Monoterpenoid Glycosides from the Leaves of Ligustrum robustum and Their Bioactivities

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Abstract: The leaves of Ligustrum robustum have been applied as Ku-Ding-Cha, a functional tea to clear heat, remove toxins, and treat obesity and diabetes, in Southwest China. The phytochemical research on the leaves of L. robustum led to the isolation and identification of eight new monoterpenoid glycosides (1–8) and three known monoterpenoid glycosides (9–11). Compounds 1–11 were tested for the inhibitory activities on fatty acid synthase (FAS), a-glucosidase, a-amylase, and the antioxidant effects. Compound 2 showed stronger FAS inhibitory activity (IC50: 2.36 ± 0.10 μM) than the positive control orlistat (IC50: 4.46 ± 0.13 μM), while compounds 1, 2, 5 and 11 displayed more potent ABTS radical scavenging activity (IC50: 6.91 ± 0.10–9.41 ± 0.22 μM) than the positive control L-(+)-ascorbic acid (IC50: 10.06 ± 0.19 μM). This study provided a theoretical basis for the leaves of L. robustum as a functional tea to treat obesity.

Keywords: Ligustrum robustum; monoterpenoid glycoside; FAS; a-glucosidase; antioxidant; anti-obesity; hypoglycemic

1. Introduction

Ku-Ding-Cha has been used widely as a functional tea to clear heat, remove toxins, and treat obesity, diabetes and so on, in Southwest China for a long time [1,2]. It was produced from the leaves of more than 30 plants from 13 genera in 12 families, in which the most common categories were from the genus Ligustrum (Oleaceae) and the genus Ilex (Aquifoliaceae) [3]. Ligustrum robustum (Roxb.) Blume, distributed widely in Southwest China, India, Burma, Vietnam and Cambodia, has been consumed as Ku-Ding-Cha in Southwest China, especially in Guizhou Province [4]. L. robustum has been classified as a food by the Chinese Ministry of Health since 2011 [5]. In the past two decades, the phytochemical studies on L. robustum led to the isolation and identification of monoterpenoid glycosides, phenylethanoid glycosides, iridoid glycosides, flavonoid glycosides and triterpenoids [1,6–11]. The biological research on L. robustum reported the anti-obesity activity of the total glycosides and the aqueous extract [2,5], the antioxidative, anti-inflammatory and hepatoprotective effects of the aqueous extract [4], and the antioxidant effect of some constituents [1,10]. In our previous study on L. robustum [12], some antioxidative and a-glucosidase inhibitory components, which might be a part of anti-diabetic ingredients of L. robustum [13–16], were discovered. However, to the best of our knowledge, the exact anti-obesity ingredients of L. robustum and their mechanisms are still unclear so far.

Studies revealed that fatty acid synthase (FAS) catalyzed the synthesis of saturated long-chain fatty acids from acetyl-coenzyme A, malonyl-CoA and NADPH; FAS expressed...
Studies revealed that fatty acid synthase (FAS) catalyzes the synthesis of saturated long-chain fatty acids from acetyl-coenzyme A, malonyl-CoA and NADPH; FAS expression is high in normal adipose, liver tissues, lactating mammary glands, and in patient tumor tissues at later stages of disease, while most normal tissues showed low levels of FAS expression [17–19]. Thus, FAS is a potential therapeutic target for anti-obesity and anti-cancer drugs. There have been no reports on the screening FAS inhibitors from the constituents of L. robustum. In this work, eight new monoterpenoid glycosides, named ligurobustosides T (1), T_1 (2), T_2 (3), T_{3,4} (4), T_5 (5), T_6 (6), T_7 (7), T_{8,9} (8), and three known monoterpenoid glycosides (9–11) (Figure 1) were isolated from the leaves of L. robustum. This paper deals with the isolation and structure elucidation of 1–11, and it describes their inhibitory activities on FAS, α-glucosidase, α-amylase, and their antioxidant effects.

Figure 1. Structures of compounds 1–11 from the leaves of L. robustum.
2. Material and Methods

2.1. General Experimental Procedure

First, 1D and 2D NMR spectra were measured on a Bruker Ascend™ 400 NMR spectrometer (Bruker, Germany) (¹H at 400 MHz, ¹³C at 100 MHz) or an Agilent 600/54 Premium Compact NMR spectrometer (Agilent, Santa Clara, CA, USA) (¹H at 600 MHz, ¹³C at 150 MHz) with CD3OD as the solvent at 25 °C. Chemical shifts are expressed in δ (ppm) with tetramethylsilane (TMS) as the internal standard, and coupling constants (J) are reported in Hz. High-resolution electrospray ionization mass spectroscopy (HRESIMS) was carried out on a Waters Q-TOF Premier mass spectrometer (Waters, Milford, MA, USA). The IR absorption spectrum was measured with a PerkinElmer Spectrum Two FT-IR spectrometer (PerkinElmer, Waltham, MA, USA). UV spectrum was recorded using a UV2700 spectrophotometer (Shimadzu, Kyoto, Japan). Optical rotation value was analyzed with an AUTOPOL VI automatic polarimeter (Rudolph, Hackettstown, NJ, USA).

UV-vis absorbance was analyzed with a Spark 10M microplate reader (Tecan Trading Co. Ltd., Shanghai, China). Preparative HPLC was performed on a GL3000-300 mL system instrument (Chengdu Gelai Precision Instruments Co., Ltd., Chengdu, China) with a GL C-18 column (particle size 5 µm, 50 × 450 mm) and a UV-3292 detector operating at 215 nm, eluting with MeOH-H2O at a flow rate of 30 mL/min. Column chromatography (CC) was performed on silica gel (SiO2: 200–300 mesh, Qingdao Ocean Chemical Industry Co., Qingdao, China), MCI-gel CHP-20P (75–150 µm, Mitsubishi Chemical Co., Tokyo, Japan), and polyamide (60–90 mesh, Jiangsu Changfeng Chemical Industry Co., Yangzhou, China). TLC was carried out on precoated HPTLC Fertigplatten Kieselgel 60 F254 plates (Merck), and the spots were visualized by spraying with α-naphthol-sulfuric acid solution or 10% sulfuric acid ethanolic solution and heating at 105 °C for 2–5 min. NADPH and acetyl-coenzyme A (Ac-CoA) were obtained from Zeye Biochemical Co., Ltd. (Shanghai, China). Methylmalonyl coenzyme A tetralithium salt hydrate (Mal-CoA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). 2,2’-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ammonium salt (ABTS) was purchased from Aladdin Industrial Co., Ltd. (Shanghai, China).

2.2. Plant Material

The leaves of L. robustum were collected from Yibin City, Sichuan Province, China, in April 2017, and identified by Professor Guo-Min Liu (Kudingcha Research Institute, Hainan University, Haikou, 570228, China). A voucher specimen (No. 201704lsh) was deposited in West China School of Pharmacy, Sichuan University, China.

2.3. Extraction and Isolation

The fresh leaves of L. robustum were stirred and dried at 120 °C for 50 min and then powdered. The dried raw powder (7.0 kg) was extracted under reflux with 70% ethanol (28 L × 1) in a multi-function extractor for 2 h. The ethanol extract was filtrated and concentrated in vacuo to obtain a dark brown paste (2.2 kg). The paste was dissolved in 95% ethanol (3 L), and then, the distilled water (3 L) was added to precipitate the chlorophyll. After filtration, the filtrate was concentrated in vacuo to gain a brown residue (1.0 kg). The residue was chromatographed on silica gel column, eluting with CH2Cl2-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 separated repeatedly by CC on silica gel, eluting with CH2Cl2-MeOH-H2O (200:10:1–40:10:1) or EtOAc-MeOH-H2O (50:2:1–50:3:1), and then subjected to polyamide column (MeOH-H2O, 3:7–7:3) and MCI column (MeOH-H2O, 3:7–7:3), and purified finally by preparative HPLC (MeOH-H2O, 40:60–65:35) or silica gel column (EtOAc-MeOH-H2O, 50:2:1–50:3:1), to yield 1 (48.5 mg), 2 (49.2 mg), 3 (11.8 mg), 4 (15.3 mg), 5 (8.2 mg), 6 (17.6 mg), 7 (8.2 mg), 8 (10.7 mg), 9 (20.0 mg), 10 (135.8 mg) and 11 (38.6 mg).

