOH-H$_2$O at 30 mL/min.

TLC was performed on precoated HPTLC Fertigplatten Kieselgel 60 F$_{254}$ plates (Merck, Rahway, NJ, USA), and the spots were visualized by spraying with 10% sulfuric acid ethanolic solution or α-naphthol-sulfuric acid solution and baking at 105 °C for 2–5 min. UV-vis absorbance was determined on a Spark 10M microplate reader (Tecan Trading Co. Ltd., Shanghai, China) or a UV2700 spectrophotometer (Shimadzu, Kyoto, Japan).

Acetyl-coenzyme A (Ac-CoA) and NADPH were purchased from Zeye Biochemical Co., Ltd. (Shanghai, China). Methylmalonyl coenzyme A tetralithium salt hydrate (Mal-CoA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ammonium salt (ABTS) was obtained from Aladdin Industrial Co., Ltd. (Shanghai, China).

2.2. Plant Material

The leaves of *L. robustum* were harvested in April 2017 from Yibin City, Sichuan Province, China, and authenticated by Professor Guo-Min Liu (Kudingcha Research Institute, Hainan University, China). A voucher specimen (No. 201704lsh) was conserved at West China School of Pharmacy, Sichuan University, China.

2.3. Extraction and Isolation

The fresh leaves of *L. robustum* were agitated and baked at 120 °C for 50 min and then smashed. The raw powder (7.0 kg) was extracted with 70% ethanol (28 L × 1) under reflux in a multi-function extractor for 2 h [13]. The ethanol extract was percolated and condensed in vacuo to gain a paste (2.2 kg). The paste was dissolved in 3 L 95% ethanol, and then 3 L purified water was infused to sediment the chlorophyll. After percolation, the filtrate was condensed in vacuo to obtain a residue (1.0 kg). The residue was separated on a silica gel column, eluting with CH$_2$Cl$_2$-MeOH (10:0–0:10), to yield Fr. I (84 g), Fr. II (145 g), Fr. III (93 g), and Fr. IV (70 g). Fr. II was isolated repeatedly by CC on silica gel, eluting with CH$_2$Cl$_2$-MeOH-H$_2$O (200:10:1–80:20:2) or EtOAc-MeOH-H$_2$O (100:4:2–100:6:2), and then separated on polyamide column (EtOH-H$_2$O, 1:9–6:4) and MCI column (MeOH-H$_2$O, 3:7–5:5), and purified finally by preparative HPLC (MeOH-H$_2$O, 40:60–65:35) and silica gel column (EtOAc-MeOH-H$_2$O, 100:4:2–100:6:2), or recrystallized in 70% methanol, to afford 1 (107.4 mg), 4 (11.2 mg), 5 (3.5 mg), 6 (8.3 mg), 7 (14.4 mg), 10 (21.3 mg), and 11 (139.4 mg). Fr. III was separated twice by CC on silica gel column, eluting with CH$_2$Cl$_2$-MeOH-H$_2$O (200:10:1–80:20:2) or EtOAc-MeOH-H$_2$O (100:4:2–100:6:2), and then subjected to polyamide column (EtOH-H$_2$O, 0:10–6:4) and MCI column (MeOH-H$_2$O, 3:7–5:5), and purified at last by preparative HPLC (MeOH-H$_2$O, 30:70–50:50) and silica gel column (EtOAc-MeOH-H$_2$O, 100:10:5), to give 2 (37.3 mg), 3 (22.4 mg), and 9 (13.5 mg).

Compound 1: white amorphous powder. [α]$^2_0$D $-$43.9 (c 0.28, MeOH); UV (MeOH) λ$_{max}$: (log ε) 212 (4.1), 227 (4.2), 317 (4.4) nm; IR (film) ν$_{max}$: 3368, 2930, 1690, 1604, 1515, 1445, 1261, 1039, 829 cm$^{-1}$; $^1$H NMR (CD$_3$OD, 400 MHz) data, see Table 1; $^{13}$C NMR (CD$_3$OD, 400 MHz) data, see Table 2; HRESIMS $m/z$ 761.2634 [M + Na]$^+$ (calculated for C$_{35}$H$_{46}$NaO$_{17}$, 761.2633).
Table 1. $^1$H NMR (400 MHz) data of compounds 1–3 from *L. robustum*.  

