

# Chemical Constituents from The Leaves and Twigs of *Magnolia Decidua*

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(Received July 26, 2022; Revised December 09, 2022; Accepted December 15, 2022)

**Abstract:** A previously undescribed phenolic glycoside (manglycoside A, **1**), as well as 16 identified compounds (**2–17**), were isolated from the leaves and twigs of the 'vulnerable' plant *Magnolia decidua* (Magnoliaceae). Comprehensive spectroscopic investigation and chemical transformations revealed the structure of the previously unknown compound **1**. Among the isolates, honokiol (**6**) and liriodenine (**14**) were discovered to exhibit anti-inflammatory activities in LPS-induced RAW 264.7 cells via reducing nitric oxide (NO) formation, with IC<sub>50</sub> values of 4.3 and 17.3 μM, correspondingly.

**Keywords:** Magnoliaceae; *Magnolia decidua*; phenolic glycoside; manglycoside A; anti-inflammatory. © 2023 ACG Publications. All rights reserved.

## 1. Introduction

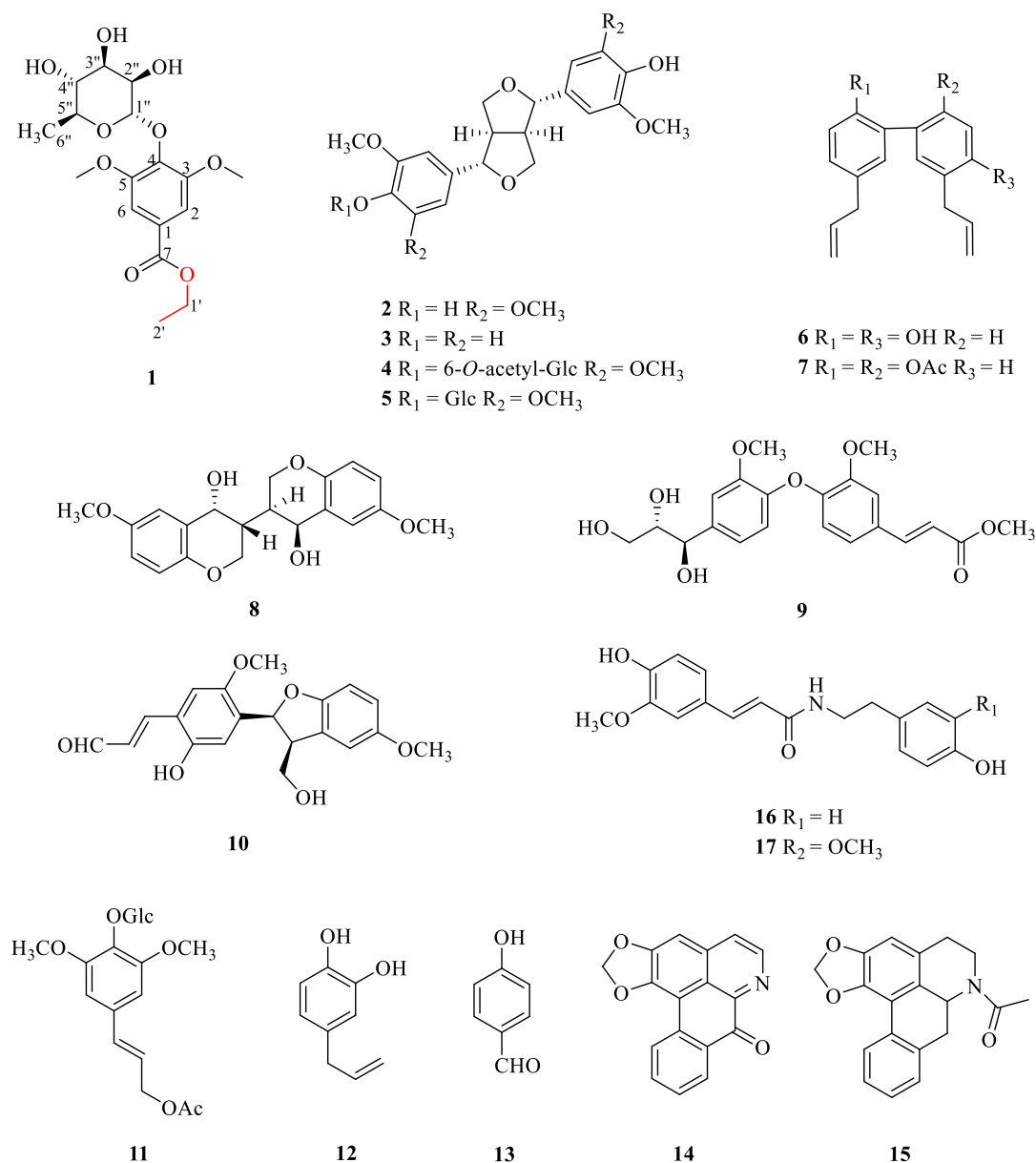
*Magnolia*, a member of the Magnoliaceae family, has approximately 40 species worldwide, with half found in China [1]. Several *Magnolia* species are produced as ornamental plants and as TCM (Traditional Chinese Medicines) for treating constipation and cough [2]. Lignans and alkaloids were often encountered from *Magnolia* species [3,4]. A few sesquiterpenoids and norsesquiterpenoids of the megastigmane-type (with PTP1B inhibitory activities) were extracted from *M. aromatica* branches and leaves [5], which is a Magnoliaceae plant classified as "endangered" and included in the CPRDB (China Plant Red Data Book) [6]. *M. decidua* Q. Y. Zheng is a deciduous shrub featuring off-white trunk bark, elliptical papery leaves, and single flowers at the extremities of its branches. Due to its limited natural