Compound 1: yellowish amorphous powder. [α]D20-91.9 (c 0.27, MeOH); UV (MeOH) λmax: (log ε) 214 (4.2), 244 (4.1), 331 (4.4) nm; IR (film) νmax: 3375, 2923, 1694, 1630, 1601,
1515, 1446, 1376, 1261, 1025, 928, 836, 811 cm$^{-1}$; $^1$H NMR (CD$_3$OD, 400 MHz) data, see Table 1; $^{13}$C NMR (CD$_3$OD, 100 MHz) data, see Table 2; HRESIMS $m/z$ 647.2679 [M + Na]$^+$ (calculated for C$_{31}$H$_{44}$NaO$_{13}$, 647.2680).

Compound 2: white amorphous powder. [$\alpha$]$^D$ -29.9 (c 0.98, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$): 209 (3.9), 230 (3.9), 314 (4.4) nm; IR (film) $\nu_{\text{max}}$: 3375, 2927, 1689, 1632, 1604, 1515, 1445, 1263, 1169, 1037, 832 cm$^{-1}$; $^1$H NMR (CD$_3$OD, 400 MHz) data, see Table 1; $^{13}$C NMR (CD$_3$OD, 100 MHz) data, see Table 2; HRESIMS $m/z$ 631.2728 [M + Na]$^+$ (calculated for C$_{31}$H$_{44}$NaO$_{12}$, 631.2730).

Compound 3: white amorphous powder. [$\alpha$]$^D$ -11.0 (c 0.47, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$): 208 (3.9), 230 (3.9), 316 (4.4) nm; IR (film) $\nu_{\text{max}}$: 3370, 2926, 2855, 1696, 1605, 1514, 1448, 1262, 1169, 1036, 833 cm$^{-1}$; $^1$H NMR (CD$_3$OD, 600 MHz) data, see Table 1; $^{13}$C NMR (CD$_3$OD, 100 MHz) data, see Table 2; HRESIMS $m/z$ 647.2680 [M + Na]$^+$ (calculated for C$_{31}$H$_{44}$NaO$_{13}$, 647.2680).

Table 1. $^1$H NMR data of compounds 1–8 from L. robustum in CD$_3$OD.$^a$

| No  | 1$^b$   | 2$^b$   | 3$^c$   | 4a$^b$   | 4b$^b$   |
|-----|--------|--------|--------|---------|---------|
| 1   | 5.22 dd (10.8, 1.2) | 5.19 dd (10.8, 1.2) | 5.19 br. d (10.8) | 5.19 dd (10.8, 2.0) | 5.19 dd (10.8, 2.0) |
| 2   | 5.26 dd (17.6, 1.2) | 5.23 dd (18.0, 1.2) | 5.24 br. d (18.0) | 5.24 dd (18.0, 2.0) | 5.24 dd (18.0, 2.0) |
| 4   | 1.58 m   | 1.56 m   | 1.22 dd (10.8, 2.4) | 1.57 m   | 1.57 m   |
| 5   | 1.62 m   | 1.60 m   | 1.0 m    | 1.90 m   | 1.90 m   |
| 6   | 2.04 m   | 2.02 m   | 1.60 m   | 1.32 m   | 1.32 m   |
| 8   | 1.67 s   | 1.62 br. s | 4.78 br. s | 1.11 s   | 1.11 s   |
| 9   | 1.60 s   | 1.56 br. s | 1.14 s   | 1.14 s   |
| 10  | 1.39 s   | 1.34 s   | 1.36 s   | 1.36 s   |

7-OCH$_3$

Glc$^a$

| 1$'$ | 4.43 d (8.0) | 4.39 d (8.0) | 4.38 d (8.4) | 4.41 d (8.0) | 4.36 d (8.0) |
| 2$'$ | 3.36 m   | 3.29 m   | 3.29 m   | 3.29 m   | 3.27 m   |
| 3$'$ | 3.77 t (9.2) | 3.49 m   | 3.48 m   | 3.50 t (8.8) | 3.46 t (8.8) |
| 4$'$ | 4.89 m   | 3.34 m   | 3.32 m   | 3.33 m   | 3.29 m   |
| 5$'$ | 3.45 m   | 3.49 m   | 3.48 m   | 3.47 m   | 3.42 m   |
| 6$'$ | 3.49 m   | 4.30 dd (12.0, 6.8) | 4.30 dd (12.0, 6.6) | 4.30 dd (12.0, 6.0) | 4.25 dd (12.0, 6.0) |
| 7$'$ | 3.57 m   | 4.45 dd (12.0, 2.4) | 4.45 dd (12.0, 2.4) | 4.45 dd (12.0, 2.4) | 4.40 dd (12.0, 2.4) |

inner- Rha$^a$

| 1$''$ | 5.18 d (1.6) | 5.17 d (2.0) | 5.17 d (1.8) | 5.18 d (2.0) | 5.15 d (2.0) |
| 2$''$ | 3.91 dd (3.2, 1.6) | 3.94 dd (3.2, 2.0) | 3.94 m   | 3.94 dd (3.2, 2.0) | 3.94 dd (3.2, 2.0) |
| 3$''$ | 3.58 m   | 3.70 dd (9.6, 3.2) | 3.70 dd (9.6, 3.6) | 3.70 dd (9.6, 3.2) | 3.70 dd (9.6, 3.2) |
| 4$''$ | 3.29 t (9.6) | 3.40 t (9.6) | 3.39 t (9.6) | 3.39 t (9.6) | 3.39 t (9.6) |
| 5$''$ | 3.56 m   | 4.00 dd (9.6, 6.4) | 4.00 m   | 3.99 dd (9.6, 6.4) | 3.99 dd (9.6, 6.4) |
| 6$''$ | 1.08 d (6.4) | 1.25 d (6.4) | 1.24 d (6.6) | 1.25 d (6.4) | 1.24 d (6.4) |

outer- Rha$^a$

| 1$'''$ | 3.64 m   | 3.58 m   | 3.39 m   | 3.38 m   | 3.36 m   |
| 2$'''$ | 3.18 m   | 3.20 m   | 3.09 m   | 3.09 m   | 3.07 m   |
| 3$'''$ | 3.12 m   | 3.14 m   | 3.14 m   | 3.14 m   | 3.14 m   |
| 4$'''$ | 3.06 m   | 3.08 m   | 3.08 m   | 3.08 m   | 3.08 m   |
| 5$'''$ | 3.02 m   | 3.04 m   | 3.04 m   | 3.04 m   | 3.04 m   |
| 6$'''$ | 2.99 m   | 3.01 m   | 3.01 m   | 3.01 m   | 3.01 m   |
### Table 1. Cont.