| No | 1b | 2a | 2b | 3 |
|----|----|----|----|---|
| 1  | 7.01 d (8.0) | 6.72 d (2.0) | 6.72 d (2.0) | 7.46 br. s |
| 2  | 6.67 d (8.0) | 6.67 d (8.0) | 6.74 d (8.0) | 6.89 d (8.4) |
| 3  | 7.01 d (8.0) | 6.83 dd (8.0, 2.0) | 6.83 dd (8.0, 2.0) | 7.47 br. d (8.4) |
| 4  | 2.83 t (7.2) | 4.75 dd (8.6, 3.2) | 4.75 dd (8.6, 3.2) | 7.48 d (16.8) |
| 5  | 3.72 m | 3.56–3.72 m | 3.56–3.72 m | 3.90–3.98 m |
| 6  | 3.96 m | 3.90–3.98 m | 3.90–3.98 m | 5.26 d (16.8) |
| Glc or Man | | | |
| 1' | 4.28 d (7.6) | 4.41 d (8.0) | 4.43 d (8.0) | 4.54 d (7.6) |
| 2' | 3.31 m | 3.46 m | 3.45 m | 3.52 m |
| 3' | 3.54 m | 3.63 m | 3.80 m | 3.87 m |
| 4' | 3.38 m | 4.95 t (8.6) | 4.90 t (9.6) | 4.96 t (9.6) |
| 5' | 3.53 m | 3.56 m | 3.51 m | 3.61 m |
| 6' | 4.29 dd (11.6, 6.4) | 3.53 m | 3.53 m | 3.54 m |
| 7 | 2.83 t (7.2) | 4.75 dd (9.6, 3.2) | 4.75 dd (9.6, 3.2) | 7.48 d (16.8) |
| 8 | 3.72 m | 3.56–3.72 m | 3.56–3.72 m | 3.90–3.98 m |
| Inner-Rha | | | |
| 1" | 5.17 d (2.0) | 5.22 d (2.0) | 5.21 d (2.0) | 5.22 br. s |
| 2" | 3.89 m | 3.88 dd (3.2, 2.0) | 3.82 dd (3.2, 2.0) | 3.87 m |
| 3" | 3.84 dd (9.6, 3.2) | 3.68 dd (9.2, 3.2) | 3.68 dd (9.2, 3.2) | 3.66 m |
| 4" | 3.53 m | 3.40 m | 3.40 m | 3.40 m |
| 5" | 4.10 m | 3.60 m | 3.60 m | 3.60 m |
| 6" | 1.28 d (6.0) | 1.09 d (6.0) | 1.08 d (6.0) | 1.10 d (6.0) |
| Outer-Rha | | | |
| 1‴ | 5.19 d (1.6) | 5.04 d (2.0) | 5.06 d (2.0) | 5.06 br. s |
| 2‴ | 3.94 m | 3.90 dd (3.2, 2.0) | 3.90 dd (3.2, 2.0) | 3.88 m |
| 3‴ | 3.60 dd (9.6, 3.2) | 3.51 m | 3.51 m | 3.49 m |
| 4‴ | 3.39 m | 3.32 m | 3.32 m | 3.32 m |
| 5‴ | 3.70 m | 3.46 m | 3.46 m | 3.46 m |
| 6‴ | 1.25 d (6.4) | 1.04 d (6.0) | 1.04 d (6.0) | 1.06 d (6.0) |
| Cou | | | |
| 2″″ | 7.62 d (8.4) | 7.49 d (8.8) | 7.72 d (8.8) | 7.54 d (8.4) |
| 3″″ | 6.75 d (8.4) | 6.82 d (8.8) | 6.77 d (8.8) | 6.87 d (8.4) |
| 5″″ | 6.75 d (8.4) | 6.82 d (8.8) | 6.77 d (8.8) | 6.87 d (8.4) |
| 6″″ | 7.62 d (8.4) | 7.49 d (8.8) | 7.72 d (8.8) | 7.54 d (8.4) |
| 7″″ | 6.86 d (12.8) | 7.67 d (16.0) | 6.99 d (12.8) | 7.68 d (16.0) |
| 8″″ | 5.79 d (12.8) | 6.33 d (16.0) | 5.76 d (12.8) | 6.37 d (16.0) |

* a Coupling constants (J values in Hz) are shown in parentheses.  
  b In CD$_3$OD.  
  c In CD$_3$OD + DMSO-d$_6$.

Table 2. $^{13}$C NMR data of compounds 1–3 from *L. robustum*.

| No | 1b | 2a | 2b | 3 |
|----|----|----|----|---|
| 1  | 130.6 | 133.6 | 133.6 | 127.9 |
| 2  | 130.9 | 119.0 | 119.0 | 115.8 |
| 3  | 116.1 | 146.3 | 146.3 | 146.7 |
| 4  | 156.7 | 146.1 | 146.1 | 152.9 |
| 5  | 116.1 | 116.2 | 116.2 | 117.0 |
| 6  | 130.9 | 114.6 | 114.6 | 122.9 |
| 7  | 36.4 | 74.2 | 74.2 | 196.4 |
| 8  | 72.3 | 76.7 | 76.7 | 72.2 |
| Glc or Man | | | | |
| 1' | 104.2 | 104.6 | 104.4 | 103.9 |
| 2' | 75.7 | 76.4 | 76.4 | 76.2 |
| 3' | 83.6 | 81.2 | 81.1 | 81.1 |
| 4' | 70.4 | 70.3 | 70.1 | 70.4 |
| 5' | 75.2 | 76.1 | 75.9 | 76.2 |
| 6' | 64.4 | 62.2 | 62.3 | 62.2 |
| Inner-Rha | | | | |
| 1" | 103.2 | 102.6 | 102.7 | 102.6 |
| 2" | 72.8 | 72.8 | 72.8 | 72.7 |
| 3" | 73.0 | 72.6 | 72.6 | 72.6 |
| 4" | 81.1 | 81.6 | 81.5 | 81.2 |
| 5" | 68.4 | 68.9 | 68.6 | 68.8 |
| 6" | 18.6 | 19.1 | 18.9 | 19.4 |
Table 2. Cont.

| No | Outer-Rha | 1b<sup>a</sup> | 2a<sup>b</sup> | 2b<sup>b</sup> | 3<sup>c</sup> |
|----|-----------|----------------|----------------|----------------|------------|
| 1<sup>″″</sup> | 102.4 | 103.5 | 103.4 | 103.3 |
| 2<sup>″″</sup> | 72.3 | 72.3 | 72.2 | 72.3 |
| 3<sup>″″</sup> | 72.3 | 72.3 | 72.2 | 72.3 |
| 4<sup>″″</sup> | 73.8 | 73.8 | 73.9 | 73.6 |
| 5<sup>″″</sup> | 70.4 | 70.3 | 70.1 | 70.3 |
| 6<sup>″″</sup> | 17.8 | 17.7 | 17.8 | 18.1 |
| Cou | 127.5 | 126.9 | 127.5 | 126.9 |
| 2<sup>″″</sup> | 133.7 | 131.5 | 134.3 | 131.5 |
| 3<sup>″″</sup> | 115.9 | 117.1 | 115.0 | 117.2 |
| 4<sup>″″</sup> | 160.1 | 161.5 | 160.3 | 161.4 |
| 5<sup>″″</sup> | 115.9 | 117.1 | 115.0 | 117.2 |
| 6<sup>″″</sup> | 133.7 | 131.5 | 134.3 | 131.5 |
| 7<sup>″″</sup> | 145.3 | 147.6 | 147.5 | 147.3 |
| 8<sup>″″</sup> | 116.3 | 114.7 | 115.7 | 114.9 |
| CO | 168.1 | 168.1 | 166.8 | 167.6 |

<sup>a</sup> At 100 MHz, in CD<sub>3</sub>OD. <sup>b</sup> At 150 MHz, in CD<sub>3</sub>OD. <sup>c</sup> At 100 MHz, in CD<sub>3</sub>OD + DMSO-d<sub>6</sub>.

