Bodiniosides S–Y, Seven New Triterpenoid Saponins from *Elsholtzia bodinieri* and Their Anti-Influenza Activities

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**Abstract:** Investigation of the n-BuOH extract of the aerial parts of *Elsholtzia bodinieri* led to the isolation of seven new triterpenoid saponins, Bodiniosides S–Y (1–7, resp.). Their structures were elucidated on the basis of spectroscopic techniques, including HSQC, HSBC, and HSQC–TOCSY experiments, together with acid hydrolysis and GC analysis. The anti-influenza activities of compounds 1–7 were evaluated against A/WSN/33/2009 (H1N1) virus in MDCK cells. The results showed that compounds 2 and 5 exhibited moderate anti-influenza activities against A/WSN/33/2009 (H1N1), with inhibition rates of 35.33% and 24.08%, respectively.

**Keywords:** *Elsholtzia bodinieri*; triterpenoid saponins; anti-influenza virus activities; bodiniosides S–Y

1. Introduction

*Elsholtzia bodinieri* Vaniot (Chinese name “Dongzisu”), belonging to the taxonomically diverse group of the family Labiatae, is a medicinal plant that grows in Yunnan and Guizhou Provinces in China. It is commonly known as “yashuacao” and is used as a traditional Chinese medicine for the treatment of cough, headache, pharyngitis, fever and hepatitis [1]. Previous studies on *E. bodinieri* led to the isolation of triterpenoid saponins [2–6], flavonoid glycosides [7,8], sesquiterpene glycosides [9], clerodane diterpenoid glycosides [10], and phenolic constituents [11] from the aerial parts of this plant. As a continuation of our work, we further systematically investigated the chemical components of the aerial parts of this plant. In our search for secondary metabolites with structural diversity and potential anti-influenza virus activity, seven new triterpenoid saponins, Bodiniosides S–Y (1–7, resp.), were obtained from *E. bodinieri*. Among them, compounds 2 and 5 exhibited moderate inhibition of influenza virus activities with inhibition rates of 35.33% and 24.08%, respectively. Herein, we report the isolation, structural elucidation and anti-influenza virus activities of the isolated compounds.

2. Results

The n-BuOH soluble fraction of the 75% aqueous acetone extract of the aerial parts of *E. bodinieri* was subjected to repeated column chromatography over silica gel, Sephadex LH-20, RP-18, and semipreparative reversed-phase HPLC, eluting with various solvent systems, to afford seven new triterpenoid saponins (Figure 1). The spectrums can be found in supplementary materials.
Compound 1 was obtained as a white amorphous powder. Its molecular formula was determined as C₆₁H₉₈O₂₈, according to the [M – H]⁻ peak at m/z 1277.6148 in the negative HR-ESI-MS, indicating 13 degrees of unsaturation. It exhibited a UV maximum at 204 nm. The IR spectrum showed the presence of hydroxyl (3441 cm⁻¹), carbonyl (1722 cm⁻¹), and olefinic (1635 cm⁻¹) groups. NMR data analysis indicated that 1 was a saponin containing a triterpene sapogenin and five monosaccharides.

In the ¹H and ¹³C NMR spectra of aglycone moiety, 6 tertiary methyl groups [δH 0.81, 0.83, 0.84, 0.88, 1.07, and 1.25 (each, 3H, s); δC 12.9, 15.9, 17.5, 23.6, 25.5, and 30.6 (each, q)], 11 methylenes containing an oxygenated one [δH 4.03 (1H, m, H₅-23), 4.12 (1H, m, H₆-23; δC 64.7 (t, C-23)], 5 methines including an oxygenated one[δH 3.73 (1H, br. s, H-3); δC 81.9 (δ, C-23)] and 1 unsaturated one [δH 5.27 (1H, m, H-12); δC 123.2 (δ, C-12)], as well as 8 quaternary carbons (including a carbonyl carbon (δC 176.4, C-28) and an unsaturated one (δC 144.0, C-15)) were observed (Tables 1 and 2). This information suggested that the aglycone moiety of compound 1 was 3β, 23-dihydroxyolean-12-en-28-oic acid [12]. Except for the signals for the aglycone, the remaining 31 signals were assigned as five sugar moieties and an acetoxyl group, due to signals of δC 172.7 and 20.9. Moreover, comparison of its ¹H and ¹³C NMR spectroscopic data with those of Bodinioside H [4], suggested that they had same 3-hydroxy-23-acetoxy-olean-12-en-28-oic acid as the aglycone, but differed in the sugar moiety.

![Figure 1. Chemical structure of compounds 1–7 from Elsholtzia bodinieri.](image_url)

