A New Flavonoid From Leaves of *Ormosia xylocarpa*

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Abstract
A new flavonoid, 5, 4′-dihydroxy-7-methoxy-6-[(2-O-β-D-glucopyranoside)ethyl]-flavanone (1), along with 7 known compounds (2-8), was separated from the leaves of *Ormosia xylocarpa* and identified by a variety of spectroscopic techniques. Compounds 1-8 were subjected to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging experiment. Among them, compounds 1-4 showed potential antioxidant activities with IC₅₀ values of 1.23, 0.83, 0.36, and 0.021 mmol/L, respectively.

Keywords
*Ormosia xylocarpa*, chemical constituents, flavonoids, 5, 4′-dihydroxy-7-methoxy-6-[(2-O-β-D-glucopyranoside)ethyl]-flavanone, antioxidant activities

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Introduction
*Ormosia xylocarpa* Chun ex L. Chen, family Leguminosae, is a precious timber tree species, which is widely distributed in southern China.¹ It has high economic value for furniture and carving.² The plant has a long growth period, and practitioners have to operate for more than 40 years to get a good price for the timber. To maintain wood quality, pruning becomes a necessary annual step, resulting in a large number of branches and leaves being discarded in woodland and unusable. We have been committed to researching the development and utilization of *Ormosia* plant resources.³⁴⁵⁶⁷ Previously, we found that the extract of *O. xylocarpa* leaves showed promising antioxidant activity. To discover compounds with strong antioxidant activity, 8 compounds (1-8) were isolated from *Ormosia* plants for the first time. Also, the antioxidant activities of compounds 1-8 were preliminarily determined for the first time.

Results and Discussion
Compound 1 was isolated as yellow gum (MeOH). Its molecular formula of C₂₄H₂₈O₁₁ was determined based on the HRESIMS ion at m/z 515.15238 [M + Na]⁺ (calcd for C₂₄H₂₈O₁₁Na 515.15271), indicating 1¹ of unsaturation. The IR (KBr) spectrum showed absorptions attributable to hydroxyl (3386 cm⁻¹) and carbonyl (1895 cm⁻¹) groups (Figure 1 and Figure 2).

The ¹H-NMR spectrum of 1 (Table 1) revealed the presence of a 1,4-disubstituted benzene ring [δ_H: 7.33 (2H, d, J = 8.8 Hz) and 6.79 (2H, d, J = 8.8 Hz)].⁹ The characteristic hydrogen proton signal of a dihydroflavonoid [δ_H: 5.49 (1H, dd, J = 3.2, 12.8 Hz), 3.88 (1H, overlapped), and 2.70 (1H, d, J = 3.2 Hz)]¹¹,¹² an isolated aromatic hydrogen signal [δ_H: 6.24 (1H, s), 2 hydroxyl hydrogen signals [δ_H: 12.31 (1H, s, -OH) and 9.62 (1H, s, -OH)], along with a methoxy proton signal [δ_H: 3.83 (3H, s)].

The ¹³C-NMR and DEPT spectra exhibited 24 carbons resonances and showed the signals of one carbonyl carbon [δ_C: 197.4], 7 quaternary carbons [δ_C: 128.9, 160.2, 104.6, 162.0, 197.4],
The compound was confirmed to have a \( \beta \)-D-glucose moiety according to carbon signals \( \delta_c: 103.1, 73.7, 77.1, 70.2, 77.0, \) and 61.2) and a terminal proton signal \( \delta_h: 4.13 (1H, d, J = 8.0 \text{ Hz}) \) by comparison with literature data.\(^{10-13}\) These \( ^1 \text{H} \) and \( ^{13} \text{C} \) spectral data suggested that compound 1 was a dihydroflavonoid with a \( \beta \)-D-glucose moiety.