| No     | Ester 2′′′′ | 3′′′′ | 4a′′ | 4b′′ |
|--------|------------|-------|------|------|
| 1      | 7.05 d (2.0) | 7.45 d (8.8) | 7.46 d (8.4) | 7.46 d (8.8) |
| 2      | 6.81 d (8.8) | 6.80 d (8.4) | 6.81 d (8.8) | 6.76 d (8.8) |
| 3      | 6.77 d (8.0) | 6.80 d (8.4) | 6.81 d (8.8) | 6.76 d (8.8) |
| 4      | 6.95 dd (8.0, 2.0) | 7.45 d (8.8) | 7.46 d (8.4) | 7.46 d (8.8) |
| 5      | 7.58 d (16.0) | 7.64 d (16.2) | 7.64 d (16.0) | 6.87 d (12.8) |
| 6      | 6.27 d (16.0) | 6.33 d (16.0) | 6.33 d (16.2) | 6.34 d (16.0) |

| No     | Glc 1′′ | 2′′ | 3′′ | 4′′ | 5′′ |
|--------|--------|-----|-----|-----|-----|
| 1      | 4.41 d (8.0) | 4.44 d (7.6) | 4.41 d (8.0) | 4.31 d (8.0) | 4.27 d (8.0) |
| 2      | 3.31 m | 3.37 m | 3.37 m | 3.33 m | 3.28 m |
| 3      | 3.50 t (8.8) | 3.77 t (9.6) | 3.77 t (9.6) | 3.51 m | 3.46 m |
| 4      | 3.35 m | 4.91 t (9.6) | 4.86 t (9.6) | 3.37 m | 3.33 m |
| 5      | 3.49 m | 3.46 m | 3.46 m | 3.51 m | 3.47 m |
| 6      | 4.32 dd (12.0, 7.2) | 3.50 m | 3.50 m | 4.35 dd (12.0, 6.0) | 4.31 dd (12.0, 6.0) |

| No     | inner- Rha 1′′ | 2′′ | 3′′ | 4′′ | 5′′ |
|--------|----------------|-----|-----|-----|-----|
| 1      | 5.17 d (2.0) | 5.19 d (2.0) | 5.29 d (2.0) | 5.17 d (2.0) | 5.16 d (2.0) |
| 2      | 3.94 dd (3.6, 2.0) | 3.86 dd (3.2, 2.0) | 3.82 dd (3.2, 2.0) | 3.94 m | 3.92 m |
| 3      | 3.70 dd (9.6, 3.6) | 3.68 dd (9.6, 3.2) | 3.68 dd (9.6, 3.2) | 3.70 dd (9.6, 3.2) | 3.68 dd (9.6, 3.2) |
| 4      | 3.40 t (9.6) | 3.39 m | 3.45 m | 3.40 m | 3.40 m |
| 5      | 4.00 dd (9.6, 6.4) | 3.59 m | 3.60 m | 4.00 dd (9.6, 6.4) | 4.00 dd (9.6, 6.4) |
| 6      | 1.25 d (6.4) | 1.08 d (6.0) | 1.21 d (6.4) | 1.24 d (6.4) | 1.23 d (6.4) |

| No     | outer- Rha 1′′′′ | 2′′′′ | 3′′′′ | 4′′′′ | 5′′′′ |
|--------|-----------------|------|------|------|------|
| 1      | 5.04 d (2.0) | 5.13 d (2.0) | 5.13 d (2.0) | 5.13 d (2.0) | 5.13 d (2.0) |
| 2      | 3.90 dd (3.2, 2.0) | 3.82 dd (3.2, 2.0) | 3.82 dd (3.2, 2.0) | 3.82 dd (3.2, 2.0) | 3.82 dd (3.2, 2.0) |
| 3      | 3.51 m | 3.51 m | 3.51 m | 3.51 m | 3.51 m |
| 4      | 3.32 m | 3.34 m | 3.34 m | 3.34 m | 3.34 m |
| 5      | 3.46 m | 3.46 m | 3.46 m | 3.46 m | 3.46 m |
| 6      | 1.04 d (6.0) | 1.21 d (6.4) | 1.21 d (6.4) | 1.21 d (6.4) | 1.21 d (6.4) |

| Ester 2′′′′ | 3′′′′ | 4′′′′ | 5′′′′ | 6′′′′ |
|-----------|------|------|------|------|
| 2′′′′ | 7.45 d (8.4) | 7.48 d (8.4) | 7.72 d (8.4) | 7.45 d (8.4) | 7.65 d (8.4) |
| 3′′′′ | 6.81 d (8.4) | 6.82 d (8.4) | 6.77 d (8.4) | 6.80 d (8.4) | 6.75 d (8.4) |
| 4′′′′ | 6.81 d (8.4) | 6.82 d (8.4) | 6.77 d (8.4) | 6.80 d (8.4) | 6.75 d (8.4) |
| 5′′′′ | 7.45 d (8.4) | 7.48 d (8.4) | 7.72 d (8.4) | 7.45 d (8.4) | 7.65 d (8.4) |
| 6′′′′ | 7.63 d (16.0) | 7.66 d (16.0) | 6.98 d (12.8) | 7.64 d (16.0) | 6.87 d (12.8) |
| 7′′′′ | 6.32 d (16.0) | 6.33 d (16.0) | 5.76 d (12.8) | 6.35 d (16.0) | 5.79 d (12.8) |

*a Coupling constants (J values in Hz) are shown in parentheses. b At 400 MHz. c At 600 MHz.*
Table 2. $^{13}$C NMR data of compounds 1–8 from L. robustum in CD$_3$OD.

| No | 1$^a$ | 2$^a$ | 3$^a$ | 4a$^a$ | 4b$^a$ |
|----|-------|-------|-------|-------|-------|
| 1  | 115.9 | 115.7 | 115.8 | 115.9 | 115.9 |
| 2  | 144.3 | 144.3 | 144.3 | 144.3 | 144.3 |
| 3  | 81.6  | 81.5  | 81.4  | 81.5  | 81.5  |
| 4  | 42.6  | 42.5  | 30.2  | 39.9  | 39.9  |
| 5  | 23.6  | 23.6  | 30.1  | 26.4  | 26.4  |
| 6  | 125.7 | 125.7 | 76.9  | 80.1  | 80.1  |
| 7  | 132.2 | 132.1 | 148.7 | 73.9  | 73.9  |
| 8  | 25.9  | 25.8  | 111.4 | 24.9  | 24.9  |
| 9  | 17.7  | 17.7  | 17.7  | 25.8  | 25.8  |
| 10 | 23.2  | 23.5  | 23.5  | 23.9  | 23.9  |

7-OCH$_3$

| Glc | 1$^b$ | 2$^b$ | 3$^b$ | 4$^b$ | 5$^b$ | 6$^b$ |
|-----|------|------|------|------|------|------|
| 1$'$| 99.4 | 99.3 | 99.4 | 99.4 | 99.3 | 99.3 |
| 2$'$| 76.3 | 75.7 | 75.8 | 75.8 | 75.8 | 75.8 |
| 3$'$| 82.0 | 84.4 | 84.4 | 84.2 | 84.2 | 84.2 |
| 4$'$| 70.7 | 70.8 | 70.8 | 70.7 | 70.7 | 70.7 |
| 5$'$| 75.7 | 75.0 | 75.1 | 75.1 | 75.0 | 75.0 |
| 6$'$| 62.5 | 65.0 | 64.9 | 64.9 | 62.7 | 62.7 |

inner-Rha

| 1$''$| 103.1 | 102.8 | 102.8 | 102.7 | 102.4 | 102.4 |
| 2$''$| 72.4  | 72.4  | 72.4  | 72.4  | 72.4  | 72.4  |
| 3$''$| 72.0  | 72.3  | 72.3  | 72.3  | 72.3  | 72.3  |
| 4$''$| 73.8  | 74.0  | 74.0  | 74.0  | 74.0  | 74.0  |
| 5$''$| 70.4  | 70.0  | 70.0  | 70.0  | 70.0  | 70.0  |
| 6$''$| 18.5  | 17.9  | 17.9  | 17.9  | 17.9  | 17.9  |