**Table 1.** ¹H NMR data of compounds 1–7 in Pyridine-d₅ (600 MHz, δH in ppm, δ in Hz).

| Position | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
|----------|-------|-------|-------|-------|-------|-------|-------|
| 1        | 1.57 (m); 0.96 (m) | 1.66 (m); 0.98 (m) | 1.56 (m); 0.82 (m) | 1.49 (m); 1.15 (m) | 1.51 (m); 1.03 (m) | 1.66 (m); 1.17 (m) | 1.66 (m); 1.23 (m) |
| 2        | 1.81 (m); 1.71 (m) | 1.92 (m); 1.76 (m) | 1.89 (m); 1.71 (m) | 2.20 (m); 2.05 (m) | 2.20 (m); 2.01 (m) | 4.24 (m) | 3.15 (m) |
| 3        | 3.73 (m) | 3.59 (m) | 3.90 (m) | 4.31 (m) | 4.32 (m) | 3.41 (m) | 3.63 (m) |
| 5        | 1.52 (m) | 1.74 (m) | 1.54 (m) | 1.69 (m) | 1.63 (m) | 1.02 (m) | 1.80 (m) |
| 6        | 1.64 (m); 1.35 (m) | 1.62 (m); 1.40 (m) | 1.73 (m); 1.33 (m) | 1.66 (m); 1.37 (m) | 1.65 (m); 1.31 (m) | 1.78 (m); 1.54 (m) | 1.68 (m); 1.44 (m) |
| 7        | 1.82 (m); 1.54 (m) | 1.71 (m); 1.55 (m) | 1.93 (m); 1.57 (m) | 1.87 (m); 1.52 (m) | 1.84 (m); 1.50 (m) | 1.67 (m); 1.35 (m) | 1.73 (m); 1.36 (m) |
| 9        | 1.66 (m) | 1.66 (m) | 1.70 (m) | 1.70 (m) | 1.70 (m) | 1.80 (m) | 1.61 (m) |
| 11       | 1.93 (m); 1.87 (m) | 1.93 (m); 1.82 (m) | 1.92 (m); 1.86 (m) | 1.97 (m); 1.82 (m) | 1.96 (m); 1.84 (m) | 2.02 (m); 1.92 (m) | 1.98 (m); 1.62 (m) |
| 12       | 5.27 (br. s) | 5.42 (br. s) | 5.44 (br. s) | 5.41 (br. s) | 5.40 (br. s) | 5.52 (br. s) | 5.55 (br. s) |
| 15       | 1.67 (m); 1.17 (m) | 1.57 (m); 1.20 (m) | 1.78 (m); 1.21(m) | 2.06 (m); 1.56 (m) | 2.04 (m); 1.55(m) | 2.05 (m); 1.69 (m) | 2.03 (m); 1.36 (m) |
| 16       | 2.03 (m); 1.72 (m) | 2.03 (m); 1.68 (m) | 2.01 (m); 1.56 (m) | 2.20 (m); 2.18 (m) | 2.12 (m); 2.07 (m) | 2.28 (m); 2.10 (m) | 2.24 (m); 2.12 (m) |
| 18       | 3.12 (dd, 13.6, 3.8) | 3.14 (dd, 13.5) | 3.17 (dd, 13.5) | 3.13 (dd, 13.7) | 3.11 (dd, 13.5, 4.0) | 2.88 (dd, 13.5, 3.4) | 4.25 (m) |
| 19       | 1.70 (m); 1.17 (m) | 2.08 (m); 1.18 (m) | 1.89 (m); 1.12 (m) | 1.74 (m); 1.15 (m) | 1.76 (m); 1.12 (m) |
| 21       | 1.28 (m); 1.19 (m) | 2.20 (m); 1.14 (m) | 1.29 (m); 1.01 (m) | 1.42 (m); 1.04 (m) | 1.30 (m); 0.94 (m) | 2.04 (m); 1.72 (m) | 2.04 (m); 1.78 (m) |
| 22       | 1.68 (m); 1.32 (m) | 1.39 (m); 1.00 (m) | 1.75 (m); 1.35 (m) | 1.68 (m); 1.35 (m) | 1.64 (m); 1.08 (m) | 2.08 (m); 1.78 (overlap) | 2.29 (m); 1.42 (m) |
### Table 1. Cont.

| Position | 1                  | 2                  | 3                  | 4                  | 5                  | 6                  | 7                  |
|----------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 23       | 4.12 (m); 4.03 (m) | 4.45 (m); 4.03 (m) | 4.30 (m); 4.10 (m) | 4.32 (m); 3.63 (m) | 4.33 (m); 3.61 (m) | 4.36 (m); 3.04 (m) | 4.33 (m); 4.02 (m) |
| 24       | 0.83 (s)           | 0.84 (s)           | 0.88 (s)           | 0.90 (s)           | 0.87 (s)           | 1.04 (s)           | 1.13 (s)           |
| 25       | 1.07 (s)           | 1.09 (s)           | 1.14 (s)           | 1.20 (s)           | 1.09 (s)           | 1.09 (s)           | 1.39 (s)           |
| 26       | 0.81 (s)           | 0.83 (s)           | 0.84 (s)           | 0.82 (s)           | 0.82 (s)           | 0.84 (s)           | 1.03 (s)           |
| 27       | 0.84 (s)           | 0.89 (s)           | 0.93 (s)           | 0.99 (s)           | 0.90 (s)           | 1.15 (s)           | 1.19 (s)           |
| 28       | 1.25 (s)           | 1.26 (s)           | 0.97 (s)           | 1.23 (s)           | 0.97 (s)           | 1.54 (s)           | 1.62 (s)           |
| 29       | 0.88 (s)           | 0.91 (s)           | 1.26 (s)           | 1.48 (s)           | 1.16 (s)           | 1.11 (s)           | 1.06 (s)           |

#### 23-AcO

| 23       | 2.15 (s)           | 2.14 (s)           | 2.08 (s)           |                    |                    |                    |                    |

#### 3-Xyl

| 23       | 4.97 (d, 7.8)      | 4.86 (d, 7.8)      | 5.02 (d, 7.4)      | 5.01 (d, 7.8)      |                    |                    |                    |
| 24       | 0.83 (s)           | 0.84 (s)           | 0.88 (s)           | 0.90 (s)           | 0.87 (s)           | 1.04 (s)           | 1.13 (s)           |
| 25       | 1.07 (s)           | 1.09 (s)           | 1.14 (s)           | 1.20 (s)           | 1.09 (s)           | 1.09 (s)           | 1.39 (s)           |
| 26       | 0.81 (s)           | 0.83 (s)           | 0.84 (s)           | 0.82 (s)           | 0.82 (s)           | 0.84 (s)           | 1.03 (s)           |
| 27       | 0.84 (s)           | 0.89 (s)           | 0.93 (s)           | 0.99 (s)           | 0.90 (s)           | 1.15 (s)           | 1.19 (s)           |
| 28       | 1.25 (s)           | 1.26 (s)           | 0.97 (s)           | 1.23 (s)           | 0.97 (s)           | 1.54 (s)           | 1.62 (s)           |
| 29       | 0.88 (s)           | 0.91 (s)           | 1.26 (s)           | 1.48 (s)           | 1.16 (s)           | 1.11 (s)           | 1.06 (s)           |

