Structural Characterization and Assessment of Anti-Inflammatory Activities of Polyphenols and Depsidone Derivatives from *Melastoma malabathricum subsp. normale*

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Abstract: The roots of *Melastoma malabathricum subsp. normale* (D. Don) Karst. Mey have been used in traditional ethnic medicine systems in China to treat inflammation-triggered ailments, such as trauma, toothache, and fever. Therefore, the aim of this study is to screen for compounds with anti-inflammatory activity in the title plant. The extract of *M. malabathricum subsp. normale* roots was separated using various chromatographic methods, such as silica gel, ODS C18, MCI gel, and Sephadex LH-20 column chromatography, as well as semi-preparative HPLC. One new complex tannin, named whiskey tannin D (1), and an undescribed tetracyclic depsidone derivative, named guanxidone B (2), along with nine known polyphenols (2–10) and three known depsidone derivatives (12–14) were obtained from this plant. The structures of all compounds were elucidated by extensive NMR and CD experiments in conjunction with HR-ESI-MS data. All these compounds were isolated from this plant for the first time. Moreover, compounds 1–4, 8, and 10–14 were obtained for the first time from the genus *Melastoma*, and compounds 1, 2, and 11–14 have not been reported from the family Melastomataceae. This is the first report of complex tannin and depsidone derivatives from *M. malabathricum subsp. normale*, indicating their chemotaxonomic significance to this plant. Compounds 1–12 were investigated for their anti-inflammatory activities on the production of the nitric oxide (NO) in lipopolysaccharide (LPS)-stimulated RAW264.7 cells, and compounds 1, 11, and 12 showed anti-inflammatory activities with IC50 values of 6.46 ± 0.23 μM, 8.02 ± 0.35 μM, and 9.82 ± 0.43 μM, respectively. The structure–activity relationship showed that the catechin at glucose C-1 in ellagitannin was the key to its anti-inflammatory activity, while CH3O- at C-16 of aromatic ring A in depsidone derivatives had little effect on its anti-inflammatory activity. The study of structure–activity relationships is helpful to quickly discover new anti-inflammatory drugs. The successful isolation and structure identification of these compounds, especially complex tannin 1, not only provide materials for the screening of anti-inflammatory compounds, but also provide a basis for the study of chemical taxonomy of the genus *Melastoma*.

Keywords: *Melastoma malabathricum subsp. normale* (D. Don) Karst. Mey; *Melastoma*; polyphenols; complex tannin; depsidone derivatives; anti-inflammatory

1. Introduction

The genus *Melastoma* (Melastomataceae), with approximately 100 species, is widespread in southern Asia, northern Oceania, and the Pacific islands, and a total of 9 species and 1 variety are found in China [1]. Some species of this genus are used for the treatment
of diarrhea, dysentery, leucorrhoea, ulcers, and wounds [2]. Flavonoids, tannins, phenylpropanoids, organic acids (esters), terpenoids, and other components were previously characterized from this genus [3]. Some of them exhibited anti-inflammatory [4], hemostatic activity [5], anticoagulant activity [6], antioxidant activity [8,9], hepatoprotective activity [10], gastroprotective activity [11], hypoglycemic activity [12], and cytotoxic activities [13]. *Melastoma malabathricum subsp. normale* (D. Don) Karst.Mey, a shrub of the family Melastomataceae, grows mainly in Xizang, Sichuan, Guangxi, and Fujian provinces of China [1]. Its roots have been used in Zhuang and Yao medicines for the treatment of inflammation-triggered ailments, such as trauma, toothache, and fever [14,15]. With the aim to find compounds with anti-inflammatory activity in the title plant, the roots of *M. malabathricum subsp. normale* were extracted by 80% aqueous acetone, and subsequently separated using silica gel, MCI, ODS C18, and Sephadex LH-20 column chromatography, as well as semi-preparative HPLC to yield ten polyphenols and four depsidone derivatives. The structures of these compounds were characterized by experimental and published spectroscopic data analyses. As we all know, complex tannin is a kind of flavono-ellagitannin, which has a unique C-C condensation structure of C-glycoside tannin (vescalagin-type or stachyurin-type) and flavane-3-alcohol. To date, these compounds have only been found in a few plant families, including Combretaceae, Myrtaceae, Melastomataceae, Fagaceae, and Theaceae [16]. Compound 1 is the only complex tannin isolated from *M. malabathricum subsp. normale*, and its analogs have also been reported from *M. malabathricum* L. in the family Melastomataceae [17], suggesting their closely chemotaxonomic relationships between *M. malabathricum subsp. normale* and *M. malabathricum* L. Depsidone derivatives have never been reported from the family Melastomataceae [3,18]. These compounds enrich the chemical diversity of *M. malabathricum subsp. normale* and provided a basis for the chemotaxonomic studies of the species of the genus *Melastoma*. Moreover, the anti-inflammatory activities of compounds 1–12 were investigated to develop polyphenols or depsidone derivatives as a novel anti-inflammatory drug. In the present study, the isolation and structural elucidation of compounds 1 and 11, as well as the anti-inflammatory activities of 1–12, are reported in detail.