Compound 2: white amorphous powder. [α]<sup>23</sup>D = −62.1 (c 0.49, MeOH); UV (MeOH) λ<sub>max</sub> (log ε): 213 (4.1), 226 (4.2), 318 (4.4) nm; IR (film) ν<sub>max</sub>: 3356, 2931, 1693, 1630, 1603, 1515, 1448, 1263, 1040, 982, 834, 803 cm<sup>−1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) data, see Table 1; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 150 MHz) data, see Table 2; HRESIMS m/z 793.2536 [M + Na]<sup>+</sup> (calculated for C<sub>35</sub>H<sub>46</sub>NaO<sub>19</sub>, 793.2531).

Compound 3: yellow amorphous powder. [α]<sup>23</sup>D = −46.0 (c 0.45, MeOH); UV (MeOH) λ<sub>max</sub> (log ε): 213 (4.1), 227 (4.2), 316 (4.4) nm; IR (film) ν<sub>max</sub>: 3402, 1652, 1604, 1048, 1029, 823, 761 cm<sup>−1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD + DMSO-d<sub>6</sub>, 400 MHz) data, see Table 1; <sup>13</sup>C NMR (CD<sub>3</sub>OD + DMSO-d<sub>6</sub>, 100 MHz) data, see Table 2; HRESIMS m/z 791.2371 [M + Na]<sup>+</sup> (calculated for C<sub>35</sub>H<sub>44</sub>NaO<sub>19</sub>, 791.2374).

Compound 4: white amorphous powder. [α]<sup>20</sup>D = −122.4 (c 0.25, MeOH); UV (MeOH) λ<sub>max</sub> (log ε): 210 (3.9), 230 (3.9), 315 (4.4) nm; IR (film) ν<sub>max</sub>: 3360, 2929, 1695, 1603, 1449, 1330, 1259, 1157, 1021, 912, 833, 741, 699 cm<sup>−1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) data, see Table 3; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz) data, see Table 4; HRESIMS m/z 585.1943 [M + Na]<sup>+</sup> (calculated for C<sub>28</sub>H<sub>34</sub>NaO<sub>12</sub>, 585.1948).

Table 3. <sup>1</sup>H NMR data of compounds 4–6 from L. robustum in CD<sub>3</sub>OD<sup>ac</sup>.

| No | 4b<sup>b</sup> | 5<sup>b</sup> | 6<sup>c</sup> |
|----|----------------|----------------|------------|
| 2  | 7.43 br. d (7.2) | 7.39 br. d (7.2) | 7.36 br. d (7.8) |
| 3  | 7.35 br. t (7.2) | 7.30 br. t (7.2) | 7.29 br. t (7.8) |
| 4  | 7.28 br. d (7.2) | 7.26 br. d (7.2) | 7.25 br. d (7.8) |
| 5  | 7.35 br. t (7.2) | 7.30 br. t (7.2) | 7.29 br. t (7.8) |
| 6  | 7.43 br. d (7.2) | 7.39 br. d (7.2) | 7.36 br. d (7.8) |
| 7  | 4.68 d (11.6) | 4.65 d (12.0) | 4.59 d (12.0) |
| 8  | 4.96 d (11.6) | 4.87 d (12.0) | 4.80 d (12.0) |

Glc or Man

| No | 4b<sup>b</sup> | 5<sup>b</sup> | 6<sup>c</sup> |
|----|----------------|----------------|------------|
| 1<sup>″</sup> | 4.42 d (8.0) | 4.38 d (8.0) | 4.33 d (7.8) |
| 2<sup>″</sup> | 3.46 dd (9.2, 8.0) | 3.38 m | 3.37 m |
| 3<sup>″</sup> | 3.76 t (9.2) | 3.52 t (8.8) | 3.49 t (9.0) |
| 4<sup>″</sup> | 4.90 m | 3.43 m | 3.37 m |
| 5<sup>″</sup> | 3.54 m | 3.52 m | 3.49 m |
| 6<sup>″</sup> | 3.56 m | 4.38 dd (12.0, 3.6) | 4.30 dd (12.0, 6.0) |
| 7<sup>″</sup> | 3.64 m | 4.52 dd (12.0, 2.0) | 4.50 dd (12.0, 1.8) |
Table 3. Cont.

| No  | 4b<sup>b</sup> | 5<sup>b</sup> | 6<sup>c</sup> |
|-----|---------------|---------------|---------------|
|     | Rha           |               |               |
| 1<sup>a</sup> | 5.16 d (1.6)  | 5.17 d (2.0)  | 5.15 d (1.8)  |
| 2<sup>a</sup> | 3.92 dd (3.2, 1.6) | 3.94 dd (3.6, 2.0) | 3.93 dd (3.0, 1.8) |
| 3<sup>a</sup> | 3.58 m         | 3.70 dd (9.6, 3.6) | 3.70 dd (9.6, 3.0) |
| 4<sup>a</sup> | 3.29 t (9.6)  | 3.39 m         | 3.39 t (9.6)   |
| 5<sup>a</sup> | 3.56 m         | 4.00 dd (9.6, 6.4) | 3.99 dd (9.6, 6.0) |
| 6<sup>a</sup> | 1.16 d (6.0)  | 1.24 d (6.4)   | 1.24 d (6.0)   |
|     | Cou           |               |               |
| 2<sup>a</sup>' | 7.73 d (8.8)  | 7.46 d (8.4)  | 7.66 d (8.4)  |
| 3<sup>a</sup>' | 6.76 d (8.8)  | 6.79 d (8.4)  | 6.76 d (8.4)  |
| 5<sup>a</sup>' | 6.76 d (8.8)  | 6.79 d (8.4)  | 6.76 d (8.4)  |
| 6<sup>a</sup>' | 7.73 d (8.8)  | 7.46 d (8.4)  | 7.66 d (8.4)  |
| 7<sup>a</sup>' | 6.95 d (12.8) | 7.66 d (16.0) | 6.90 d (13.2) |
| 8<sup>a</sup>' | 5.80 d (12.8) | 6.38 d (16.0) | 5.82 d (13.2) |

<sup>b</sup> Coupling constants (J values in Hz) are shown in parentheses. <sup>a</sup> At 400 MHz. <sup>c</sup> At 600 MHz.