#### 28-O-sugar

| 23       | 0.81 (s)           | 0.83 (s)           | 0.84 (s)           | 0.82 (s)           | 0.82 (s)           | 0.84 (s)           | 1.03 (s)           |
| 24       | 0.84 (s)           | 1.07 (s)           | 1.09 (s)           | 1.14 (s)           | 1.20 (s)           | 1.09 (s)           | 1.39 (s)           |
| 25       | 0.84 (s)           | 1.07 (s)           | 1.09 (s)           | 1.14 (s)           | 1.20 (s)           | 1.09 (s)           | 1.39 (s)           |
| 26       | 0.81 (s)           | 0.83 (s)           | 0.84 (s)           | 0.82 (s)           | 0.82 (s)           | 0.84 (s)           | 1.03 (s)           |
| 27       | 0.84 (s)           | 0.89 (s)           | 0.93 (s)           | 0.99 (s)           | 0.90 (s)           | 1.15 (s)           | 1.19 (s)           |
| 28       | 1.25 (s)           | 1.26 (s)           | 0.97 (s)           | 1.23 (s)           | 0.97 (s)           | 1.54 (s)           | 1.62 (s)           |
| 29       | 0.88 (s)           | 0.91 (s)           | 1.26 (s)           | 1.48 (s)           | 1.16 (s)           | 1.11 (s)           | 1.06 (s)           |

### Table 2. C NMR data of compounds 1–7 in pyridine-$d_5$ (150 MHz, $\delta_C$ in ppm).

| Position | 1                  | 2                  | 3                  | 4                  | 5                  | 6                  | 7                  |
|----------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 1        | 39.8               | 38.5               | 38.6               | 39.2               | 38.6               | 47.7               | 47.9               |
| 2        | 26.0               | 26.4               | 26.8               | 26.2               | 26.1               | 68.9               | 68.8               |
| 3        | 81.9               | 81.9               | 78.3               | 82.1               | 81.9               | 78.5               | 79.9               |
| 4        | 42.4               | 42.1               | 42.2               | 43.6               | 43.3               | 43.7               | 43.5               |
| 5        | 48.3               | 48.3               | 48.3               | 48.0               | 47.0               | 48.2               | 48.0               |
| 6        | 18.5               | 18.4               | 18.6               | 18.4               | 18.1               | 18.9               | 18.6               |
| 7        | 33.7               | 32.2               | 32.3               | 33.2               | 32.2               | 33.2               | 33.3               |
| 8        | 41.8               | 39.8               | 41.8               | 40.1               | 39.8               | 40.3               | 40.5               |
| 9        | 48.2               | 48.3               | 48.3               | 48.3               | 47.6               | 48.5               | 41.7               |
| 10       | 36.7               | 36.8               | 37.1               | 37.1               | 36.8               | 38.6               | 38.3               |
| 11       | 23.6               | 23.7               | 23.2               | 23.2               | 25.1               | 23.7               | 24.6               |
| 12       | 123.2              | 123.3              | 123.2              | 123.1              | 122.6              | 123.5              | 128.2              |
| 13       | 144.0              | 144.0              | 143.9              | 144.2              | 143.9              | 144.5              | 139.2              |
| 14       | 42.1               | 41.8               | 42.3               | 42.3               | 41.8               | 42.5               | 42.2               |
| 15       | 30.6               | 28.8               | 28.7               | 28.4               | 28.5               | 28.8               | 29.4               |
| 16       | 23.1               | 23.3               | 23.8               | 23.7               | 23.3               | 24.4               | 26.1               |
| 17       | 47.1               | 47.1               | 48.6               | 47.5               | 48.1               | 46.7               | 48.0               |
| 18       | 42.1               | 42.4               | 42.1               | 42.3               | 42.0               | 45.0               | 54.6               |
| 19       | 46.2               | 46.6               | 46.3               | 46.3               | 46.3               | 81.3               | 72.5               |
Table 2. Cont.

| Position | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
|----------|------|------|------|------|------|------|------|
| 20       | 30.3 | 30.6 | 30.6 | 30.8 | 30.6 | 29.5 | 42.2 |
| 21       | 32.9 | 34.2 | 34.4 | 36.7 | 33.8 | 35.4 | 26.7 |
| 22       | 32.1 | 33.8 | 33.1 | 32.9 | 32.8 | 32.7 | 37.4 |
| 23       | 64.7 | 65.6 | 65.2 | 64.3 | 64.3 | 66.7 | 66.6 |
| 24       | 12.9 | 13.0 | 12.7 | 13.7 | 13.5 | 14.3 | 14.2 |
| 25       | 15.9 | 15.9 | 16.0 | 16.4 | 16.1 | 17.7 | 17.5 |
| 26       | 17.5 | 17.4 | 17.5 | 17.7 | 17.4 | 17.5 | 17.4 |
| 27       | 25.5 | 25.6 | 25.7 | 26.1 | 25.7 | 28.0 | 24.1 |
| 28       | 176.4| 176.5| 176.5| 176.7| 176.4| 177.2| 176.8|
| 29       | 30.6 | 33.0 | 33.0 | 33.2 | 33.0 | 33.2 | 26.9 |
| 30       | 23.6 | 23.7 | 23.7 | 23.7 | 23.7 | 24.7 | 16.6 |

23-Ac-O

|       | 171.2| 171.1| 171.1|      |      |      |      |
|-------|------|------|------|------|------|------|------|
| 3-Xyl | 20.7 | 20.7 | 20.7 |      |      |      |      |