### 2. Results and Discussion

The EtOAc fractionation and purification of the 80% aqueous acetone extract of *M. malabathricum subsp. normale* roots using various chromatographic methods yielded ten polyphenols (1–10) and four depsidone derivatives (11–14). The known compounds 2–10 and 12–14 were identified by analysis of mass spectral data, the NMR spectral data, specific rotations, and/or melting point data as whiskey tannin B (2) [19]; castalagin (3) [20]; 3,3′-dimethoxy ellagic acid (4) [21]; 3,3′,4-trimethoxyellagic acid (5) [22]; 3,3′,4′-trimethoxyellagic acid-4-O-β-D-glucopyranoside (6) [23]; 3,3′-dimethoxy ellagic acid-4-O-α-D-xylopyranoside (7) [24]; 1,2,4-benzenetriol (8) [25]; 1,4,6-tri-O-galloyl-glucose (9) [26]; 6-O-galloyl-glucose (10) [27]; guanxidone A (12) [28]; excelsione (13) [29]; and dioxepin-11-one (14) [30]. The structures of 1–14 are shown in Figure 1. All these compounds were obtained from this species for the first time. Moreover, compounds 1–4, 8, and 10–14 were isolated for the first time from the genus *Melastoma*, and compounds 1, 2, and 11–14 were reported from the family Melastomataceae for the first time.
Figure 1. Structures of compounds 1–14.
2.1. Structure Elucidation