Table 4. 13C NMR data of compounds 4–6 from *L. robustum* in CD3OD.

| No  | 4b<sup>a</sup> | 5<sup>b</sup> | 6<sup>a</sup> |
|-----|---------------|---------------|---------------|
|     | Glc or Man    |               |               |
| 1<sup>a</sup> | 103.2         | 103.1         | 103.1         |
| 2<sup>a</sup> | 76.2          | 75.7          | 75.6          |
| 3<sup>a</sup> | 81.6          | 83.9          | 84.1          |
| 4<sup>a</sup> | 70.6          | 70.4          | 70.5          |
| 5<sup>a</sup> | 76.1          | 75.5          | 75.4          |
| 6<sup>a</sup> | 62.4          | 64.6          | 64.5          |
|     | Rha           |               |               |
| 1<sup>a</sup> | 103.0         | 102.7         | 102.8         |
| 2<sup>a</sup> | 72.3          | 72.3          | 72.4          |
| 3<sup>a</sup> | 72.0          | 72.2          | 72.3          |
| 4<sup>a</sup> | 73.8          | 74.0          | 74.0          |
| 5<sup>a</sup> | 70.4          | 70.0          | 70.0          |
| 6<sup>a</sup> | 18.2          | 17.9          | 17.9          |
|     | Cou           |               |               |
| 1<sup>a</sup>' | 127.5         | 126.7         | 127.4         |
| 2<sup>a</sup>' | 134.2         | 131.3         | 133.8         |
| 3<sup>a</sup>' | 115.8         | 117.1         | 116.1         |
| 4<sup>a</sup>' | 160.4         | 162.2         | 160.6         |
| 5<sup>a</sup>' | 115.8         | 117.1         | 116.1         |
| 6<sup>a</sup>' | 134.2         | 131.3         | 133.8         |
| 7<sup>a</sup>' | 147.3         | 147.0         | 145.3         |
| 8<sup>a</sup>' | 115.8         | 114.5         | 116.1         |
|     | CO            |               |               |
|     | 166.9         | 169.2         | 168.2         |

<sup>a</sup> At 100 MHz. <sup>b</sup> At 150 MHz.

Compound 5: yellowish amorphous powder. [α]<sub>D</sub><sup>20</sup> = −18.5 (c 0.18, MeOH); UV (MeOH) λ<sub>max</sub> (log ε): 210 (3.9), 230 (3.9), 315 (4.4) nm; IR (film) ν<sub>max</sub>: 3369, 2925, 2854, 1706, 1605, 1512, 1452, 1164, 1038, 836, 700 cm<sup>−1</sup>; 1H NMR (CD3OD, 400 MHz) data, see Table 3; 13C NMR (CD3OD, 150 MHz) data, see Table 4; HRESIMS m/z 585.1947 [M + Na]<sup>+</sup> (calculated for C<sub>28</sub>H<sub>34</sub>NaO<sub>12</sub>, 585.1948).
Compound 6: white amorphous powder. [α]D20 −18.5 (c 0.18, MeOH); UV (MeOH) λmax (log ε): 210 (3.9), 230 (3.9), 316 (4.4) nm; IR (film) νmax: 3369, 2925, 2854, 1706, 1605, 1512, 1452, 1164, 1038, 836, 700 cm⁻¹; 1H NMR (CD3OD, 600 MHz) data, see Table 3; 13C NMR (CD3OD, 100 MHz) data, see Table 4; HRESIMS m/z 585.1949 [M + Na]+ (calculated for C25H34NaO12, 585.1948).

2.4. Acid Hydrolysis of Compounds 1–6

Compounds 1–6 (2 mg), dissolved in 0.1 mL MeOH, were injected into 2 mL H2SO4 aqueous solution (1 M) and hydrolyzed at 95 °C for 6 h, respectively. Then, 2 mL Ba(OH)2 solution (1 M) was added. The hydrolyzed solution was filtered and condensed. The monosaccharides in the condensed solution were affirmed by TLC (EtOAc:MeOH-HOAc-H2O, 8:1:1:0.7, 2 developments) with authentic samples [13]. The Rf values of D-mannose, D-glucose, and L-rhamnose were 0.46, 0.43, and 0.73, respectively.

2.5. Enzymatic Hydrolysis of Compounds 2

Compound 2 (20 mg) and cellulase (30 mg) were added to 12 mL HOAc-NaOAc buffer solution (pH 5.0) and kept at 37 °C for 6 h. The hydrolyzed product was extracted with EtOAc and purified on a silica gel column (eluting with EtOAc) to afford (R)-(−)-(3,4-dihydroxyphenyl)ethane-1,2-diol and (S)-(+)-(3,4-dihydroxyphenyl)ethane-1,2-diol (9:11) confirmed by [α]27D +4.8 (c 0.15, EtOAc) [17].

2.6. Determination of Bioactivities

The inhibitory effects on FAS, α-glucosidase and α-amylase, and the DPPH and ABTS radical scavenging activities of compounds 1–11 were determined by the reported methods [12,13,18,19], while orlistat, acarbose, and L-(+)-ascorbic acid were applied as the positive controls, respectively (Supplementary Material S1).

2.7. Statistical Analyses

Statistical analyses were performed on GraphPad Prism 5.01. All samples were determined in triplicate. The IC50 (the ultimate concentration of sample needed to inhibit 50% of enzyme activity or clear away 50% of free radicals) was acquired by plotting the inhibition or scavenging percentage of every sample against its concentration. The results are recorded as mean ± standard deviation (SD). Differences of means between several groups were analyzed by one-way analysis of variance (ANOVA) on the statistical package SPSS 25.0. The differences between groups were deemed to be significant when p < 0.05.