28-O-sugar

| Glc   |       |      |      |      |      |      |      |
|-------|------|------|------|------|------|------|------|
| 1     | 94.5 | 94.4 | 94.5 | 94.7 | 94.6 | 95.1 | 94.8 |
| 2     | 76.3 | 76.3 | 76.3 | 77.6 | 76.2 | 75.7 | 75.1 |
| 3     | 78.5 | 79.2 | 78.3 | 78.5 | 78.4 | 79.8 | 78.9 |
| 4     | 71.4 | 71.5 | 71.1 | 71.1 | 71.8 | 71.4 | 72.5 |
| 5     | 77.6 | 77.3 | 77.4 | 77.6 | 77.9 | 79.1 | 78.3 |
| 6     | 68.2 | 68.3 | 68.3 | 68.6 | 69.7 | 62.1 | 62.2 |

| Rha   |       |      |      |      |      |      |      |
|-------|------|------|------|------|------|------|------|
| 1     | 101.0| 101.2| 101.2| 101.5| 101.3| 101.4| 101.3|
| 2     | 71.3 | 71.6 | 71.6 | 71.3 | 70.9 | 72.2 | 71.4 |
| 3     | 72.4 | 72.4 | 72.3 | 72.1 | 72.3 | 72.4 | 72.2 |
| 4     | 85.7 | 85.7 | 85.7 | 85.6 | 74.6 | 73.8 | 73.7 |
| 5     | 70.9 | 71.5 | 70.9 | 71.1 | 71.8 | 69.7 | 69.6 |
| 6     | 18.6 | 18.5 | 18.7 | 18.8 | 18.6 | 18.6 | 18.6 |

| Glc'/Ara/Xyl |       |      |      |      |      |      |      |
|--------------|------|------|------|------|------|------|------|
| 1            | 105.3| 109.7| 105.5| 105.7| 105.7| 105.4|      |
| 2            | 75.3 | 81.9 | 74.7 | 74.9 | 74.7 | 75.5 |      |
| 3            | 78.5 | 76.3 | 77.9 | 78.7 | 77.8 |      |      |
| 4            | 71.3 | 86.0 | 72.4 | 71.8 | 72.0 |      |      |
| 5            | 78.2 | 67.2 | 66.9 | 67.2 | 66.8 |      |      |
| 6            | 62.7 |      |      |      |      |      |      |

| Glc''/Glc' |       |      |      |      |      |      |      |
|------------|------|------|------|------|------|------|------|
| 1          | 107.2| 107.0| 107.1| 106.7|      |      |      |
| 2          | 76.3 | 76.3 | 75.8 | 75.5 |      |      |      |
| 3          | 79.2 | 79.2 | 79.3 | 78.8 |      |      |      |
| 4          | 71.0 | 71.5 | 71.6 | 71.8 |      |      |      |
| 5          | 78.5 | 78.2 | 77.4 | 78.7 |      |      |      |
| 6          | 62.5 | 62.7 | 62.8 | 62.9 |      |      |      |

The $^1$H NMR spectrum showed five signals for anomeric protons at $\delta_H$ 6.45 (1H, br. s), 6.09 (1H, d, $J = 7.8$ Hz), 5.16 (1H, d, $J = 7.8$ Hz), 4.97 (1H, d, $J = 7.8$ Hz) and 4.86 (1H, d, $J = 7.8$ Hz), which correlated with anomeric carbon signals at $\delta_C$ 101.0, 94.5, 107.2, 107.1 and 105.3 in the HSQC spectrum, respectively, suggesting the presence of five sugar moieties. Acid hydrolysis of 1 with 1 M HCl produced L-rhamnose (Rha), D-glucose (Glc), and D-xylose (Xyl) as sugar residues by GC chromatography with the corresponding trimethylsilylated L-cysteine derivatives. Since NMR signals of five sugar units have undesirable overlapped effects, the HMOC-TOSCY experiment was successfully
used to distinguish and assign the $^1$H and $^{13}$C NMR signals of each sugar moiety. The correlations from the anomeric proton signal at $\delta_{\text{H}}$ 4.97 to three carbon signals at $\delta_{\text{C}}$ 75.3, 78.4, and 67.1, as well as from three proton signals at $\delta_{\text{H}}$ 4.22, 4.06, and 3.75 to the anomeric carbon, suggested the presence of D-xylopyranose. In a similar way, the $^1$H and $^{13}$C NMR signals for D-glucopyranosyl and L-rhamnopyranosyl were assigned. In addition, the $J_{\text{H1, H2}}$ coupling constants of four anomeric proton signals at $\delta_{\text{H}}$ 6.09 (1H, d, $J = 7.8$ Hz), 5.16 (1H, d, $J = 7.8$ Hz), 4.97 (1H, d, $J = 7.8$ Hz) and 4.86 (1H, d, $J = 7.8$ Hz) suggested the $\beta$ anomeric configuration for the xylopyranosyl and glucopyranosyl units. The inspection of the anomeric proton (6.45, br. s) deduced the $\alpha$ anomeric configuration for the L-rhamnopyranosyl unit [4].

The sequence of the glycoside chains connected to C-3 and C-28 was established by analysis of the HMBC correlations (Figure 2). The absence of any glycosidation shift for Xyl was further confirmed by HMBC correlation of HXyl-1 ($\delta_{\text{H}}$ 5.62) to CGlc-6 ($\delta_{\text{C}}$ 68.3), and from HGlc-1 ($\delta_{\text{H}}$ 6.46) to C-3 ($\delta_{\text{C}}$ 82.8) of the aglycone, which was further confirmed by HMBC correlation of HXyl-1 ($\delta_{\text{H}}$ 4.97) of C-3. A series of HMBC correlations from HGlc-1 ($\delta_{\text{H}}$ 6.09) to C-28, from HRha-1 ($\delta_{\text{H}}$ 6.45) to CGlc-2 ($\delta_{\text{C}}$ 76.3), from HGlc-1 ($\delta_{\text{H}}$ 4.86) to CGlc-6 ($\delta_{\text{C}}$ 68.2), and from HGlc-1 ($\delta_{\text{H}}$ 5.16) to CRha-4 ($\delta_{\text{C}}$ 85.7) enabled the sugar chain of C-28 to be assigned as $\beta$-D-glucopyranosyl(1$\rightarrow$6)-[\beta-D-glucopyranosyl(1$\rightarrow$4)-$\alpha$-L-rhamnopyranosyl(1$\rightarrow$2)]-$\beta$-D-glucopyranoside. Thus, the structure of compound 1 was elucidated to be 3-O-$\beta$-D-xylopyranosyl-23-acetoxyolean-12-en-28-oic acid 28-O-$\beta$-D-glucopyranosyl(1$\rightarrow$6)-[\beta-D-glucopyranosyl(1$\rightarrow$4)-$\alpha$-L-rhamnopyranosyl(1$\rightarrow$2)]-$\beta$-D-glucopyranoside, a new oleanane triterpenoid saponin, named Bodinioside S.