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reproduction capacity, the IUCN (the International Union for Conservation of Nature and Natural Resources) has listed it as a Chinese "vulnerable" species. Additionally, *M. decidua* is listed in the "second grade" of List of National Key Protected Wild Plants in China [7].

Current scientific studies on rare and endangered plants (REPs) have revealed that the complex molecular structures of REPs' original natural products continue to offer medicinal chemists excellent chemical types for drug discovery [8,9]. Therefore, it is imperative to protect and properly utilize these REPs before they become extinct. As far as we know, *M. decidua* has never been studied in terms of phytochemical or pharmacological properties. Hence, in the current study, the chemical constituents of *M. decidua*'s leaves and twigs were examined as part of ongoing phytochemical research on REPs endemic to China [10-12]. As a result, an unknown phenolic glycoside (**1**) and 16 recognized compounds (**2-17**) were purified and identified (see Figure 1). Their isolation, structural characterization, and anti-inflammatory activities against generating nitric oxide induced by lipopolysaccharide (LPS) in mice macrophage RAW 264.7 cells are discussed in the present work.



**Figure 1.** Chemical structures of compounds **1-17**.

## 2. Experimental

### 2.1. General Experimental Procedures

The U.V. and I.R. spectra were acquired with a Beckman DU 640 spectrophotometer and Nicolet 5700 spectrophotometer using KBr discs, respectively. An SGW-1 digital polarimeter was used to measure optical revolutions. Solvent residual signals served as controls, and the Bruker 400 MHz spectrometers were used to obtain 1D/2D NMR spectra. Using a Waters Quattro Micro API mass spectrometer, HRESIMS spectra were collected. An SPD-20A PAD detector and a Shimadzu CBMA-20A were used for semi-preparative HPLC. A TSK-amide Gel column (5  $\mu$ m, 4.6 x 250 mm) and an X-Bridge C18 column (5  $\mu$ m and 10 x 250 mm) were used. For column chromatography (CC), Sephadex LH-20 gel (Amersham Biosciences; 40-70  $\mu$ m) and Silica gel (Qingdao Haiyang Chemical Group China.; 200-300 mesh) were utilized. G 60 F254-coated glass (Yantai Zi Fu Chemicals Co. Yantai, China) TLC plates were utilized for TLC (Thin-layer chromatography) analysis.

### 2.2. Plant Material

*M. decidua* leaves and twigs were collected in August 2019 from Hutian, Yuanzhou District, Yichun, Jiangxi Province, China. Another co-author (Mr Zhi-Yong Xiao) performed the plant identification. The specimen (Voucher No. TZU20190803) was deposited to the Herbarium of Institute of Natural Medicine and Health Products, School of Pharmaceutical Sciences at Taizhou University.