In the HMBC spectrum (Figure 2), the phenolic hydroxyl \( \delta_h: 12.31 (1H, s, 5-OH) \) was long-range coupled to C-5 \( \delta_c: 160.2) \), C-6 \( \delta_c: 104.6) \) and C-10 \( \delta_c: 102.5) \); H-8 \( \delta_h: 6.24 \) (1H, s]) to C-6 \( \delta_c: 165.6) \) and C-9 \( \delta_c: 162.0) \); the methoxy proton \( \delta_h: 3.83 (3H, s, 7-\text{OCH}_3) \) to \( \delta_c: 91.6) \) (C-8) and \( \delta_c: 165.6) \) (C-7); the phenolic hydroxyl \( \delta_h: 9.62 (1H, s, 4'-\text{OH}) \) to \( \delta_c: 158.0) \) (C-4') and \( \delta_c: 115.4) \) (C-3'); H-3' \( \delta_h: 6.79 (1H, d, J = 8.8 \text{ Hz, H-3'}) \) was long-range coupled to \( \delta_c: 128.9) \) (C-1'); H-6' \( \delta_h: 7.33 (1H, d, J = 8.8 \text{ Hz}) \) to \( \delta_c: 158.0) \) (C-4'); and H-2 \( \delta_h: 5.49 (1H, d, J = 3.2, 12.8 \text{ Hz}) \) to C-1' \( \delta_c: 128.9) \), C-2 \( \delta_c: 128.6) \) and C-9 \( \delta_c: 162.0) \). These HMBC signals and \( ^1 \text{H} \text{-}^1 \text{H} \text{ COSY correlation of H-2/H-3 \) (Figure 2) confirmed the structure of the dihydroflavonoid.

The connection of the glucose moiety was established based on the HMBC correlations (Figure 2) from the anomeric protons of \( \beta \)-glucopyranosyl moieties \( \delta_h: 4.13 (1H, d, J = 8.0 \text{ Hz, H-1'}) \) to \( \delta_c: 67.3) \) (C-12); from H-12 \( \delta_h: 3.73 (1H, d, d, J = 8.4, 10 \text{ Hz}) \) to the anomeric carbon of \( \beta \)-glucopyranosyl moieties at C-1" \( \delta_c: 103.1) \); from H-11 \( \delta_h: 2.78 (2H, m) \) to C-12 \( \delta_c: 67.3) \), C-6 \( \delta_c: 104.6) \), and C-7 \( \delta_c: 165.6) \); and \( ^1 \text{H} \text{-}^1 \text{H} \text{ COSY correlation of H-1"/H-2, H-2"/H-3", H-3"/H-4", H-4"/H-5", H-5"/H-6" and H-11/ H-12). Finally, compound 1 was deduced as 5, 4'-dihydroxy-7-methoxy-6-[(2-O-\( \beta \)-D-glucopyranoside) ethyl]-flavanone, a new compound named xylocarside. The structure of compound 1 is shown in Figure 1, and the \( ^1 \text{H} \text{-NMR and} \text{ } ^{13} \text{C-NMR data in Table 1.}

Seven known compounds (2-8) were also obtained from \( O. \text{ xylocarpa} \), and identified as zhebeiresinol (2),\(^{14} \) 2, 3-dihydroxy-1-
(4-hydroxy-3-methoxyphenyl)-propan-1-one (3), syringaresino-4'-O-β-D-glucoside (4), linaironoside A (5), alangionoside (6), 3β-hydroxyolean-12-en-28-al (7), and ursolic acid (8) by comparing their NMR data with those in the literature (Figure 1).

Compounds 1-8 and vitamin C (positive control) were evaluated for their antioxidant activities in vitro by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging assay. The results in Table 2 indicate that compounds 1-4 have significant antioxidant activity with IC₅₀ values of 1.23, 0.83, 0.36, and 0.021 mmol/L, respectively, while the IC₅₀ value of vitamin C was 0.055 mmol/L, suggesting that lignans are important constituents related to the antioxidant properties of O. xylocarpa.