**Figure 2.** Key HMBC correlation of compound 1.

Compound 2 was obtained as white amorphous powder. Its positive HR-ESI-MS spectrum indicated the molecular formula to be $\text{C}_{60}\text{H}_{95}\text{O}_{27}$ by the observation quasi-molecular ion peak $[\text{M} - \text{H}]^-$ at $m/z$ 1247.6070 and with the help of the NMR spectroscopic date, indicating 13 degrees of unsaturation. Detailed comparison of the $^1$H and $^{13}$C NMR spectral data (Tables 1 and 2) of 2 with those of 1 revealed that they were highly structural similar, except for the replacement of signals for the Glc’ at C-28 in 1 by the Ara in 2. Acid hydrolysis of 2 yielded L-rhamnose, D-glucose, L-arabinose and D-xylose as sugar residues as determined by GC analysis. In the $^1$H NMR spectrum of 2, five anomeric H-atom at $\delta_{\text{H}}$ 6.47 (br. s), 6.14 (d, $J = 7.8$ Hz), 5.62 (br. s), 5.12 (d, $J = 7.8$ Hz) and 4.86 (d, $J = 7.5$ Hz) correlated with anomeric carbon signals at $\delta_{\text{C}}$ 101.2, 94.4, 109.7, 107.0, and 107.2 in the HSQC spectrum, respectively, suggesting the presence of five sugar residues: one rhamnopyranosyl (Rha), one arabinopyranosyl (Ara), one xylopyranosyl (Xyl) and two glucopyranosyl (Glc and
Glc'-) units. The Xyl unit was still linked to C-3 ($\delta_C$ 82.8) of the aglycone based on the HMBC correlation between H$_{\text{Xyl}}$-1 (δ$_H$ 4.86) of Xyl and C-3 (δ$_C$ 82.8). A series of HMBC correlations from H$_{\text{Glc}}$-1 (δ$_H$ 6.14) to C-28, from H$_{\text{Rha}}$-1 (δ$_H$ 6.47) to C$_{\text{Glc}}$-2 (δ$_C$ 76.3), from H$_{\text{Ar}}$-1 (δ$_H$ 5.62) to C$_{\text{Glc}}$-6 (δ$_C$ 68.3), and from H$_{\text{Glc}}$-1 (δ$_H$ 5.12) to C$_{\text{Rha}}$-4 (δ$_C$ 85.7) enabled the sugar chain of C-28 to be assigned as α-L-arabinopyranosyl[(1→6)→β-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside. From the foregoing evidence, the structure of 1 was unequivocally determined to be 3-O-β-D-xylopyranosyl-23-acetoxy-olean-12-en-28-oic acid 28-O-α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside, and named Bodinioside T.

Compound 3 was isolated as white amorphous powder. The molecular formula was established as C$_{32}$H$_{58}$O$_{23}$ by positive HR-ESI-MS ($m/z$ 1139.5614, [M + Na]$^+$) and NMR spectral data, indicating 12 degrees of unsaturation. In the $^1$H NMR spectrum of 3, four anomeric H-atoms at δ$_H$ 6.45 (br. s), 6.15 (d, $J = 7.8$ Hz), 5.13 (d, $J = 7.8$ Hz) and 4.89 (d, $J = 7.8$ Hz) correlated with anomeric carbon signals at δ$_C$ 101.2, 94.5, 105.5 and 107.1 in the HSQC spectrum, respectively, suggesting the presence of four sugar residues: one rhamnopyranosyl (Rha), one xylopyranosyl (Xyl), and two glucopyranosyl (Glc and Glc') units. Detailed comparison of the $^1$H and $^{13}$C NMR spectra of 3 (Tables 1 and 2) with those of Bodinioside H [4] revealed that they were identical, except for the absence of signals for Xyl moiety on C-3. GC analysis after acid hydrolysis of 3 revealed that they were identical, except for the absence of Xyl and named Bodinioside V.

Compound 4 was obtained as white amorphous powder. It exhibited a quasi-molecular ion peak at $m/z$ 1205.5950 [M − H]$^+$ in the negative HR-ESI-MS spectrum, suggesting the molecular formula C$_{35}$H$_{60}$O$_{26}$, indicating 12 degrees of unsaturation. Besides one hydroxyl taking the place of an acetoxy group substituent on C-23, most NMR signals (1 and 2) of 4 were nearly identical to those of Bodinioside H [4]. Five anomeric H-atoms at δ$_H$ 6.38 (br. s), 6.11 (d, $J = 7.9$ Hz), 5.15 (d, $J = 7.6$ Hz), 5.02 (d, $J = 7.4$ Hz) and 4.85 (d, $J = 7.4$ Hz) in the $^1$H NMR spectrum were ascribed to D-xylose, D-glucose, and L-rhamnose, respectively, in combination with acid hydrolysis and GC analysis. The Xyl unit was assigned to C-3 (δ$_C$ 82.1) of the aglycone on the basis of the long-range correlation between H-1 (δ$_H$ 5.02) of Xyl and C-3. Meanwhile, a series of HMBC correlations from H$_{\text{Glc}}$-1 (δ$_H$ 6.11) to C-28 (δ$_C$ 176.7), from H$_{\text{Rha}}$-1 (δ$_H$ 6.38) to C$_{\text{Glc}}$-2 (δ$_C$ 77.6), from H$_{\text{Xyl}}$-1 (δ$_H$ 4.85) to C$_{\text{Glc}}$-6 (δ$_C$ 68.6), and from H$_{\text{Glc}}$-1 (δ$_H$ 5.15) to C$_{\text{Rha}}$-4 (δ$_C$ 85.6) clarified the linkage of Glc, Xyl and Rha units at C-28 as shown. The structure of compound 4 was, therefore, concluded to be 3-O-β-D-xylopyranosyl-23-hydroxy-olean-12-en-28-oic acid 28-O-α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside, and named Bodinioside V.