### 2.3. Extraction and Isolation

7 kg of *M. decidua* air-dried branches and leaves were crushed, and after being immersed five times in 30 L of 95% MeOH at room temperature, 424 g of crude extract was prepared. With n-hexane and EtOAc, the crude extract was subsequently separated. Eight major fractions (Fr. 1–8) were produced by chromatographing the EtOAc extract (240 g) using gradient mixtures of petroleum ether-EtOAc and silica gel column (1:0→0:1, v/v). Fr. 4 (10 g) was exposed to a silica gel CC and treated with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (1:0 to E.A. neat) to recover five fractions (Fr. 4a-4e). Compounds **5**, **14**, and **15** (14, 4, and 5 mg, respectively) were purified from Fr. 4b (800 mg) using semi-preparative HPLC and Sephadex LH-20 gel CC with MeOH. Compounds **3** and **16** (both 5 mg) were separated from Fr. 4c (150 mg) using semipreparative HPLC (40% MeCN/H<sub>2</sub>O, v/v, 3 mL/min). Fr. 7 (3.5 g) was divided into six subfractions (Fr. 7a–7f) using a Sephadex LH-20 column and MeOH elution. Compounds **1**, **2**, and **13** (4, 5, and 6 mg, respectively) were acquired by preparative HPLC (30% MeCN/H<sub>2</sub>O, v/v, 3 mL/min) after further purification of Fr. 7c (210 mg). With the help of semipreparative HPLC (65% MeCN/H<sub>2</sub>O, v/v, 3 mL/min), the following compounds were obtained from Fr. 7d (190 mg): **17**, **9**, **8**, **7**, and **4** (4, 2, 3, 8 and 10 mg respectively). Fr. 7e (90 mg) was further filtered by semipreparative HPLC (CH<sub>3</sub>CN-H<sub>2</sub>O, 60:40, v/v, 3 mL/min), which resulted in the identification of compounds **12**, **11**, **10**, and **6** (5, 4, 6, and 21 mg respectively).

*Manglycoside A* (**1**): white amorphous powder;  $[\alpha]_D^{25}$  -104 (*c* 0.1, MeOH); HR-EIS-MS, *m/z*: 395.1326 [M + Na]<sup>+</sup> (Calcd. for C<sub>17</sub>H<sub>24</sub>O<sub>9</sub>Na<sup>+</sup>, 395.1318); I.R. (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3427, 1684, 1598, 1548, 1454, 1115, 1027, and 568; U.V. (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 270 (3.9), 218 (3.3). <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$ : 3.80 (6H, s, 3,5-OCH<sub>3</sub>), 1.06 (3H, d, *J* = 6.4 Hz, H-6''), 3.99 (1H, dd, *J* = 6.4, 9.6 Hz, H-5''), 3.24 (1H, t, *J* = 9.6 Hz, H-4''), 3.65 (1H, dd, *J* = 3.2, 9.6 Hz, H-3''), 3.90 (1H, br d, *J* = 3.2 Hz, H-2''), 5.19 (1H, br s, H-1''), 1.32 (3H, t, *J* = 7.2 Hz, H-2'), 4.31 (2H, q, *J* = 7.2 Hz, H-1'), 7.24 (2H, s, H-2, 6); <sup>13</sup>C NMR (100 MHz, DMSO)  $\delta$ : 56.6 (3,5-OCH<sub>3</sub>), 18.2 (C-6''), 70.6 (C-5''), 72.0 (C-4''), 70.7 (C-3''), 70.9 (C-2''), 102.6 (C-1''), 14.7 (C-2'), 61.3 (C-1'), 165.8 (C-7), 138.7 (C-4), 153.4 (C-3,5), 106.8 (C-2,6), 125.9 (C-1).