outer-Rha

| 1$'''$| 127.6 | 127.1 | 126.9 | 127.1 | 127.6 | 127.6 |
| 2$'''$| 115.2 | 131.1 | 131.2 | 131.2 | 133.8 | 133.8 |
| 3$'''$| 146.8 | 116.9 | 117.0 | 116.9 | 115.9 | 115.9 |
| 4$'''$| 149.8 | 161.5 | 161.9 | 161.9 | 160.2 | 160.2 |
| 5$'''$| 116.5 | 116.9 | 117.0 | 116.9 | 115.9 | 115.9 |
| 6$'''$| 123.2 | 131.1 | 131.2 | 131.2 | 133.8 | 133.8 |
| 7$'''$| 148.0 | 146.7 | 148.7 | 146.8 | 145.3 | 145.3 |
| 8$'''$| 114.7 | 115.0 | 114.8 | 115.0 | 116.2 | 116.2 |
| CO  | 168.3 | 169.0 | 169.0 | 169.0 | 168.1 | 168.1 |

| No | 5$^a$ | 6$^b$ | 7$^b$ | 8a$^a$ | 8b$^a$ |
|----|------|------|------|-------|-------|
| 1  | 116.0 | 115.9 | 115.9 | 66.3  | 66.3  |
| 2  | 144.0 | 144.3 | 144.3 | 122.3 | 122.3 |
| 3  | 81.2  | 81.6  | 81.6  | 140.9 | 140.9 |
| 4  | 45.5  | 42.6  | 42.6  | 43.5  | 43.5  |
| 5  | 127.4 | 23.6  | 23.7  | 129.3 | 129.3 |
| 6  | 139.2 | 125.7 | 125.7 | 138.1 | 138.1 |
| 7  | 76.5  | 132.2 | 132.2 | 76.4  | 76.4  |
| 8  | 26.1  | 25.9  | 25.9  | 26.2  | 26.2  |
| 9  | 26.1  | 17.7  | 17.7  | 26.2  | 26.2  |
| 10 | 23.5  | 23.2  | 23.1  | 16.6  | 16.6  |
Table 2. Cont.

| No  | 5<sup>a</sup> | 6<sup>b</sup> | 7<sup>b</sup> | 8a<sup>a</sup> | 8b<sup>a</sup> |
|-----|-------------|-------------|-------------|-------------|-------------|
| 7-OCH<sub>3</sub> | 50.7 | 50.6 | 50.6 |  |
| Glc | 99.3 | 99.4 | 99.4 | 102.6 | 102.6 |
| 1'  | 75.8 | 76.3 | 76.5 | 75.6 | 75.6 |
| 2'  | 84.2 | 81.9 | 79.8 | 84.0 | 84.0 |
| 3'  | 70.8 | 70.6 | 70.4 | 70.5 | 70.4 |
| 4'  | 75.0 | 75.7 | 75.6 | 75.5 | 75.4 |
| 5'  | 64.9 | 62.4 | 62.5 | 64.7 | 64.5 |
| inner-Rha | 102.8 | 102.7 | 101.9 | 102.7 | 102.8 |
| 2'' | 72.4 | 72.7 | 72.9 | 72.4 | 72.4 |
| 3'' | 72.3 | 72.9 | 73.0 | 72.2 | 72.2 |
| 4'' | 74.0 | 81.7 | 80.6 | 74.0 | 74.0 |
| 5'' | 70.0 | 68.9 | 68.6 | 70.0 | 70.3 |
| 6'' | 17.9 | 19.2 | 18.9 | 17.9 | 17.9 |
| outer-Rha | 103.5 | 103.2 |  |
| 2''' | 72.3 | 73.2 | 72.3 |  |
| 3''' | 72.3 | 73.2 | 72.3 |  |
| 4''' | 73.8 | 73.9 | 73.9 |  |
| 5''' | 70.3 | 70.3 | 70.3 |  |
| 6''' | 17.7 | 17.8 | 17.8 |  |
| Ester | 127.1 | 127.0 | 127.5 | 126.9 | 127.5 |
| 2'''' | 131.2 | 131.4 | 134.3 | 131.2 | 133.8 |
| 3'''' | 116.9 | 117.0 | 116.0 | 117.0 | 116.0 |
| 4'''' | 161.4 | 161.5 | 160.3 | 161.7 | 160.3 |
| 5'''' | 116.9 | 117.0 | 116.0 | 117.0 | 116.0 |
| 6'''' | 131.2 | 131.4 | 134.3 | 131.2 | 133.8 |
| 7'''' | 146.7 | 147.5 | 147.4 | 146.9 | 145.3 |
| 8'''' | 115.0 | 114.8 | 115.8 | 114.8 | 116.2 |
| CO  | 168.9 | 168.2 | 166.9 | 169.1 | 168.1 |

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

Compound 4: white amorphous powder. [α]<sup>23</sup> D-20.9 (c 0.31, MeOH); UV (MeOH) λ<sub>max</sub> (log ε): 208 (3.9), 229 (3.9), 315 (4.4) nm; IR (film) ν<sub>max</sub>: 3369, 2924, 2854, 1695, 1632, 1604, 1515, 1448, 1262, 1170, 833 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, 100 MHz) data, see Table 2; HRESIMS m/z 665.2784 [M + Na]<sup>+</sup> (calculated for C<sub>31</sub>H<sub>46</sub>NaO<sub>14</sub>, 665.2785).

Compound 5: white amorphous powder. [α]<sup>23</sup> D-29.3 (c 0.16, MeOH); UV (MeOH) λ<sub>max</sub> (log ε): 209 (3.9), 228 (3.9), 315 (4.4) nm; IR (film) ν<sub>max</sub>: 3375, 2926, 1694, 1632, 1605, 1515, 1377, 1260, 1169, 1038, 833 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, 100 MHz) data, see Table 2; HRESIMS m/z 661.2833 [M + Na]<sup>+</sup> (calculated for C<sub>32</sub>H<sub>46</sub>NaO<sub>13</sub>, 661.2836).

Compound 6: white amorphous powder. [α]<sup>23</sup> D-71.0 (c 0.35, MeOH); UV (MeOH) λ<sub>max</sub> (log ε): 209 (3.9), 230 (3.9), 315 (4.4) nm; IR (film) ν<sub>max</sub>: 3410, 2973, 1696, 1604, 1515, 1381, 1260, 1168, 1046, 834 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 777.3312 [M + Na]<sup>+</sup> (calculated for C<sub>37</sub>H<sub>46</sub>NaO<sub>16</sub>, 777.3310).

Compound 7: white amorphous powder. [α]<sup>23</sup> D-71.0 (c 0.35, MeOH); UV (MeOH) λ<sub>max</sub> (log ε): 208 (3.9), 230 (3.9), 316 (4.4) nm; IR (film) ν<sub>max</sub>: 3410, 2973, 1696, 1604, 1515, 1381, 1260, 1168, 1046, 834 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 777.3312 [M + Na]<sup>+</sup> (calculated for C<sub>37</sub>H<sub>46</sub>NaO<sub>16</sub>, 777.3310).
Compound 8: white amorphous powder. $[\alpha]^2_D = -17.8$ (c 0.21, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 208 (3.9), 230 (3.9), 316 (4.4) nm; IR (film) $\nu_{max}$: 3391, 2925, 1697, 1632, 1605, 1515, 1446, 1264, 1169, 1041, 834 cm$^{-1}$; $^1$H NMR (CD$_3$OD, 400 MHz) data, see Table 1; $^{13}$C NMR (CD$_3$OD, 100 MHz) data, see Table 2; HRESIMS $m/z$ 661.2831 [M + Na]$^+$ (calculated for C$_{32}$H$_{46}$NaO$_{13}$, 661.2836).