The molecular formula of Bodinioside W (5) was established as C$_{52}$H$_{84}$O$_{21}$ on the basis of the negative HR-ESI-MS from the quasi molecular ion peak at $m/z$ 1089.5454 [M + COOH]$^-$, indicating 11 degrees of unsaturation. In the $^1$H NMR spectrum of 5, four anomeric H-atoms at δ$_H$ 6.51 (br. s), 6.11 (d, $J = 8.0$ Hz), 5.01 (d, $J = 7.8$ Hz) and 4.85 (d, $J = 7.4$ Hz) correlated with anomeric carbon signals at δ$_C$ 101.3, 94.6, 106.7 and 105.4 in the HSQC spectrum, respectively. Acid hydrolysis of 5 yielded two D-xylose (Xyl), L-rhamnose (Rha) and D-glucose (Glc) as sugar residues by GC chromatography. Interpretation of its NMR data (Tables 1 and 2) revealed that the structure of compound 5 was closely related to compound 4, except for the presence of an additional Glc at C-28 of 4. Thus, the structure of compound 5 was 3-O-β-D-xylopyranosyl-23-hydroxy-olean-12-en-28-oic acid 28-O-β-D-xylopyranosyl(1→6)-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside, and named Bodinioside W.
Compound 6 was obtained as white amorphous powder. It exhibited a quasi-molecular ion peak at m/z 835.4459 [M + Na]⁺ in the positive HR-ESI-MS spectrum, suggesting the molecular formula of C₄₂H₆₈O₁₅, indicating nine degrees of unsaturation. The ¹H NMR spectrum (Tables 1 and 2) of compound 6 showed two signals for anomic protons at δ 1H 6.60 (1H, br. s) and 6.23 (1H, d, J = 8.1 Hz), which correlated with anomic carbon signals at δc 101.6 and 95.1 in the HSQC spectrum, respectively, suggesting the presence of two sugar moieties. Acid hydrolysis of 6 yielded L-rhamnose (Rha) and D-glucose (Glc) as sugar residues by GC chromatography. The NMR data of 6 were highly analogous to the sericoside [13], suggested that they had same 2, 3, 19, 23-tetrahydroxy-olean-12-en-28-oic acid as the aglycone, except for the presence of an additional Rha at C-28 of 6. The HMBC correlations from H₆[Glc]-1 (δH 6.23) to C-28, and from H₆[Rha]-1 (δH 6.60) to C₆[Glc]-2 (δc 75.7), adequately illustrated the structure of 6 as 2α, 3β, 19α, 23- tetrahydroxy-olean-12-en-28-oic acid 28-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, named Bodinioside X.

Compound 7 was obtained as white amorphous powder. It exhibited a quasi-molecular ion peak at m/z 835.4456 [M + Na]⁺ in the positive HR-ESI-MS spectrum, suggesting a molecular formula C₄₂H₆₈O₁₅, indicating nine degrees of unsaturation. The ¹H NMR spectrum (Tables 1 and 2) of 7 revealed six methyl signals at δ 1H 1.13 (3H, s), 1.39 (3H, s), 1.03 (3H, s), 1.19 (3H, s), 1.62 (3H, s), and 1.06 (3H, s) in correlation with carbons at δc 14.2 (C-24), 17.5 (C-25), 17.4 (C-26), 24.1 (C-27), 26.9 (C-29), and 16.6 (C-30) in the HSQC spectrum, respectively. The signal at δ 1H 5.55 (1H, br. s), corresponding to the carbon at δc 128.2 (C-12), coupled with δc 139.2 (C-13) in the ¹³C NMR spectrum. On the basis of the above spectroscopic data, compound 7 was suggested to possess an ursane-12-ene skeleton [6]. Comparison of its NMR spectroscopic data with those of niga-ichigoside F1 [14], suggested that they had the same 2, 3, 19, 23-tetrahydroxy-urs-12-en-28-oic acid as the aglycone. Detailed comparison of ¹H and ¹³C NMR spectral data of 7 with those of niga-ichigoside F1 indicated that they are highly structurally similar, except for the presence of an additional Rha at C-28 of 7. Acid hydrolysis of 7 yielded D-glucose (Glc) and L-rhamnose (Rha) as sugar residues by GC chromatography. The structure of compound 7 was, therefore, concluded to be 2α, 3β, 19α, 23-tetrahydroxy-urs-12-en-28-oic acid 28-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, and named Bodinioside Y.

The anti-influenza A virus activity of compounds 1–7 against strain A/WSN/33/2009 was evaluated in MDCK cells, and their cytotoxicity was measured in parallel with the determination of antiviral activity, using oseltamivir as a positive control. It was found that compounds 1–7 displayed no significant cytotoxicity at 50 μM concentration. Then, under this concentration, the in vitro potential anti-influenza A virus effects of all isolates were investigated. The results showed that compounds 2 and 5 exhibited moderate inhibition of influenza virus activities with inhibition rates of 35.33% and 24.08%, while the inhibition rate of the positive control (oseltamivir) was 71.20%.