3. Results and Discussion

3.1. Identification of Compounds 1–11

Compound 1 was analyzed as C35H46O17 by HRESIMS (m/z 761.2634 [M + Na]+, calculated 761.2633 for C35H46NaO17). The NMR spectra of 1 showed 2 stereoisomers 1a and 1b (5:1). The 1H and 13C NMR data of 1a (Supplementary Material S2.) was in agreement with those of 2-(4-hydroxyphenyl)ethyl 3-O-[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl]-6-O-(trans-p-coumaroyl)-O-β-D-mannopyranoside (ligurobustoside R) [12]. The NMR data of 1b (Tables 1 and 2) were similar to those of 1a, except the trans-p-coumaroyl [δH 7.62, 6.35 (1H each, d, J = 16.0 Hz, H-7″″, H-8″″)] in 1a was replaced by the cis-p-coumaroyl [δH 6.86, 5.79 (1H each, d, J = 12.8 Hz, H-7″″, H-8″″)] in 1b. The acid hydrolysis experiment of 1 gave D-mannose, and L-rhamnose was affirmed by TLC. The HMBC experiment of 1b (Figure 2) displayed the long-distance correlations: between δH 4.28 (H-1′ of mannosyl) and δC 72.3 (C-8 of aglycone), between δH 5.17 (H-1″ of inner rhamnose) and δC 83.6 (C-3′ of mannosyl), between δH 5.19 (H-1‴ of outer rhamnose) and δC 81.1 (C-4′ of outer rhamnose), and between δH 4.29 (H-6′a of mannosyl), 4.46 (H-6′b of mannosyl) and δC 168.1 (carbonyl of coumaroyl). The 1H and 13C NMR signals of 1b were assigned by the HMBC experiment (Figure S1). So 1b was identified as 2-(4-hydroxyphenyl)ethyl 3-O-[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl]-6-O-(cis-
p-coumaroyl)-O-β-D-mannopyranoside. It is a novel phenylethanoid glycoside named ligurobustoside R1. In conclusion, compound 1 is a mixture of ligurobustosides R and R1.

Figure 2. Key HMBC correlations of compounds 1–6.

Compound 2 was analyzed as C35H46O19 by HRESIMS (m/z 793.2536 [M + Na]+, calculated 793.2531 for C35H46NaO19). The NMR spectra of 2 showed 2 stereoisomers 2a and 2b (10:3). The 1H NMR spectrum of 2a (Table 1) revealed the following signals: (1) a 4-substituted phenyl at δH 6.82, 7.49 (2H each, d, J = 8.8 Hz); (2) a 3,4-disubstituted phenyl at δH 6.72 (1H, d, J = 2.0 Hz), 6.74 (1H, d, J = 8.0 Hz), and 6.83 (1H, dd, J = 8.0, 2.0 Hz); (3) a trans double bond at δH 7.67 and 6.33 (1H each, d, J = 16.0 Hz); (4) three anomeric protons at δH 4.41 (1H, dd, J = 8.0 Hz), 5.04 (1H, d, J = 2.0 Hz), and 5.22 (1H, d, J = 2.0 Hz); (5) a methylene at δH 3.56–3.72 (1H, m) and 3.90–3.98 (1H, m), a methyne at δH 4.75 (1H, dd, J = 9.6, 3.2 Hz), and two methyl groups at δH 1.04 (3H, d, J = 6.0 Hz) and 1.09 (3H, d, J = 6.0 Hz). The
$^{13}$C NMR spectrum of 2a (Table 2) showed a carbonyl at $\delta_C$ 168.1, 2 phenyl groups at $\delta_C$ 114.6–161.5, a double bond at $\delta_C$ 114.7 and 147.6, 3 anomic carbons at $\delta_C$ 102.6–104.6, 13 sugar carbons at $\delta_C$ 62.2–81.6, a methylene at $\delta_C$ 76.7, a methyne at $\delta_C$ 74.2, and 2 methyl groups at $\delta_C$ 17.7 and 19.1. The above $^1$H and $^{13}$C NMR features of 2a were related closely to those of (2R)-2-hydroxy-2-(3,4-dihydroxyphenyl)ethyl 3-O-[a-L-rhamnopyranosyl-(1→4)-a-L-rhamnopyranosyl]-4-O-(trans-cafeoyl)-O-β-D-glucopyranoside (ligurobustoside P) [7], except that the trans-cafeoyl in ligurobustoside P was replaced by the trans-p-coumaroyl in 2a. The acid hydrolysis experiment of 2 gave D-glucose, and L-rhamnose was affirmed by TLC. Furthermore, the HMBC experiment of 2a (Figure 2) displayed the long-distance correlations: between $\delta_H$ 4.41 (H-1′ of glucosyl) and $\delta_C$ 76.7 (C-8 of aglycone), between $\delta_H$ 5.22 (H-1″ of inner rhamnosyl) and $\delta_C$ 81.2 (C-3′ of glucosyl), between $\delta_H$ 5.04 (H-1‴ of outer rhamnosyl) and $\delta_C$ 81.6 (C-4″ of inner rhamnosyl), and between $\delta_H$ 4.95 (H-4′ of glucosyl) and $\delta_C$ 168.1 (carbonyl of coumaroyl). Thus, the plane structure of 2a was elucidated as 2-hydroxy-2-(3,4-dihydroxyphenyl)ethyl 3-O-[a-L-rhamnopyranosyl-(1→4)-a-L-rhamnopyranosyl]-4-O-(trans-p-coumaroyl)-O-β-D-glucopyranoside.

The NMR data of 2b (Tables 1 and 2) were similar to those of 2a, except the trans-p-coumaroyl in 2a was replaced by the cis-p-coumaroyl ($\delta_H$ 6.99, 5.76 (1H each, d, $J = 12.8$ Hz, H-7‴, H-8‴)) in 2b. The HMBC experiment of 2b (Figure 2) displayed the long-distance correlations: between $\delta_H$ 4.43 (H-1′ of glucosyl) and $\delta_C$ 76.7 (C-8 of aglycone), between $\delta_H$ 5.21 (H-1″ of inner rhamnosyl) and $\delta_C$ 81.1 (C-3′ of glucosyl), between $\delta_H$ 5.06 (H-1‴ of outer rhamnosyl) and $\delta_C$ 81.5 (C-4″ of inner rhamnosyl), and between $\delta_H$ 4.90 (H-4′ of glucosyl) and $\delta_C$ 166.8 (carbonyl of coumaroyl). Therefore, the plane structure of 2b was identified as 2-hydroxy-2-(3,4-dihydroxyphenyl)ethyl 3-O-[a-L-rhamnopyranosyl-(1→4)-a-L-rhamnopyranosyl]-4-O-(cis-p-coumaroyl)-O-β-D-glucopyranoside.