Compound 1, a pale brown amorphous powder, shows the positive coloration characteristic of complex tannin when reacting with anisaldehyde-sulfuric acid (pink) and NaNO₂-AcOH (brown) reagent. A deprotonated molecular ion peak at \( m/z \) 1249.1580 [M – H]⁻ (calcld, 1249.1586) was observed in the HR-ESI-MS spectrum, indicating that the molecular formula of 1 is \( \text{C}_{28}\text{H}_{42}\text{O}_{32} \). The \(^1\)H NMR data (Table 1) revealed at least two hexahydroxydiphenoyl (HHDP) groups at \( \delta \) 6.63 (s), 6.80 (s), and 7.02 (s); an ethoxyl at \( \delta \) 4.21 (q, 7.1, 2H) and 1.20 (t, 7.1, 3H). As shown in Figure 1, the \(^1\)H-COSY correlations among methylene (\( \delta \) 3.86 and 4.81) and five methine protons (\( \delta \) 4.36–5.53) revealed a polyalcohol unit, which exhibited similar NMR data to the open-chain glucose core of stenophyllanine B [31]. The \(^13\)C NMR data (Table 1) revealed six ester carbonyl groups at \( \delta \) 170.3, 168.2, 168.0, 167.8, 167.4, and 164.2. Five downfield signals at \( \delta \)C 63.6–83.2 suggested that the hydroxyl at C-2–C-6 was esterified. The large difference in chemical shifts between H-6a (\( \delta \) 4.81) and Hb (\( \delta \) 3.86) suggested one of the HHDP moieties was located at C-4 and C-6, which can be explained by the anisotropic effect of a C-6 ester carbonyl group. It is restrained to be rigidly coplanar with one of the C-6 methylene protons in the eleven-membered diester ring, so the proton was placed in a strongly deshielding environment [32]. This was also supported by the correlations of H-4 and H-6 with carbonyl carbons C\(^{\text{HHDP-7}}\) (\( \delta \) 164.2) of the HHDP group and C\(^{\text{HHDP-7′′′′}}\) (\( \delta \) 167.4) of the HHDP group in the HMBC spectrum (Figure 2). The carbon signals at \( \delta \) 201.4 (C\(^{\text{3′}}\)), 170.3 (C\(^{\text{7′}}\)), 155.9 (C\(^{\text{5′}}\)), 146.8 (C\(^{\text{4′}}\)), 84.1 (C\(^{\text{2′}}\)), 45.1 (C\(^{\text{1′}}\)), 62.9 (OCH\(_2\)), and 14.3 (CH\(_3\)) were assignable to a cyclopentenone ring bearing an ethoxycarbonyl moiety. This was confirmed by the correlations of methylene protons (\( \delta \) 4.21) with C\(^{\text{7′}}\) and H\(^{\text{C-1′′′′′′}}\) (\( \delta \) 5.60) with C\(^{\text{2′}}\), C\(^{\text{3′}}\), C\(^{\text{4′}}\), C\(^{\text{5′}}\), and C\(^{\text{7′}}\) in the HMBC spectrum. In addition, the HMBC spectrum showed correlations of H-1 with C\(^{\text{3′}}\), C\(^{\text{4′}}\), and C\(^{\text{5′}}\), indicating the linkages of C-1 with C\(^{\text{4′}}\). The carbonyl signals at \( \delta \) 164.2 (C\(^{\text{6′}}\)) suggested that this carbonyl was connected by a double bond and formed a \( \delta \)-lactone ring with glucose O-2. This was confirmed by the correlation of H-2 with carbonyl carbon (\( \delta \) 164.2) in the HMBC spectrum. The correlations of H-3 with C\(^{\text{HHDP-7′′′′′′}}\) (\( \delta \) 168.2) of the HHDP group and C\(^{\text{HHDP-7′′′′′′}}\) (\( \delta \) 167.4) of the HHDP group in the HMBC spectrum (Figure 2). The carbon signals at \( \delta \) 201.4 (C\(^{\text{3′}}\)), 170.3 (C\(^{\text{7′}}\)), 155.9 (C\(^{\text{5′}}\)), 146.8 (C\(^{\text{4′}}\)), 84.1 (C\(^{\text{2′}}\)), 45.1 (C\(^{\text{1′}}\)), 62.9 (OCH\(_2\)), and 14.3 (CH\(_3\)) were assignable to a cyclopentenone ring bearing an ethoxycarbonyl moiety. This was confirmed by the correlations of methylene protons (\( \delta \) 4.21) with C\(^{\text{7′}}\) and H\(^{\text{C-1′′′′′′}}\) (\( \delta \) 5.60) with C\(^{\text{2′}}\), C\(^{\text{3′}}\), C\(^{\text{4′}}\), C\(^{\text{5′}}\), and C\(^{\text{7′}}\) in the HMBC spectrum. In addition, the HMBC spectrum showed correlations of H-1 with C\(^{\text{3′}}\), C\(^{\text{4′}}\), and C\(^{\text{5′}}\), indicating the linkages of C-1 with C\(^{\text{4′}}\). The carbonyl signals at \( \delta \) 164.2 (C\(^{\text{6′}}\)) suggested that this carbonyl was connected by a double bond and formed a \( \delta \)-lactone ring with glucose O-2. This was confirmed by the correlation of H-2 with carbonyl carbon (\( \delta \) 164.2) in the HMBC spectrum. The correlations of H-3 with C\(^{\text{HHDP-7′′′′′′}}\) (\( \delta \) 168.0) of the HHDP group and H-5 with C\(^{\text{HHDP-7′′′′′′}}\) (\( \delta \) 167.8) of the HHDP group in the HMBC spectrum indicated that these two carbonyl carbons (C\(^{\text{HHDP-7′′′′}}\) and C\(^{\text{HHDP-7′′′′′′}}\)) were connected to glucose O-3 and glucose O-5, respectively. The correlations of H\(^{\text{C-1′′′′′′}}\) with C\(^{\text{HHDP-2′}}\) (\( \delta \) 124.7) and C\(^{\text{HHDP-3′}}\) (\( \delta \) 112.8) of the HHDP group in the HMBC spectrum indicated that C\(^{\text{p-1′}}\) was linked to C\(^{\text{HHDP-3}}\) of the HHDP group. Comparison between the NMR data of whiskey tannin B [19] and 1 (Table 1) revealed that -OH at C-1 in whiskey tannin B was replaced by a 5,7,3′,4′-tetrahydroxy flavan-3-ol moiety in 1. This moiety could be constructed by analysis of the \(^1\)H NMR data of a 1,2,4-trisubstituted aromatic ring at \( \delta \) 6.94 (d, 0.9), 6.86 (dd, 8.2, 0.9), and 6.78 (d, 8.2) and a phloroglucinol aromatic ring at \( \delta \) 6.00 (s), as well as the C-ring characteristic protons of a 2,3-trans flavan-3-ol at \( \delta \) 7.42 (br s), 4.11 (m), 2.57 (dd, 16.1, 7.7), and 2.83 (d, 16.1) [33]. This is further supported by the \(^1\)H-COSY correlations of catechin H-3−− with catechin H-2−−− and catechin H-4−−− (Figure 1), and by the correlations of catechin H-2−−−− with aromatic carbons (\( \delta \) 144.2 and 120.6) and catechin H-3−−−− with aromatic carbon (\( \delta \) 131.3) in the HMBC spectrum. This moiety has also been found in stenophyllanine B [31]. To determine the C-6 or C-8 linkage between catechin and C-glycosylated ellagitannin moieties, methylation of 1 was carried out, giving 1a, and its \(^13\)C-NMR data showed an unsubstituted A-ring carbon signal at \( \delta \) 89.6, indicating the presence of a substituent at C-8 of the flavan-3-ol moiety [34]. The side-chain moiety linked to C-1 was confirmed by the correlations of H-1 with aromatic carbons (\( \delta \) 102.9, 154.9, and 156.2) in the HMBC spectrum and by the upfield chemical shift (\( \delta \) 33.9). Compound 1 was refluxed in 20% acetic acid ethanol, and then chromatographed on Sephadex LH-20 to obtain a crystalline compound [m.p. 170–171 °C; \([\alpha]_{D}^{25} +14^\circ\) (acetone)] that was identical with (+)-catechin [34]. Thus, the planar of 1 was identified.
Table 1. $^1$H (500 MHz) and $^{13}$C NMR (125 MHz) spectroscopic data for 1 in acetone-$d_6$. 