3. Discussion

In summary, seven new triterpenoid saponins, including six oleanane triterpenoid saponins and a ursane one, named Bodiniosides S–Y, were isolated from the aerial parts of Elsholtzia bodinieri. Elucidation of their structures was performed based on extensive spectroscopic analyses. The anti-influenza activities of the isolates against A/WSN/33/2009 (H1N1) virus were investigated. The results demonstrated that compounds 2 and 5 exhibited potent inhibition of influenza virus activities, with inhibition rates of 35.33% and 24.08%; meanwhile, compounds 1, 3, 4, 6 and 7 were found to be inactive, with an inhibition rate lower than 10%. A previous study revealed that pentacyclic triterpenoids, including ursane, oleanane, and lupane types, have anti-influenza virus activity [15]. Our results further confirmed that the pentacyclic triterpenoids were active against influenza virus. This investigation should provide valuable information for further understanding of E. bodinieri.
4. Materials and Methods

4.1. General Experimental Procedures

Optical rotations were recorded using a Jasco DIP-370 digital polarimeter (Jasco, Tokyo, Japan). UV spectra were performed on a UV-210A spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Bio-Rad FTS-135 spectrophotometer (Bio-Rad Laboratories, California, CA, USA) with KBr pellets. The 1D- and 2D NMR spectra were run on Bruker DRX-600 instruments (Bruker BioSpin Group, Rheinstetten, Germany) with TMS as an internal standard. ESI-MS and HR-ESI-MS were measured with an API-Qstar-TOF instrument (Applied Biosystem/MSD Scieix, Concord, ON, Canada). GC analysis was taken on Agilent Technologies HP5890 gas chromatograph (Agilent Technologies Inc., Massy, France) with flame ionization detector. Semi-preparative HPLC was run on an Agilent 1200 liquid chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) with a ZORBAX SB-C18 (5 Å°, 9.4 × 250 mm) column. Column chromatography (CC) was carried out on silica gel (200–300 mesh, 80–100 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Diaion HP-20SS (63–150 mm, Mitsubishi Fine Chemical Industries Co., Ltd., Tokyo, Japan), ODS-C₁₈ (75 µm, YMC Co., Ltd., Tokyo, Japan), and Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden); thin-layer chromatography (TLC) was monitored by TLC plates (Si gel GF₂₅₄, Qingdao Marine Chemical Factory, Qingdao, China), and spots were visualized by spraying with 5% H₂SO₄–EtOH, followed by heating on a hot plate.

The purity (>95%) of compounds 1–7 was determined by HPLC.

4.2. Materials

The aerial parts of *E. bodinieri* were collected in Yuxi city, Yunnan Province, P. R. China, in May 2016, and identified by Dr. Jindong Zhong. A voucher specimen (KMUST 20160005) was deposited at the Laboratory of Phytochemistry, Faculty of Life Science and Technology, Kunming University of Science and Technology.

4.3. Extraction and Isolation

The powered air-dried aerial parts of *E. bodinieri* (15 kg) were extracted with 75% aq. Me₂CO (3 × 35 L, 24 h, each) at room temperature and filtered. The filtrate was concentrated in vacuo, and the resulting residue was extracted successively with CHCl₃, AcOEt and n-BuOH, respectively.

The n-BuOH extract (300.0 g) was separated over macroporous resin CC (Diaion HP-20SS) eluting with MeOH/H₂O (gradient30, 60, 90, and 100%, each 15 L) to obtain four fractions (Fr. A–D). Fr. C (eluted with 60% MeOH/H₂O, 86.5 g) was chromatographed successively over Sephadex LH-20 gel column (20%, 30%, 40%, 50%, 60%, and 100% MeOH/H₂O, each 8 L) to obtain subfractions Fr. C-1–C-6. Fr. C-1 (31 g) was isolated by ODS CC (eluted with 10%, 30%, 60%, and 100% MeOH/H₂O) to obtain subfractions Fr. C-1-1–C-1-4. Fr. C-1-3 (13 g) was chromatographed over silica gel CC (eluted with CHCl₃/MeOH 15: 1 to 0: 1) to yield Fr. C-1-3-1–C-1-3-5. Compounds 1 (tᵣ = 15.0 min, 5.6 mg) and 2 (tᵣ = 20.1 min, 6.2 mg) were purified from Fr. C-1-3-4 (167 mg) via semi-preparative HPLC (58% MeOH, 3 mL/min). Compounds 3 (tᵣ = 17.4 min, 4.3 mg) and 6 (tᵣ = 22.1 min, 4.8 mg) were purified from Fr. C-1-3-3 (103 mg) via semi-preparative HPLC (56% MeOH, 3 mL/min). Compound 4 (tᵣ = 14.6 min, 5.6 mg) was purified from Fr. C-1-3-2 (84 mg) via semi-preparative HPLC (52% MeOH, 3 mL/min). Fr. C-1-4 (2.5 g) was chromatographed over silica gel CC (eluted with CHCl₃/MeOH 15: 1 to 0: 1) to yield Fr. C-1-4-1–C-1-4-3. Compounds 5 (tᵣ = 15.6 min, 4.8 mg) and 7 (tᵣ = 17.6 min, 7.3 mg) were purified from Fr. C-1-4-2 (163 mg) via semi-preparative HPLC (55% MeOH, 3 mL/min).

4.3.1. Bodinioside S (1)

White amorphous powder. [α]D²⁶ = −30.43 (c = 0.23, MeOH), IR (KBr): 3441, 2942, 1722, 1635, 1384, 1255, 1045 cm⁻¹. UV λmax (MeOH) nm (logε): 204 (2.8). ESI-MS (neg.) m/z: 1277 [M − H]⁰, HR-ESI-MS (neg.) m/z: 1277.6148 [M − H]⁻, (Calcld. for C₆₁H₉₈O₂₈, 1278.6245). ¹H and ¹³C NMR: Tables 1 and 2.
4.3.2. Bodinioside T (2)

White amorphous powder. $\left[a\right]_{D}^{21.7} = -11.95 (c = 0.15, \text{MeOH}),$ ESI-MS (neg.) $m/z$: 1247 [M − H]$^-$, HR-ESI-MS (neg.) $m/z$: 1247.6070 [M − H]$^-$, (Calcd. for C$_{60}$H$_{96}$O$_{27}$, 1248.6139). $^1$H and $^{13}$C NMR: Tables 1 and 2.