In addition, the enzymatic hydrolysis experiment of 2 gave (R)-(−)-L-(3,4-dihydroxyphenyl)ethane-1,2-diol and (S)-(−)-L-(3,4-dihydroxyphenyl)ethane-1,2-diol (9:11), meaning that R/S (9:11) was not equal to 2a/2b (10:3). Based on the above evidence, compound 2 was characterized as a mixture of (2R/S)-2-hydroxy-2-(3,4-dihydroxyphenyl)ethyl 3-O-[a-L-rhamnopyranosyl-(1→4)-a-L-rhamnopyranosyl]-4-O-(trans-p-coumaroyl)-O-β-D-glucopyranoside and (2R/S)-2-hydroxy-2-(3,4-dihydroxyphenyl)ethyl 3-O-[a-L-rhamnopyranosyl-(1→4)-a-L-rhamnopyranosyl]-4-O-(cis-p-coumaroyl)-O-β-D-glucopyranoside. It is a novel phenylethanoid glycoside named ligurobustoside R2-3.

Compound 3 was determined as C$_{35}$H$_{44}$O$_{19}$ by HRESIMS (m/z 791.2371 [M + Na]$^+$, calculated 791.2374 for C$_{35}$H$_{44}$NaO$_{19}$). The $^1$H NMR spectrum of 3 (Table 1) showed the following signals: (1) a 4-substituted phenyl at $\delta_H$ 6.87, 7.54 (2H each, d, $J = 8.4$ Hz); (2) a 3,4-disubstituted phenyl at $\delta_H$ 6.89 (1H, d, $J = 8.4$ Hz), 7.46 (1H, br. s) and 7.47 (1H, br. d, $J = 8.4$ Hz); (3) a trans double bond at $\delta_H$ 7.68 and 6.37 (1H each, d, $J = 16.0$ Hz); (4) three anomic protons at $\delta_H$ 4.54 (1H, d, $J = 7.6$ Hz), 5.06 (1H, br. s) and 5.22 (1H, br. s); (5) a methylene at $\delta_H$ 4.98 and 5.26 (1H each, d, $J = 16.8$ Hz), and two methyl groups at $\delta_H$ 1.06 (3H, d, $J = 6.0$ Hz) and 1.10 (3H, d, $J = 6.0$ Hz). The $^{13}$C NMR spectrum of 3 (Table 2) revealed 2 carbonyl groups at $\delta_C$ 167.6 and 196.4, 2 phenyl groups at $\delta_C$ 115.8–161.4, 1 double bond at $\delta_C$ 114.9 and 147.3, 3 anomic carbons at $\delta_C$ 102.6–103.9, 13 sugar carbons at $\delta_C$ 62.2–81.2, a methylene at $\delta_C$ 72.2, and 2 methyl groups at $\delta_C$ 18.1 and 19.4. The above $^1$H and $^{13}$C NMR characteristics of 3 were similar to those of 2a, except that the methylene (C-7 of aglycone) linking with hydroxy in 2a was replaced by the carbonyl in 3. The acid hydrolysis experiment of 3 afforded D-glucose and L-rhamnose affirmed by TLC. Additionally, the HMBC experiment of 3 (Figure 2) displayed the long-distance correlations: between $\delta_H$ 7.46 (H-2), 7.47 (H-6), 4.98 (H-8a), 5.26 (H-8b) and $\delta_C$ 196.4 (C-7), between $\delta_H$ 4.54 (H-1′ of glucosyl) and $\delta_C$ 167.6 (C-8 of aglycone), between $\delta_H$ 5.22 (H-1‴ of inner rhamnosyl) and $\delta_C$ 81.1 (C-3′ of glucosyl), between $\delta_H$ 5.06 (H-1‴ of outer rhamnosyl) and $\delta_C$ 81.2 (C-4″ of inner rhamnosyl), and between $\delta_H$ 4.96 (H-4′ of glucosyl) and $\delta_C$ 167.6 (carbonyl of coumaroyl). The $^1$H and $^{13}$C NMR signals of 3 were assigned by $^1$H-$^1$H COSY, HSQC, and HMBC experiments (Figure S3). Therefore, compound 3 was determined to be 2-(3,4-
Molecules 2022, 27, 7390
dihydroxyphenyl)-2-oxoethyl 3-O-[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl]-4-O-(trans-p-coumaroyl)-O-β-D-glucopyranoside. It is a novel phenylethanoid glycoside named ligurobustoside R4.