2.4. Acid Hydrolysis of Compounds 1–8

Compounds 1–8 (2 mg) in MeOH (0.1 mL) were added to 1 M H$_2$SO$_4$ aqueous solution (2 mL) and heated in 95 °C water bath for 6 h, respectively. The hydrolyzed solution was neutralized with 1 M Ba(OH)$_2$, filtered and concentrated to a small amount. The monosaccharides in the concentrated solution were identified by TLC with authentic standards, developing with EtOAc-MeOH-HOAc-H$_2$O (8:1:1:0.7, 2 developments). The $R_f$ values of D-glucose, D-mannose and L-rhamnose were 0.43, 0.46 and 0.73, respectively.

2.5. Enzymatic Hydrolysis of Compounds 1–2

Compound 1 or 2 (20 mg) was hydrolyzed with cellulase (30 mg) in HOAc-NaOAc buffer solution (pH 5.0, 12 mL) at 37 °C for 12 h. The hydrolyzed product was extracted with EtOAc and purified on silica gel column (eluting with CH$_2$Cl$_2$), to give (R)-linalool and (S)-linalool (4:6) confirmed by $[\alpha]^2_D +3.5$ (c 0.09, EtOAc) or +2.8 (c 0.07, EtOAc).

2.6. Determination of Bioactivities

The inhibitory activities on FAS, $\alpha$-glucosidase and $\alpha$-amylase, and the DPPH and ABTS radical scavenging effects of compounds 1–11 were evaluated according to the methods described in the literature [12,18,20], while orlistat, acarbose and L-(+)-ascorbic acid were used as the positive controls, respectively (S1).

2.7. Statistical Analyses

Statistical analyses were carried out on GraphPad Prism 5.01. All samples were measured in triplicate. The IC$_{50}$ (the final concentration of sample needed to inhibit 50% of enzyme activity or scavenge 50% of free radical) was obtained by plotting the inhibition or scavenging percentage of each sample against its concentration. The results are reported as mean ± standard deviation (SD). Differences of means between groups were analyzed by one-way analysis of variance (ANOVA) on statistical package SPSS 13.0. The differences between groups were believed to be significant when $p < 0.05$.

3. Results and Discussion

3.1. Identification of Compounds 1–11

Compound 1 was analyzed as C$_{31}$H$_{44}$O$_{13}$ by HRESIMS ($m/z$ 647.2679 [M + Na]$^+$, calculated 647.2680 for C$_{31}$H$_{44}$NaO$_{13}$). The $^1$H NMR spectrum of 1 (Table 1) revealed the following signals: (1) a 3,4-disubstituted phenyl at $\delta_H$ 7.05 (1H, d, $J = 2.0$ Hz), 6.95 (1H, dd, $J = 8.0, 2.0$ Hz) and 6.77 (1H, d, $J = 8.0$ Hz); (2) a trans double bond at $\delta_H$ 7.58 and 6.27 (1H each, $J = 16.0$ Hz); (3) a monosubstituted double bond at $\delta_H$ 5.22 (1H, dd, $J = 10.8, 1.2$ Hz), 5.26 (1H, dd, $J = 17.6, 1.2$ Hz) and 5.93 (1H, dd, $J = 17.6, 10.8$ Hz); (4) an olefinic proton at $\delta_H$ 5.10 (1H, tt, $J = 7.2, 1.6$ Hz); (5) two anomeric protons at $\delta_H$ 4.43 (1H, d, $J = 8.0$ Hz) and 5.18 (1H, d, $J = 1.6$ Hz); (6) two methylene groups at $\delta_H$ 2.04 (2H, m), 1.58, 1.62 (1H each, m), and four methyl groups at $\delta_H$ 1.67, 1.60, 1.39 (3H each, s), and 1.08 (3H, d, $J = 6.4$ Hz). The $^{13}$C NMR spectrum of 1 (Table 2) showed a carbonyl at $\delta_C$ 168.3, three double bonds at $\delta_C$ 114.7–148.0, a benzene ring at $\delta_C$ 115.2–149.8, two anomic carbons at $\delta_C$ 99.4 and 103.1, nine sugar carbons at $\delta_C$ 62.5–82.0, a quaternary carbon at $\delta_C$ 81.6, two methylene groups at $\delta_C$ 23.6 and 42.6, and four methyl groups at $\delta_C$ 17.7–25.9. The $^1$H and $^{13}$C NMR features of 1 were related closely to those of linaloyl-(3-O-$\alpha$-L-rhamnopyranosyl)-(4-O-trans-p-coumaroyl)-$\beta$-D-glucopyranoside (lipedoside B-III) [21], except that the 4-substituted phenyl in lipidoside B-III was replaced by the 3,4-disubstituted phenyl in 1. The acid hydrolysis experiment of 1 gave D-glucose and L-rhamnose identified by TLC.
Furthermore, the HMBC experiment of 1 (Figure 2) displayed the long-distance correlations: between \( \delta_H 4.33 (H-1^\prime \text{ of glucosyl}) \) and \( \delta_C 81.6 (C-3 \text{ of aglycone}) \), between \( \delta_H 5.18 (H-1^{\prime \prime} \text{ of rhamnosyl}) \) and \( \delta_C 82.0 (C-3' \text{ of glucosyl}) \), between \( \delta_H 7.58 (H-7^{\prime \prime\prime} \text{ of caffeoyl}) \) and \( \delta_C 127.6 (C-1^{\prime \prime\prime} \text{ of caffeoyl}) \), and between \( \delta_H 4.89 (H-4' \text{ of glucosyl}) \) and \( \delta_C 168.3 \) (carbonyl of caffeoyl). In addition, the enzymatic hydrolysis experiment of 1 gave (R)-linalool and (S)-linalool (4:6). The \(^1\text{H}\) and \(^{13}\text{C}\) NMR signals of 1 were assigned by \(^1\text{H}-\text{H COSY}, \text{HSQC}\) and HMBC experiments (Figure S1). Based on the above evidence, compound 1 was characterized as a mixture (\( R:S = 4:6 \)) of 3-(R)-linaloyl-(3-O-\( \alpha-L\)-rhamnopyranosyl)-(4-O-trans-caffeoyl)-O-\( \beta-D\)-glucopyranoside. It is a novel monoterpenoid glycoside, named ligurobustoside T.

![Figure 2](image-url)  
**Figure 2.** Key HMBC, \(^1\text{H}-\text{H COSY}\) and NOEDS correlations of compounds 1–8.

Compound 2 was determined as \( C_{31}H_{44}O_{12} \) by HRESIMS (\( m/z \) 631.2728 [M + Na]\(^+\), calculated 631.2730 for \( C_{31}H_{44}NaO_{12} \)). The \(^1\text{H}\) and \(^{13}\text{C}\) NMR data of 2 (Tables 1 and 2) were similar to those of 1, except the 4-O-trans-caffeoyl in 1 was replaced by a trans-\( p\)-coumaroyl [\( \delta_H 6.81, 7.45 \) (2H each, d, \( J = 8.8 \) Hz)] at a different position in 2. The acid hydrolysis experiment of 2 gave d-glucose and L-rhamnose identified by TLC. The HMBC experiment of 2 (Figure 2) showed the long-distance correlations: between \( \delta_H 4.39 \) (H-1' of glucosyl) and \( \delta_C 81.5 \) (C-3 of aglycone), between \( \delta_H 5.17 \) (H-1'' of rhamnosyl) and \( \delta_C 84.4 \) (C-3' of glucosyl), and between \( \delta_H 4.30, 4.45 \) (H-6' of glucosyl) and \( \delta_C 169.0 \)
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(carbonyl of coumaroyl). Additionally, the enzymatic hydrolysis experiment of 2 gave (R)-linalool and (S)-linalool (4:6). The \(^1\)H and \(^{13}\)C NMR signals of 2 were assigned by \(^1\)H-\(^1\)H COSY, HSQC and HMBC experiments (Figure S2). Thus, compound 2 was confirmed as a mixture (R:S = 4:6) of 3(R)- and 3(S)-linalool-(3-O-a-L-rhamnopyranosyl)-(6-O-trans-p-coumaroyl)-O-b-D-glucopyranoside, which is a new monoterpenoid glycoside and named ligurobustoside T1.