Chemical Constituents from *Magnolia Decidua*2.4. Acid Hydrolysis of Compound **1**

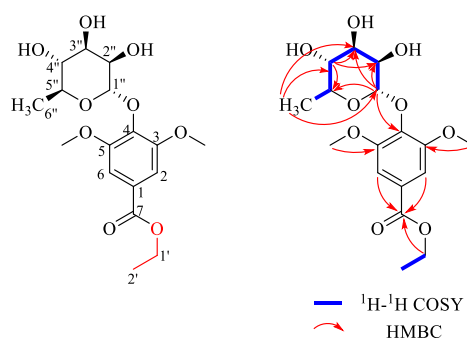
In 10 mL of 4.0 M HCl, 3 mg of compound **1** was dissolved before refluxing at 105 °C for 6 hours. After reaching room temperature, the solution was extracted with CHCl<sub>3</sub>. With the help of a TSK-gel Amide-80 column and a reference sample of *L*-rhamnose (CAS#10030-85-0), the supernatant liquid was purified to yield glucoside (0.8 mg), which was then determined by HPLC-ELSD. Then its optical rotation value of  $[\alpha]_{\text{D}}^{20} +10$  (*c* 0.08, H<sub>2</sub>O) {reference:  $[\alpha]_{\text{D}}^{20} +9$  (*c* 0.06, H<sub>2</sub>O)} [13]}, the *L*-configuration, was validated.

## 2.5. Nitric Oxide Production Analysis

Incubation conditions for the RAW 264.7 cells consisted of 1% penicillin-streptomycin (100 U/mL), 10% FBS (fetal bovine serum), and DMEM (Dulbecco's modified Eagle's medium). The cells were preincubated for 24 hours in a 96-well plate before being administered with the compounds and for 24 h treated with LPS (500 ng/mL). The quantity of nitrite in the supernatant was determined using a Griess test to quantify NO (nitrogen oxide) production. At 540 nm, absorbance was measured with curcumin as a control sample.

## 3. Results and Discussion

The *Manglycoside A* (**1**) was obtained as a white, amorphous powder. Through analysis of the HRESIMS at *m/z* 395.1326 ( $[M + Na]^+$ ; calculated for C<sub>17</sub>H<sub>24</sub>O<sub>9</sub>Na<sup>+</sup>, 395.1318) and <sup>13</sup>C NMR data, its chemical formula was established to be C<sub>17</sub>H<sub>24</sub>O<sub>9</sub>, suggesting six degrees of unsaturation. Carbonyl (1684 cm<sup>-1</sup>) and hydroxyl (3427 cm<sup>-1</sup>) groups were detected in the I.R. spectrum. 17 carbon atoms included seven sp<sup>2</sup> carbon atoms, four methyl groups, one sp<sup>3</sup> methylene, and five sp<sup>3</sup> methines, each of which was attributed to a sugar moiety, an ethoxy group, and a benzoate group. The above data indicated that the chemical structure of **1** was similar to that of the known methyl syringate *α*-*L*-rhamnoside [14], except that the methoxy group at C-7 was substituted by the ethoxy group in **1**, which was supported by the key HMBC correlations of H-1' to C-7 ( $\delta_{\text{C}}$  165.8) and H-2' to C-1' ( $\delta_{\text{C}}$  61.3), as well as the <sup>1</sup>H-<sup>1</sup>H COSY correlations from H-1' to H-2' (see Figure 2). Furthermore, the small coupling constant ( $J_{\text{H-1'}/\text{H-2'}}$ ) of the anomeric proton resonating at  $\delta_{\text{H}}$  5.19 (1H, br s, H-1'") suggested that the sugar moiety is *α*-rhamnoside. The mono sugar residue was confirmed by the acid hydrolysis of **1**, followed by a chiral HPLC analysis. The *L*-configuration was confirmed based on the optical rotation value of the mono sugar  $[\alpha]_{\text{D}}^{20} +10$  (*c* 0.08, H<sub>2</sub>O) {reference:  $[\alpha]_{\text{D}}^{20} +9$  (*c* 0.06, H<sub>2</sub>O)} [13]}. Thus, compound **1** was established as ethyl syringate *α*-*L*-rhamnoside.