Table 1. ¹H and ¹³C-NMR Spectroscopic Data of Compound 1 (400/100 MHz, DMSO-d₆).

| Position | δC | δH | Position | δC | δH |
|----------|----|----|----------|----|----|
| 2        | 78.9 | 5.49 (1H, dd, J = 3.2, 12.8 Hz) | 2',6' | 128.6 | 7.33 (2H, d, J = 8.8 Hz) |
| 3        | 42.2 | 3.38 (1H, overlapped) | 3',5' | 115.4 | 6.79 (2H, d, J = 8.8 Hz) |
| 4        | 197.4 |  | 4' | 158.0 |  |
| 5        | 160.2 |  | 1'' | 103.1 | 4.13 (1H, d, J = 8.0 Hz) |
| 6        | 104.6 |  | 2'' | 73.7 | 2.95 (1H, m) |
| 7        | 165.6 |  | 3'' | 77.1 | 3.12 (1H, m) |
| 8        | 91.6 | 6.24 (1H, s) | 4'' | 70.2 | 3.05 (1H, overlapped) |
| 9        | 162.0 |  | 5'' | 77.0 | 3.05 (1H, overlapped) |
| 10       | 102.5 |  | 6'' | 61.2 | 3.64 (1H, d, J = 5.6, 11.6 Hz) |
| 11       | 22.9 | 2.76 (2H, br s) | 5-OH | 12.31 | (1H, s) |
| 12       | 67.3 | 3.73 (1H, dd, J = 8.4, 10 Hz) | 3.41 (1H, d, J = 8.4 Hz) |
| 1'       | 128.9 |  |

Table 2. Antioxidant Activity Values of Compounds 1-8.

| Compounds | IC₅₀ (mmol/L) |
|------------|--------------|
| 5, 4'-dihydroxy-7-methoxy -6-[(2-O-β-D-glucopyranoside)ethyl] -flavanone (1) | 1.23 |
| Zhebeir esinol (2) | 0.83 |
| 2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one (3) | 0.36 |
| Syringaresino- 4'-O-β-D-glucose (4) | 0.021 |
| Linaironoside A (5) | >100 |
| Alangionoside (6) | >100 |
| 3β-hydroxyolean-12-en-28-al (7) | >100 |
| Ursolic acid (8) | >100 |
| Vitamin C | 0.055 |

Experimental

General Experimental Procedures

¹H-NMR, ¹³C-NMR, HMBC, HMQC, and ¹H-¹H COSY spectra were obtained using a Bruker-400 spectrometer, and high resolution electrospray-ionization mass spectra with an Agilent 6520 HPLC-Q-TOF. High-performance liquid chromatography-diode array detection-mass spectrometric analysis was performed using a Waters 2690/5-W2998 series system with a Dikma Diamonsil (C18 250 × 4.6 mm, 5 µm) column. For preparative HPLP, a Shimadzu LC-8AD instrument with a SPD-20A detector was used. Column chromatography was performed with silica gel (200-300 mesh, Yantai Jiangyou Silicone Development Co., Ltd), PRP-512A macroporous adsorbent resin (Beijing Sunflower Technology Development Co.), and Sephadex LH-20 (GE).

Plant Material

The leaves of O. xylocarpa, collected from Shaxian, Sanming City, Fujian Province, People’s Republic of China (PRC), were identified by Professor Xiaoxing Zou from Fujian Agriculture and Forestry University (FAFU), PRC. The voucher sample of the plant (NO.20190812) is stored in the Engineering Research Institute of Conservation, Utilization of Natural Bioresources, FAFU, Fuzhou, PRC.