Compound 3 was analyzed as C\(_{31}\)H\(_{44}\)O\(_{13}\) by HRESIMS (m/z 647.2680 [M + Na]\(^+\), calculated 647.2680 for C\(_{31}\)H\(_{44}\)NaO\(_{13}\)). The \(^1\)H and \(^{13}\)C NMR data of 3 (Tables 1 and 2) are similar to those of 2 except for some data of the aglycone. The HSQC experiment of 3 displayed the correlations between \(\delta_H 4.78\) (H-8a of aglycone), 4.88 (H-8b of aglycone) and \(\delta_C 111.4\) (C-8 of aglycone), meaning that the C-6 double bond in 2 was replaced by the C-7 double bond in 3. The \(^1\)H-\(^1\)H COSY experiment of 3 (Figure 2) displayed the correlations between \(\delta_H 1.22\) (H-4 of aglycone), 3.95 (H-6 of aglycone) and \(\delta_H 1.60\) (H-5 of aglycone), meaning that a hydroxy was linked at C-6 in 3. Thus, the aglycone of 3 was 3,7-dimethyl-octa-1,7-diene-3,6-diol. The acid hydrolysis experiment of 3 gave D-glucose and L-rhamnose identified by TLC. The HMBC experiment of 3 (Figure 2) displayed the long-distance correlations: between \(\delta_H 4.38\) (H-1 of glucosyl) and \(\delta_C 81.4\) (C-3 of aglycone), between \(\delta_H 5.17\) (H-1\(''\) of rhamnosyl) and \(\delta_C 84.4\) (C-3\(''\) of glucosyl), and between \(\delta_H 4.30, 4.45\) (H-6\(''\) of glucosyl) and \(\delta_C 169.0\) (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 3-(3,6-dihydroxy-3,7-dimethyl-octa-1,7-diaryl)-(3-O-a-L-rhamnopyranosyl)-(6-O-trans-p-coumaroyl)-O-b-D-glucopyranoside. It is a novel monoterpenoid glycoside named ligurobustoside T2.

Compound 4 was analyzed as C\(_{31}\)H\(_{46}\)O\(_{14}\) by HRESIMS (m/z 665.2784 [M + Na]\(^+\), calculated 665.2785 for C\(_{31}\)H\(_{46}\)NaO\(_{14}\)). The NMR spectra of 4 showed two stereoisomers 4a and 4b (2:1). The \(^1\)H NMR spectrum of 4a (Table 1) displayed the following signals: (1) a 4-substituted phenyl at \(\delta_H 6.81, 7.46\) (2H each, d, \(J = 8.8\) Hz); (2) a trans double bond at \(\delta_H 6.34, 7.64\) (1H each, d, \(J = 16.0\) Hz); (3) a monosubstituted double bond at \(\delta_H 5.19\) (1H, dd, \(J = 10.8, 2.0\) Hz), 5.24 (1H, dd, \(J = 18.0, 2.0\) Hz), 5.92 (1H, dd, \(J = 18.0, 10.8\) Hz); (4) two anomic protons at \(\delta_H 4.41\) (1H, d, \(J = 8.0\) Hz), 5.18 (1H, d, \(J = 2.0\) Hz); (5) a methenyl at \(\delta_H 3.21\) (1H, dd, \(J = 10.4, 2.0\) Hz); (6) two methylene groups at \(\delta_H 1.32–1.90\) (4H, m); (7) four methyl groups at \(\delta_H 1.11, 1.14, 1.36\) (3H each, s), 1.25 (3H, d, \(J = 6.4\) Hz). The \(^{13}\)C NMR spectrum of 4a (Table 2) revealed a carbonyl at \(\delta_C 169.0\), two double bonds at \(\delta_C 115.0–146.8\), a 4-substituted phenyl at \(\delta_C 116.9–161.4\), two anomeric carbons at \(\delta_C 99.4\) and 102.7, nine sugar carbons at \(\delta_C 64.9–84.2\), four quaternary carbons at \(\delta_C 73.9\) and 81.5, a methenyl at \(\delta_C 80.1\), two methylene groups at \(\delta_C 26.4\) and 39.9, and four methyl groups at \(\delta_C 17.9–25.8\). The above \(^1\)H and \(^{13}\)C NMR data of 4a were similar to those of 3-(6,7-dihydroxy-3,7-dimethyloct-1-ene)-(3-O-a-L-rhamnopyranosyl)-(4-O-trans-p-coumaroyl)-O-b-D-glucopyranoside (lipedesoside B-VI) [21], except the trans-p-coumaroyl was linked at different positions. The acid hydrolysis experiment of 4 gave D-glucose and L-rhamnose identified by TLC. The HMBC experiment of 4a (Figure 2) displayed the long-distance correlations: between \(\delta_H 4.41\) (H-1\(''\) of glucosyl) and \(\delta_C 81.5\) (C-3 of aglycone), between \(\delta_H 5.18\) (H-1\(''\) of rhamnosyl) and \(\delta_C 84.2\) (C-3\(''\) of glucosyl), and \(\delta_H 4.30, 4.45\) (H-6\(''\) of glucosyl) and \(\delta_C 169.0\) (carbonyl of coumaroyl). The \(^1\)H and \(^{13}\)C NMR signals of 4 were assigned by \(^1\)H-\(^1\)H COSY, HSQC and HMBC experiments (Figure S4). So, 4a was identified as 3-(3,6,7-trihydroxy-3,7-dimethyloct-1-ene)-(3-O-a-L-rhamnopyranosyl)-(6-O-trans-p-coumaroyl)-O-b-D-glucopyranoside.

The NMR data of 4b (Tables 1 and 2) are similar to those of 4a, except the trans-p-coumaroyl in 4a was replaced by the cis-p-coumaroyl (\(\delta_H 6.87, 5.78\) (1H each, d, \(J = 12.8\) Hz, H-7\(''\), H-8\(''\))) in 4b. The HMBC experiment of 4b (Figure 2) showed the long-distance correlations: between \(\delta_H 4.36\) (H-1\(''\) of glucosyl) and \(\delta_C 81.5\) (C-3 of aglycone), between \(\delta_H 5.15\) (H-1\(''\) of rhamnosyl) and \(\delta_C 84.2\) (C-3\(''\) of glucosyl), and between \(\delta_H 4.25, 4.40\) (H-6\(''\) of glucosyl) and \(\delta_C 168.1\) (carbonyl of coumaroyl). So, 4b was identified as 3-(3,6,7-trihydroxy-3,7-dimethyloct-1-ene)-(3-O-a-L-rhamnopyranosyl)-(6-O-cis-p-
Coumaroyl)-O-β-D-glucopyranoside. In conclusion, compound 4 is a mixture of novel monoterpenoid glycosides 4a and 4b, named ligurobustoside T3-4.