**Figure 2.** Key <sup>1</sup>H-<sup>1</sup>H COSY and HMBC correlations of compound **1**.

The veracity of compound **1**, an ethoxy-substituted phenolic acid, as a natural product might be suspicious. From a structural point of view, **1** could be readily generated from the esterification between syringic acid-4-*O*-*α*-*L*-rhamnoside and ethanol. Indeed, compound **1** was detectable in the MeOH crude extract by HPLC and TLC analyses, providing conclusive evidence that it is a naturally occurring compound rather than a purification artefact. Naturally occurring compounds with ethoxy functionality

were often reported, such as (7-methoxy-8-[1'-ethoxy-2'-hydroxy-3'-methyl-but-3'eny]-coumarin) [15] and 4-hydroxy-6-ethoxy-2-[(10'Z,13'Z)-10',13',16'-heptadecatrienelresorcinol [16].

Other isolates were identified to be (-)-syringaresinol (**2**) [17], (+)-pinoresinol (**3**) [18], pharsyringaresinol (**4**) [19], syringaresinol  $\beta$ -D-glucoside (**5**) [20], honokiol (**6**) [21], magnolol diacetate (**7**) [22], *rel*-(3*R*,3'*S*,4*R*,4'*S*)-3,3',4,4'-tetrahydro-6,6'- dimethoxy[3,3'-bi-2H-benzopyran]-4,4'-diol (**8**) [23], rhemaneolignan A (**9**) [24], melianoninol (**10**) [25], 9-acetoxy syringin (**11**) [26], 4-allylcatechol (**12**) [27], *p*-hydroxybenzaldehyde (**13**) [28], liriodenine (**14**) [29], *N*-acetyldehydroanonnaine (**15**) [30], *N-trans* feruloyltyramine (**16**) [31], and *N-trans*-feruloyl-3-methoxytyramine (**17**) [32] by contrasting their physiological and spectroscopic characteristics with those that have been previously reported.

The methanol extract of *M. decidua* leaves and twigs demonstrated anti-inflammatory action (IC<sub>50</sub> of 39.79  $\mu$ g/mL) by inhibiting NO formation in macrophage RAW 264.7 cells of mice induced by lipopolysaccharide. Consequently, the designated compounds (**1**, **6**, **8–10**, **14**, **15**, **17**) were examined for their anti-inflammatory activities. As a result, compounds **6** (IC<sub>50</sub> = 4.3  $\mu$ M) and **14** (IC<sub>50</sub> = 17.3  $\mu$ M) exhibited considerable anti-NO production activities, which are comparable to the previously reported data (IC<sub>50</sub> = 3.3  $\mu$ M for **6**; IC<sub>50</sub> = 10.5  $\mu$ M for **14**) [33,34]. The rest did not show detectable activity at 20  $\mu$ M (see Table 1). The IC<sub>50</sub> value for the positive control, curcumin, was determined to be 2.6  $\mu$ M.

**Table 1.** Curcumin and compounds **1**, **6**, **8–10**, **14**, **15**, and **17** inhibit nitrogen oxide production in LPS-induced RAW264.7 cells<sup>a</sup>

Compound	Activity IC <sub>50</sub> ( $\mu$ M)	Compound	Activity IC <sub>50</sub> ( $\mu$ M)
<b>1</b>	>20	<b>10</b>	>20
<b>6</b>	4.3 $\pm$ 0.87	<b>14</b>	17.3 $\pm$ 0.64
<b>8</b>	>20	<b>15</b>	>20
<b>9</b>	>20	<b>17</b>	>20
Curcumin	2.6 $\pm$ 0.54		

#### 4. Conclusions

This is a preliminary phytochemical work on the endangered plant *M. decidua* (leaves and twigs). A novel naturally occurring phenolic glycoside (manglycoside A, **1**) and sixteen known compounds (**2–17**) were obtained and identified in the present study. Compound **1** could be used as a chemotaxonomic marker within the genus *Magnolia*. One of the isolates, honokiol (**6**) as a major constituent, reduced the amount of NO that RAW264.7 cells produced after being stimulated by LPS. Similar to the previous works [10,11], the above results showed the significant contribution of plant diversity protection in supporting chemical diversity and the prospective development of new therapeutics.

#### Acknowledgements

This work was supported by the Science and Technology Projects of Taizhou [Nos.1901ny11 and 21ywb80], the Open Foundation of Key Laboratory of Tropical Medicinal Plant Chemistry of Hainan Province [No. RDZH2021S002], and Zhejiang Provincial Natural Science Foundation of China [No. LQ22H280014].

#### Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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