Compound 4 was determined as C28H34O12 by HRESIMS (m/z 585.1943 [M + Na]+, calculated 585.1948 for C28H34NaO12). The NMR spectra of 4 exhibited 2 stereoisomers 4a and 4b (2:1). The 1H and 13C NMR data of 4a (Supplementary Material S2) was in accordance with those of benzyl 3-O-(α-L-rhamnopyranosyl)-4-O-(trans-p-coumaroyl)-O-β-D-mannopyranoside (ligurobustoside S) [12]. The NMR data of 4b (Tables 3 and 4) were very similar to those of 4a, except the trans-p-coumaroyl [δH 7.67, 6.35 (1H each, d, J = 16.0 Hz, H-7″″, H-8″″) in 4a was replaced by the cis-p-coumaroyl [δH 6.95, 5.80 (1H each, d, J = 12.8 Hz, H-7″″, H-8″″)] in 4b. The acid hydrolysis experiment of 4 offered D-mannose and L-rhamnose confirmed by TLC. The HMBC experiment of 4b (Figure 2) displayed the long-distance correlations: between δC 134.3 (C-6 of aglycone), between δC 134.3 and δC 117.1–162.2, a double bond at δC 114.5 and 147.0, two anomic carbons at δC 72.0 and a methyl at δC 17.9. The above 1H and 13C NMR characteristics of 5 were similar to those of benzyl 6-O-[(E)-3-(3,4-dihydroxyphenyl)-prop-2-enoyl]-3-O-(α-L-rhamnopyranosyl)-O-β-D-glucopyranoside (salsaside A) [20], except the trans-coumaroyl in salsaside A was replaced by the trans-p-coumaroyl in 5. The acid hydrolysis experiment of 5 yielded D-glucose and L-rhamnose identified by TLC. The HMBC experiment of 5 (Figure 2) displayed the long-distance correlations: between δH 4.38 (H-1″ of glucosyl) and δC 72.0 (C-7 of aglycone), between δH 5.17 (H-1″ of rhamnosyl) and δC 83.9 (C-3′ of glucosyl), and between δH 4.38 (H-6′a of glucosyl), 4.52 (H-6′b of glucosyl) and δC 169.2 (carbonyl of coumaroyl). The 1H and 13C NMR signals of 5 were assigned by the HMBC experiment (Figure S4). So 4b was identified as benzyl 3-O-(α-L-rhamnopyranosyl)-4-O-(cis-p-coumaroyl)-O-β-D-mannopyranoside. It is a new phenylethanoid glycoside named ligurobustoside S1. In sum, compound 4a is a mixture of ligurobustosides S and S1.

Compound 5 was determined as C28H34O12 by HRESIMS (m/z 585.1947 [M + Na]+, calculated 585.1948 for C28H34NaO12). The 1H NMR spectrum of 5 (Table 3) showed the following signals: (1) a 4-substituted phenyl at δH 6.79, 7.46 (2H each, d, J = 8.4 Hz); (2) a phenyl at δH 7.76 (1H, br. d, J = 7.2 Hz), 7.30 (2H, br. t, J = 7.2 Hz), and 7.39 (2H, br. d, J = 7.2 Hz); (3) a trans double bond at δH 7.66 and 6.38 (1H each, d, J = 16.0 Hz); (4) two anomic protons at δH 4.38 (1H, d, J = 8.0 Hz) and 5.17 (1H, d, J = 2.0 Hz); (5) a methylene at δH 4.65 and 4.87 (1H each, d, J = 12.0 Hz), and a methyl at δH 1.24 (3H, d, J = 6.4 Hz). The 13C NMR spectrum of 5 (Table 4) revealed a carbonyl at δC 169.2, two phenyl groups at δC 117.1–162.2, a double bond at δC 114.5 and 147.0, two anomic carbons at δC 102.7 and 103.1, nine sugar carbons at δC 64.6–83.9, a methylene at δC 72.0, and a methyl at δC 17.9. The above 1H and 13C NMR characteristics of 5 were similar to those of benzyl 6-O-[(E)-3-(3,4-dihydroxyphenyl)-prop-2-enoyl]-3-O-(α-L-rhamnopyranosyl)-O-β-D-glucopyranoside (salsaside A) [20], except the trans-coumaroyl in salsaside A was replaced by the trans-p-coumaroyl in 5. The acid hydrolysis experiment of 5 yielded D-glucose and L-rhamnose identified by TLC. The HMBC experiment of 5 (Figure 2) displayed the long-distance correlations: between δH 4.38 (H-1″ of glucosyl) and δC 72.0 (C-7 of aglycone), between δH 5.17 (H-1″ of rhamnosyl) and δC 83.9 (C-3′ of glucosyl), and between δH 4.38 (H-6′a of glucosyl), 4.52 (H-6′b of glucosyl) and δC 169.2 (carbonyl of coumaroyl). The 1H and 13C NMR signals of 5 were assigned by 1H-1H COSY, HSQC, and HMBC experiments (Figure S5). Therefore, compound 5 was elucidated to be benzyl 3-O-(α-L-rhamnopyranosyl)-6-O-(trans-p-coumaroyl)-O-β-D-glucopyranoside. It is a new phenylethanoid glycoside named ligurobustoside S2.

Compound 6 was analyzed as C28H34O12 by HRESIMS (m/z 585.1949 [M + Na]+, calculated 585.1948 for C28H34NaO12). The 1H and 13C NMR data of 6 (Tables 3 and 4) were related closely to those of 5, except the trans-p-coumaroyl [δH 7.66, 6.38 (1H each, d, J = 16.0 Hz, H-7″″, H-8″″)] in 5 was replaced by the cis-p-coumaroyl [δH 6.90, 5.82 (1H each, d, J = 13.2 Hz, H-7″″, H-8″″)] in 6. The acid hydrolysis experiment of 6 yielded D-glucose and L-rhamnose identified by TLC. The HMBC experiment of 6 (Figure 2) showed the long-distance correlations: between δH 4.33 (H-1″ of glucosyl) and δC 72.0 (C-7 of aglycone), between δH 5.15 (H-1″ of rhamnosyl) and δC 84.1 (C-3′ of glucosyl), and between δH 4.30 (H-6′a of glucosyl), 4.50 (H-6′b of glucosyl) and δC 168.2 (carbonyl of coumaroyl). The 1H and 13C NMR signals of 6 were assigned by 1H-1H COSY, HSQC, and HMBC experiments (Figure S6). Thus, compound 6 was identified as benzyl 3-O-(α-L-rhamnopyranosyl)-6-O-(cis-p-coumaroyl)-O-β-D-glucopyranoside. It is a new phenylethanoid glycoside named ligurobustoside S3.
Compounds 7–11 (1H, 13C NMR data see S2.) were identified as reported ligupurpurside B (7) [21], cis-ligupurpurside B (8) [21,22], ligurobustoside N (9) [1], osmanthuside D (10) [23], and (Z)-osmanthuside B9 (11) [23,24], by comparison with published NMR data and 2D-NMR experiments (1H–1H COSY, HSQC, and HMBC). Compounds 8, 10, and 11 were isolated from this plant for the first time.