Compound 5 was analyzed as C_{32}H_{46}O_{13} by HRESIMS (m/z 661.2833 [M + Na]^+, calculated 661.2836 for C_{32}H_{46}NaO_{13}). The ^1H and ^13C NMR data of 5 (Tables 1 and 2) are similar to those of 2 except for some data of the aglycone. The ^1H-^1H COSY experiment of 5 (Figure 2) displayed the correlations between δ_H 2.36 (2H, d, J = 7.2 Hz, H-4 of aglycone), 5.40 (1H, d, J = 16.0 Hz, H-6 of aglycone) and δ_H 5.64 (1H, dt, J = 16.0, 7.2 Hz, H-5 of aglycone), meaning that the C-6 double bond in 2 was replaced by the C-5(E) double bond in 5. The HMBC experiment of 5 (Figure 2) displayed the correlation between δ_H 3.09 (OCH_3) and δ_C 76.5 (C-7 of aglycone). Hence, the aglycone of 5 was (5E)-7-methoxy-3,7-dimethyl-octa-1,5-dien-3-ol. The acid hydrolysis experiment of 5 gave D-glucose and l-rhamnose identified by TLC. The HMBC experiment of 5 (Figure 2) displayed the long-distance correlations: between δ_H 4.41 (H-1′′′′ of glucosyl) and δ_C 81.2 (C-3 of aglycone), between δ_H 5.17 (H-1″′ of rhamnosyl) and δ_C 84.2 (C-3′′′ of glucosyl), and between δ_H 4.32, 4.45 (H-6′′ of glucosyl) and δ_C 168.9 (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 determined to be (5E)-3-(3-hydroxy-7-methoxy-3,7-dimethyl-octa-1,5-dienyl)-(3-O-α-L-rhamnopyranosyl)-(6-O-trans-p-coumaroyl)-O-β-D-glucopyranoside. It is a novel monoterpenoid glycoside, named ligurobustoside T5.

Compound 6 was determined as C_{37}H_{54}O_{16} by HRESIMS (m/z 777.3312 [M + Na]^+, calculated 777.3310 for C_{37}H_{54}NaO_{16}). The ^1H and ^13C NMR data of 6 (Tables 1 and 2) are similar to those of lipedoside B-III [21], except there was another rhamnoscyl 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 δ_H 4.44 (H-1′ of glucosyl) and δ_C 81.6 (C-3 of aglycone), between δ_H 5.19 (H-1″ of inner rhamnosyl) and δ_C 81.9 (C-3′′ of glucosyl), between δ_H 5.04 (H-1‴″ of outer rhamnosyl) and δ_C 81.7 (C-4″ of inner rhamnosyl), and between δ_H 4.91 (H-4′ of glucosyl) and δ_C 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 confirmed as linaloyl-[3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl]-4-(O-trans-p-coumaroyl)-O-β-D-glucopyranoside, which is a new monoterpenoid glycoside and named ligurobustoside T6.

Compound 7 was determined as C_{37}H_{54}O_{16} by HRESIMS (m/z 777.3312 [M + Na]^+, calculated 777.3310 for C_{37}H_{54}NaO_{16}). The ^1H and ^13C NMR data of 7 (Tables 1 and 2) are related closely to those of 6, except the trans-p-coumaroyl (δ_H 7.66, 6.33 (1H each, d, J = 16.0 Hz, H-7‴‴, H-8‴‴)) in 6 was replaced by the cis-p-coumaroyl (δ_H 7.98, 5.76 (1H each, d, J = 12.8 Hz, H-7‴‴, H-8‴‴)) in 7. The acid hydrolysis experiment of 7 yielded D-glucose and L-rhamnose identified by TLC. The HMBC experiment of 7 (Figure 2) showed the long-distance correlations: between δ_H 4.41 (H-1′ of glucosyl) and δ_C 81.6 (C-3 of aglycone), between δ_H 5.29 (H-1″′′′ of inner rhamnosyl) and δ_C 79.8 (C-3′′′′ of glucosyl), between δ_H 5.13 (H-1‴‴″ of outer rhamnosyl) and δ_C 80.6 (C-4‴‴″ of inner rhamnosyl), and between δ_H 4.86 (H-4′′′′ of glucosyl) and δ_C 166.9 (carbonyl of coumaroyl). The ^1H and ^13C NMR signals of 7 were assigned by ^1H-^1H COSY, HSQC and HMBC experiments (Figure S7). Thus, compound 7 was identified as linaloyl-[3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl]-4-(O-cis-p-coumaroyl)-O-β-D-glucopyranoside. It is a new monoterpenoid glycoside, named ligurobustoside T7.

Compound 8 was analyzed as C_{32}H_{46}O_{13} by HRESIMS (m/z 661.2831 [M + Na]^+, calculated 661.2836 for C_{32}H_{46}NaO_{13}). The NMR spectra of 8 exhibited two stereoisomers 8a and 8b (2:1). The ^1H NMR spectrum of 8a (Table 1) displayed the following signals: (1) a 4-substituted phenyl at δ_H 6.80, 7.45 (2H each, d, J = 8.4 Hz); (2) two trans double bonds at δ_H 6.35, 7.64 (1H each, d, J = 16.0 Hz), 5.44 (1H, d, J = 15.6 Hz), 5.55 (1H, m); (3) an olefinic proton at δ_H 5.41 (1H, t, J = 8.0 Hz); (4) two anomic protons at δ_H 4.31 (1H, d, J = 8.0 Hz), 5.17 (1H, d, J = 2.0 Hz); (5) two methylene groups at δ_H 4.27 (2H, d, J = 8.0 Hz), 2.76 (2H, d, J = 10.2 Hz); (6) four methyl groups at δ_H 1.23, 1.23, 1.65 (3H each, s), 1.24 (3H,
d, J = 6.4 Hz); and (7) a methoxy at δH 3.12 (3H, s). The 13C NMR spectrum of 8a (Table 2) revealed a carbonyl at δC 169.1, three double bonds at δC 114.8–146.9, a 4-substituted phenyl at δC 117.0–161.7, two anomic carbons at δC 102.6 and 102.7, nine sugar carbons at δC 64.7–84.0, a quaternary carbon at δC 76.4, two methylene groups at δC 66.3, 43.5, a methoxy at δC 50.6, and four methyl groups at δC 16.6-26.2. The above 1H and 13C NMR data of 8a were similar to those of (2E,5E)-1-(1,7-dihydroxy-3,7-dimethyl-2,5-octadienyl)-(3-O-α-L-rhamnopyranosyl)-(4-O-trans-p-coumaroyl)-O-β-D-glucopyranoside (ligurobustoside I) [8], except the trans-p-coumaroyl was linked at different positions, and there was another methyl in 8a. The HMBC experiment of 8a (Figure 2) showed the correlation between δH 3.12 (OCH3) and δC 76.4 (C-7 of aglycone). The NOEDS experiment of 8a (Figure 2) displayed the long-distance correlations: between δH 4.31 (H-1 of aglycone) and δH 5.17 (H-1′ of rhamnosyl) and δC 66.3 (C-1 of aglycone), between δH 5.17 (H-1′ of rhamnosyl) and δC 84.0 (C-3′ of glucosyl), and between δH 4.35, 4.50 (H-6′ of glucosyl) and δC 169.1 (carbonyl of coumaroyl). The 1H and 13C NMR signals of 8 were assigned by 1H-1H COSY, HSQC and HMBC experiments (Figure S8). Consequently, the structure of 8a was determined to be (2E,5E)-1-(1-hydroxy-7-methoxy-3,7-dimethyl-octa-2,5-dienyl)-(3-O-α-L-rhamnopyranosyl)-(6-O-trans-p-coumaroyl)-O-β-D-glucopyranoside.