4.3.3. Bodinioside U (3)

White amorphous powder. $\left[a\right]_{D}^{21.8} = -8.24 (c = 0.34, \text{MeOH}),$ ESI-MS (pos.) $m/z$: 1140 [M + Na]$^+$, HR-ESI-MS (pos.) $m/z$: 1139.5614 [M + Na]$^+$, (Calcd. for C$_{53}$H$_{88}$O$_{23}$, 1116.5716). $^1$H and $^{13}$C NMR: Tables 1 and 2.

4.3.4. Bodinioside V (4)

White amorphous powder. $\left[a\right]_{D}^{26.4} = -23.17 (c = 0.12, \text{MeOH}),$ ESI-MS (pos.) $m/z$: 1230 [M + Na]$^+$, HR-ESI-MS (neg.) $m/z$: 1205.5950 [M − H]$^-$, (Calcd. for C$_{58}$H$_{93}$O$_{26}$, 1205.5955). $^1$H and $^{13}$C NMR: Tables 1 and 2.

4.3.5. Bodinioside W (5)

White amorphous powder. $\left[a\right]_{D}^{26.5} = -23.01 (c = 0.23, \text{MeOH}),$ ESI-MS (neg.) $m/z$: 1044 [M − H]$^-$, HR-ESI-MS (pos.) $m/z$: 1089.5454 [M + COOH]$^-$, (Calcd. for C$_{52}$H$_{84}$O$_{21}$, 1044.5505). $^1$H and $^{13}$C NMR: Tables 1 and 2.

4.3.6. Bodinioside X (6)

White amorphous powder. $\left[a\right]_{D}^{24.6} = -20.43 (c = 0.16, \text{MeOH}),$ ESI-MS (pos.) $m/z$: 835 [M + Na]$^+$, HR-ESI-MS (pos.) $m/z$: 835.4459 [M + Na]$^+$, (Calcd. for C$_{42}$H$_{68}$O$_{15}$, 812.4558). $^1$H and $^{13}$C NMR: Tables 1 and 2.

4.3.7. Bodinioside Y (7)

White amorphous powder. $\left[a\right]_{D}^{24.2} = -10.69 (c = 0.26, \text{MeOH}),$ ESI-MS (pos.) $m/z$: 835 [M + Na]$^+$, HR-ESI-MS (pos.) $m/z$: 835.4456 [M + Na]$^+$, (Calcd. for C$_{42}$H$_{68}$O$_{15}$, 812.4558). $^1$H and $^{13}$C NMR: Tables 1 and 2.

4.4. Acid Hydrolysis for Sugar Analysis

Compounds 1–7 (1.0 mg for each compound) were hydrolyzed with 1 M HCl (0.4 mL) and heated at 90–100 °C for 5 h. The mixture was neutralized by the addition of Amberlite IRA400 (OH$^-$ form) and then filtered. The filtrate was dried in vacuo, dissolved in 0.2 mL of pyridine containing L-cysteine methyl ester (10 mg/mL) and reacted at 60 °C for 1 h. To this mixture, a solution (0.2 mL) of trimethylsilyl imidazole in pyridine (10 mg/mL) was added, and then heated at 60 °C for 1 h. The final mixture was directly analyzed by GC [30QC2/AC-5 quartz capillary column (30 m × 0.32 mm) with the following conditions: column temperature: 180/280 °C; programmed increase 3 °C/min; carrier gas: N$_2$ (1 mL/min); injection and detector temperature: 250 °C; injection volume: 4 µL; split ratio: 1/50]. The authentic samples D- and L-glucose, D- and L-xylose, L-arabinose, and L-rhamnose were treated in the same manner. Under these conditions, the retention times of authentic samples D- and L-glucose, D- and L-xylose, L-arabinose and L-rhamnose were 18.29, 18.87, 13.35, 14.01, 14.30 and 14.97 min, respectively. During our studies, identical retention times observed between the different hydrolysates and authentic standards.

4.5. Anti-Influenza Virus Activity

The anti-influenza virus activities of compounds 1–7 were evaluated, using influenza strain A/WSN/33/2009 (H1N1). For the inhibitory activity assays, compounds 1–7 were dissolved and then diluted with DMSO, using Oseltamivir as a positive control. MDCK cells were seeded into 96-well plates, incubated overnight and infected with influenza virus (MOI $\frac{1}{2}$ 0.1). The cells were suspended in DMEM supplemented with 1% fetal bovine serum (FBS), containing test compound and 2 mg/mL TPCK-treated trypsin, and a final
DMSO concentration of 1% was added in each well. After 40 h of incubation, Cell Titer-Glo reagent was added, and the plates were read, using a plate reader [15]. The inhibition rate was calculated by the following formula: inhibition rate (%) = \[1 - \left(\frac{\text{luminescence with compounds} - \text{luminescence with compounds and virus}}{\text{luminescence with DMSO} - \text{luminescence with DMSO and virus}}\right) \times 100\%\]. Assessment of anti-influenza virus activity was performed as described previously [16].

**Supplementary Materials:** The following are available online. The IR, HR-ESI-MS, and 1D and 2D NMR spectra of the seven compounds are available in the supplementary materials.

**Author Contributions:** L.Y., J.D., R.L. and J.Z. conceived and designed the experiments; L.Y. and J.D. isolated the compounds; L.Y., J.D. and J.Z. elucidated the structures; F.Y. carried out the biological assay and helped with the preparation of the manuscript; L.Y., J.D. and J.Z. wrote the paper; and J.Z. managed the research project. All authors have read and agreed to the published version of the manuscript.

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