3.2. The Bioactivities of Compounds 1–11

Compounds 1–11 from the leaves of *L. robustum* were measured for the inhibitory effects on FAS, α-glucosidase, α-amylase, and antioxidant activities. The results of the bioactivity assays are shown in Table 5. As shown in Table 5, the FAS inhibitory effect of compound 11 (IC₅₀: 4.55 ± 0.35 µM) was as strong as the positive control orlistat (IC₅₀: 4.46 ± 0.13 µM), while the FAS inhibitory effects of compounds 4 (IC₅₀: 6.49 ± 0.27 µM) and 9 (IC₅₀: 5.61 ± 0.44 µM) were weaker than orlistat; the α-glucosidase inhibitory effects of compounds 3 and 5 were moderate and weaker than the positive control acarbose; the α-amylase inhibitory effects of compounds 10 and 11 were moderate and weaker than the positive control acarbose; the DPPH radical scavenging activities of compounds 2, 3, and 9 (IC₅₀: 23.83 ± 0.89–43.17 ± 1.06 µM) were weaker than the positive control L-(+)-ascorbic acid (IC₅₀: 13.66 ± 0.13 µM); the ABTS radical scavenging activities of compounds 3, 5, and 7–11 (IC₅₀: 2.68 ± 0.05–4.86 ± 0.06 µM) were stronger than the positive control L-(+)-ascorbic acid (IC₅₀: 10.06 ± 0.19 µM).

Table 5. Results of the bioactivity assays of compounds 1–11 from *L. robustum*.

| Compounds       | FAS IC₅₀ (µM) b | α-Glucosidase Inhibition at 0.1 mM (%) | α-Amylase Inhibition at 0.1 mM (%) | DPPH IC₅₀ (µM) b | ABTS*+ IC₅₀ (µM) b |
|-----------------|----------------|----------------------------------------|-----------------------------------|-----------------|-------------------|
| 1               | NA c           | — d                                    | —                                 | —               | —                 |
| 2               | NA             | 42.3 ± 8.7 bc                          | 10.6 ± 2.3 f                      | 43.17 ± 1.06 d  | 10.62 ± 0.48 f    |
| 3               | NA             | 45.1 ± 2.5 b                           | NA                                | 23.83 ± 0.89 b  | 4.13 ± 0.06 c     |
| 4               | 6.49 ± 0.27 c  | —                                      | —                                 | —               | —                 |
| 5               | NA             | 36.5 ± 1.5 c                           | NA                                | >250            | 4.86 ± 0.06 d     |
| 6               | NA             | 19.9 ± 1.8 d                           | NA                                | >250            | 2.73 ± 0.09 a     |
| 7               | NA             | 25.4 ± 4.1 d                           | NA                                | >250            | 4.17 ± 0.06 c     |
| 9               | 5.61 ± 0.44 b  | 26.1 ± 1.9 c                           | 15.9 ± 3.1 e                      | 29.21 ± 0.37 c  | 2.68 ± 0.05 a     |
| 10              | NA             | 23.5 ± 1.7 c                           | NA                                | >250            | 3.34 ± 0.02 b     |
| 11              | 4.55 ± 0.35 a  | 93.2 ± 0.1 a                           | 51.8 ± 2.5 a                      | 13.66 ± 0.13 a  | 10.06 ± 0.19 e    |

**Data** are recorded as mean ± SD (n = 3). Means with the same letter are not significantly different (one-way analysis of variance, α = 0.05). b IC₅₀: the ultimate concentration of sample needed to inhibit 50% of enzyme activity or clear away 50% of free radicals. c NA: no activity. d It was not measured. e Positive control.

The previous study revealed that FAS was a potential therapeutic target for anti-obesity drugs [13,18]; α-glucosidase and α-amylase were two important targets to prevent diabetes and obesity [12,25]; and reactive oxygen species played an important role in the initiation and progression of diabetes [12,26]. Consequently, antioxidants 3–5, 9, and 10, with some FAS, α-glucosidase, and α-amylase inhibitory activities [12], might be a part of the effective ingredients for *L. robustum* to prevent diabetes and obesity.

4. Conclusions

In summary, the phytochemical investigation on the leaves of *L. robustum* resulted in the isolation of eight phenylethanoid glycosides (1–3, 7–11) and three phenylmethanoid glycosides (4–6), including six novel compounds (1b,2,3,4b,5,6) identified with spectroscopic method (1H, 13C NMR, 1H–1H COSY, HSQC, HMBC, HRESIMS), and physical and chemical methods. The biological assays showed that the FAS inhibitory effect of compound 11 (IC₅₀: 4.55 ± 0.35 µM) was as strong as the positive control orlistat (IC₅₀: 4.46 ± 0.13 µM); the α-glucosidase inhibitory effects of compounds 3 and 5, and the α-amylase inhibitory effects of compounds 10 and 11 were moderate; the DPPH radical scavenging activities of
compounds 2, 3, and 9 (IC50: 23.83 ± 0.89–43.17 ± 1.06 μM) were weaker than the positive control L-(+)-ascorbic acid (IC50: 13.66 ± 0.13 μM); the ABTS radical scavenging activities of compounds 3, 5, and 7–11 (IC50: 2.68 ± 0.05–4.86 ± 0.06 μM) were stronger than the positive control L-(+)-ascorbic acid (IC50: 10.06 ± 0.19 μM). Together this work and previous studies [12,13], phenylethanoid, phenylmethanoid, and monoterpenoid glycosides were believed as the main anti-obesity and anti-diabetes components of L. robustum. This research offered a theoretical basis for the leaves of L. robustum as a functional tea to prevent obesity and diabetes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27217390/s1, 1H NMR, 13C NMR, HMBC, HRES-IMS, and IR spectra of compounds 1 (Figure S1) and 4 (Figure S4); 1H NMR, 13C NMR, 1H-1H COSY, HSQC, HMBC, HRESIMS and IR spectra of compounds 2 (Figure S2), 3 (Figure S3), 5 (Figure S5), and 6 (Figure S6); determination of bioactivities (S1.); 1H NMR and 13C NMR data of 1a, 4a, and 7–11 (S2.).

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Sample Availability: Samples of the compounds are not available from the authors.

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