The NMR data of 8b (Tables 1 and 2) are similar to those of 8a, except the trans-p-coumaroyl in 8a was replaced by the cis-p-coumaroyl (δH 6.87, 5.79 (1H each, d, J = 12.8 Hz, H-7′′, H-8′′′′)) in 8b. The HMBC experiment of 8b (Figure 2) displayed the long-distance correlations: between δH 4.27 (H-1′ of glucosyl) and δC 66.3 (C-1 of aglycone), between δH 5.16 (H-1′ of rhamnosyl) and δC 84.0 (C-3′ of glucosyl), and between δH 4.31, 4.48 (H-6′ of glucosyl) and δC 168.1 (carbonyl of coumaroyl). So, 8b was identified as (2E,5E)-1-(1-hydroxy-7-methoxy-3,7-dimethyl-octa-2,5-dienyl)-(3-O-α-L-rhamnopyranosyl)-(6-O-cis-p-coumaroyl)-O-β-D-glucopyranoside. In conclusion, compound 8 is a mixture of novel monoterpene glycosides 8a and 8b, named ligurobustoside T8-s.

Compounds 9–11 (NMR data see Tables S1–S3) were identified as ligurobustosides G (9a) and H (9b), ligurobustoside C (10), ligurobustosides K (11a) and L (11b), respectively, by direct comparison with published spectral data (1H, 13C NMR) [8,9].

3.2. The Bioactivities of Compounds 1–11

Compounds 1–11 from the leaves of L. robustum were tested for the inhibitory activities on FAS, α-glucosidase, α-amylase, and the antioxidant effects. The results of bioactivity assays are shown in Table 3. As shown in Table 3, compound 2 revealed stronger FAS inhibitory activity (IC50: 2.36 ± 0.10 μM) than the positive control orlistat (IC50: 4.46 ± 0.13 μM); compound 2 showed weaker α-glucosidase inhibitory effect than the positive control acarbose; compounds 2–6, 8, 9 and 11 displayed weaker α-amylase inhibitory effect than the positive control acarbose; compounds 1, 2, 5 and 11 exhibited more potent ABTS radical scavenging activity (IC50: 6.91 ± 0.10–9.41 ± 0.22 μM) than the positive control L-(+)-ascorbic acid (IC50: 10.06 ± 0.19 μM), while compound 1 displayed weaker DPPH radical scavenging activity (IC50: 19.74 ± 0.23 μM) than L-(+)-ascorbic acid (IC50: 13.66 ± 0.13 μM).
Table 3. The results of bioactivity assays of compounds 1–11 from *L. robustum*.

| Compounds | FAS IC$_{50}$ (µM)$^b$ | α-Glucosidase Inhibition at 0.1 mM (%) | α-Amylase Inhibition at 0.1 mM (%) | DPPH IC$_{50}$ (µM)$^b$ | ABTS*+ IC$_{50}$ (µM)$^b$
|-----------|-----------------|---------------------------------|-----------------|-----------------|-----------------|
| 1         | NA$^c$          | NA                              | 19.74 ± 0.23 b  | 6.91 ± 0.10 a   | 13.66 ± 0.13 a  | 10.06 ± 0.19 d |
| 2         | 2.36 ± 0.10 a   | 48.1 ± 4.3 b                    | 31.5 ± 0.5 b    | >250            | 9.41 ± 0.22 c   | 4.46 ± 0.13 b   |
| 3         | 21.77 ± 0.38 c  | 73.0 ± 2.0 c                    | 32.5 ± 6.3 b    | NA              | 16.00 ± 0.69 g  | 0.10 ± 9.41 b   |
| 4         | >100            | NA                              | 28.2 ± 3.9 b    | NA              | 9.66 ± 0.17 cd  | 0.22 ± 0.13 c   |
| 5         | 23.71 ± 0.45 d  | 13.8 ± 2.0 d                    | 35.6 ± 2.0 b    | NA              | 6.93 ± 0.01 a   | 0.10 ± 9.41 b   |
| 6         | 4.78 ± 0.14 b   | 12.0 ± 1.7 d                    | 26.1 ± 3.0 b    | NA              | 11.30 ± 0.16 e  | 0.10 ± 9.41 b   |
| 7         | >100            | NA                              | NA              | NA              | 20.21 ± 0.33 j  | 0.10 ± 9.41 b   |
| 8         | 25.83 ± 0.47 e  | 24.7 ± 3.5 c                    | 31.4 ± 1.9 b    | NA              | 19.50 ± 0.46 i  | 0.10 ± 9.41 b   |
| 9         | 21.67 ± 0.46 c  | 12.4 ± 5.6 d                    | 29.2 ± 8.4 b    | NA              | 18.66 ± 0.47 h  | 0.10 ± 9.41 b   |
| 10        | 4.68 ± 0.16 b   | 28.7 ± 2.1 c                    | NA              | NA              | 15.10 ± 0.10 t  | 0.10 ± 9.41 b   |
| 11        | 61.74 ± 0.45 f  | NA                              | 31.3 ± 1.3 b    | NA              | 7.92 ± 0.23 b   | 0.10 ± 9.41 b   |
| Orlistat$^d$ | 4.46 ± 0.13 b  | 93.2 ± 0.1 a                    | 51.8 ± 2.5 a    | 13.66 ± 0.13 a  | 10.06 ± 0.19 d  | 0.10 ± 9.41 b   |
| Acarbose$^d$ |              |                                 |                 |                 |                 |                |
| L-(+)-Ascorbic acid$^d$ |              |                                 |                 |                 |                 |                |

$^a$ Data are expressed as mean ± SD (n = 3). Means with the same letter are not significantly different (one-way analysis of variance, a = 0.05). $^b$ IC$_{50}$: the final concentration of sample needed to inhibit 50% of enzyme activity or scavenge 50% of free radical. $^c$ NA: no activity. $^d$ Positive control.

Because FAS is a potential therapeutic target for anti-obesity drugs [17–19], compounds 2, 6 and 10 with strong FAS inhibitory activity might be a part of the constituents with anti-obesity activity in *L. robustum*. In addition, the results suggested that the FAS inhibitory activity would reduce or disappear when the monoterpene unit of glycoside was substituted with hydroxyl, or the *trans*-p-coumaroyl of glycoside was replaced by other groups.

4. Conclusions

In summary, the phytochemical research on the leaves of *L. robustum* resulted in the separation of eleven monoterpenoid glycosides (1–11), including eight new compounds (1–8) identified with spectroscopic method ($^1$H, $^{13}$C NMR, $^1$H-$^1$H COSY, HSQC, HMBC, NOEDS, HRESIMS), and physical and chemical methods. The biological study showed that compound 2 revealed stronger FAS inhibitory activity (IC$_{50}$: 2.36 ± 0.10 µM) than the positive control orlistat (IC$_{50}$: 4.46 ± 0.13 µM); compounds 1, 2, 5 and 11 displayed more potent ABTS radical scavenging activity (IC$_{50}$: 6.91 ± 0.10–9.41 ± 0.22 µM) than the positive control L-(+)-ascorbic acid (IC$_{50}$: 10.06 ± 0.19 µM); compound 2 revealed also moderate α-glucosidase and α-amylase inhibitory activities. This study provided a theoretical basis for the leaves of *L. robustum* as a functional tea to treat obesity.

Supplementary Materials: The following are available online https://www.mdpi.com/article/10.3390/molecules27123709/s1. $^1$H NMR, $^{13}$C NMR, $^1$H-$^1$H COSY, HSQC, HMBC, HRESIMS and IR spectra of compounds 1 (Figure S1) and 3–6 (Figures S3–S6); $^1$H NMR, $^{13}$C NMR, HMBC, HRESIMS and IR spectra of compounds 2 (Figure S2) and 7 (Figure S7); $^1$H NMR, $^{13}$C NMR, $^1$H-$^1$H COSY, HSQC, HMBC, NOEDS, HRESIMS and IR spectra of compound 8 (Figure S8); $^1$H NMR and $^{13}$C NMR data of 9–11 (Tables S1–S3); determination of bioactivities (S1).

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