Extraction and Isolation

The naturally dried leaves of O. xylocarpa (10 kg) were smashed through a 20-mesh sieve and reflux extracted with 70% EtOH (2 × 100 L) to give an extract (1.86 kg). This was uniformly dispersed in methanol and mixed with diatomite (2.7 kg) and successively reflux extracted twice with ethyl acetate (EtOAc) and ethanol (EtOH). The EtOAc fraction (407 g) was subjected...
to column chromatography on polyamide (60-90 mesh) and eluted with an EtOH – H2O gradient (0, 30%, 50%, 60%, 70%, and 95%) to yield 18 fractions (Fr.1-Fr.18). Fr.1 (87.3 g) was subjected to column chromatography on silica gel (200-300 mesh) and eluted with a CH2Cl2- MeOH gradient (30:1-0:1) to yield 10 fractions (Fr.1.1-Fr.1.10). Fr.1.1 (3.1 g) was eluted with a gradient of ethanol-water (10%, 30%, 60%, 90%, 95% ethanol) through a PRP-512A macroporous adsorption resin, to afford 6 fractions (Fr.1.1.1-Fr.1.1.6) and obtain compound 2 (20 mg). Fr.1.1.3.3 (7.8 mg) was purified using semi-preparative HPLC (MeOH - H2O (27:73), vol/vol, 3.0 mL/min, 210 nm) resulting in the isolation of compound 3 (2.9 mg). Fr.1.2 (4.46 g) was eluted with a gradient of ethanol-water (10%, 30%, 60%, 90%, 95% ethanol) through a PRP-512A macroporous adsorption resin to afford 6 fractions (Fr.1.2.1-Fr.1.2.6). Fraction 1.2.2 (168 mg) was purified by semi-preparative HPLC (MeOH - H2O (50:50), vol/vol, 3.0 mL/min, 210 nm) to yield compounds 4 (4.1 mg) and 5 (3.4 mg). Fr.1.2.4 (4.68 g) was eluted with a gradient of ethanol-water (10%, 30%, 60%, 90%, 95% ethanol) through a PRP-512A macroporous adsorption resin to afford 6 fractions (Fr.1.2.1-Fr.1.2.6). Fr.1.2.5 (23 mg) was purified by semi-preparative HPLC (MeOH - H2O (45:55), vol/vol, 8.0 mL/min, 210 nm) to yield compound 6 (1.1 g). Fr.1.2.6 (0.57 g) was eluted with a PE (light petroleum) - EtOAc (ethyl acetate) gradient (50:1-0:1) through a silica gel column (200-300 mesh) to yield 23 fractions (Fr.10.1-Fr.10.23). Fr.10.10 (1.1 g) was obtained with a CH2Cl2-EtOAc gradient (100:1) to yield 5 fractions (Fr.10.10.1-Fr.10.10.5). Fr.10.10.2 (86.4 mg) was purified using semi-preparative HPLC (CH2CN - H2O (27:73), vol/vol, 3.0 mL/min, 210 nm) resulting in the isolation of compound 7 (9 mg). Fr.10.12 (604.2 mg) was eluted with a n-hexane- EtOAc gradient (10:1) through a silica gel column to obtain 8 fractions (Fr.10.12.1-Fr.10.12.8). Fr.10.12.5 (23 mg) was eluted with a cyclohexane-EtOAc gradient (8:1) through a silica gel column to obtain compound 8 (2.9 mg).

**DPPH Free Radical Scavenging Assay**

Methanol solutions of compounds 1-8 and vitamin C were prepared in concentrations of 0.01, 0.02, 0.05, 0.1, 0.25, and 0.5 mg/mL, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) in a concentration of 0.2 mmol/L DPPH. One hundred microliter DPPH working solution was mixed with 100 μL of either vitamin C solution or test compound solution and stored at room temperature away from light for 30 min. Then, the absorbance was measured at 517 nm. DPPH free radical scavenging rate = [1-(A1-A2)/ A0] × 100%, where A0 is the absorbance of DPPH working solution + absorbance of methanol; A1 is the absorbance of DPPH working solution + absorbance of samples; A2 is the absorbance of methanol + absorbance of the sample.

**Conclusions**

A new flavonoid (1) and 7 known compounds (2-8) were discovered from the 70% ethanol extract of *Ormosia xylocarpa* leaves. All compounds were obtained from the *Ormosia* genus for the first time. In addition, all compounds were screened for their antioxidant activities. Compounds 1-4 exhibited significant in vitro antioxidant activities, and compound 4 showed equivalent DPPH radical scavenging ability to the positive control.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Supplemental Material**

Supplemental material for this article is available online.

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