| Pos. | $\delta_H$ | $\delta_C$ | Pos. | $\delta_H$ | $\delta_C$ | Pos. | $\delta_H$ | $\delta_C$ |
|------|------------|------------|------|------------|------------|------|------------|------------|
| Glc-1 | 4.36 s | 33.9 | HHDP-1"""" | 114.1 | Catechin-2"""" | 4.72 br s | 82.4 |
| 2 | 4.96 s | 83.2 | 2"""" | 125.9 | 3"""" | 4.11 m | 67.5 |
| 3 | 5.29 s | 74.4 | 3"""" | 6.80 s | 108.9 | 4"""" | 2.83 d (16.1) | 28.8 |
| 4 | 5.53 d (6.7) | 68.7 | 4"""" | 144.3 | 2.57 dd (16.1, 7.7) | |
| 5 | 5.35 s | 71.7 | 5"""" | 137.2 | 4""""a | 100.5 |
| 6a | 4.81 dd (11.8, 6.2) | 63.6 | 6"""" | 145.2 | 5"""" | 154.0 |
| 6b | 3.86 m | 167.8 | 7"""" | 6"""" | 6.00 s | 96.4 |
| Cp-1' | 5.60 s | 45.1 | HHDP-1"""" | 115.1 | H | 7"""" | 154.9 |
| 2' | 84.1 | 2"""" | 125.8 | 8"""" | 102.9 |
| 3' | 201.4 | 3"""" | 7.02 s | 108.8 | 8""""a | 156.2 |
| 4' | 146.8 | 4"""" | 144.8 | 9"""" | 131.3 |
| 5' | 155.9 | 5"""" | 136.7 | 10"""" | 6.94 d (0.9) | 114.2 |
| 6' | 164.2 | 6"""" | 146.0 | 11"""" | 145.6 |
| 7' | 170.3 | 7"""" | 168.2 | 12"""" | 145.1 |
| HHDP-1"""" | 115.8 | HHDP-1"""" | 114.2 | 13"""" | 6.86 dd (8.2, 0.9) | 115.9 |
| 2"""" | 124.7 | 2"""" | 125.0 | 14"""" | 12.78 d (8.2) | 120.6 |
| 3"""" | 112.8 | 3"""" | 108.5 | OH2 | 4.21q (7.1) | 62.9 |
| 4"""" | 144.2 | 4"""" | 144.1 | CH3 | 1.20 t (7.1) | 14.3 |
| 5"""" | 136.5 | 5"""" | 135.4 |
| 6"""" | 145.5 | 6"""" | 146.0 |
| 7"""" | 168.0 | 7"""" | 167.4 |

Figure 2. Key HMBC (arrows), $^1$H-$^1$H COSY (bonds), and NOE (double arrows) correlations of 1.

The coupling constant between H-1 and H-2 is 0 Hz (<2.0 Hz), indicating that the configuration at C-1 of the glucose core in 1 is the same as that of vescalagin ($J = 2.0$ Hz) [35] and different from that of whisky tannin B ($J = 6.4$ Hz) [19]. This is also evidenced by the nuclear Overhauser effect (NOE) correlations of H-1 with H-3. Assuming that 1 is derived from vescalagin, inspection of a Dreiding model of 1 showed that the proton Hc$_{p,1'}$ of the cyclopentenone ring must be $\beta$ oriented because its fusion ring system is so rigid that it is impossible to build an alternative model [35]. No proton was correlated with Hc$_{p,1'}$ in the ROESY spectrum indicated that the ethoxycarbonyl in 1 is $\alpha$-orientation. The 2R- and 3S- configurations of the flavan C-ring were deduced from the absence of the NOE cross peaks between catechin H-2"""" and catechin H-3"""" in the ROESY spectrum of 1, as well as acid hydrolysis of 1 gave (+)-catechin. The atropisomerism of the HHDP group in 1 was...
The 1D NMR data of 11 (Table 2) revealed high structural similarity to the co-isolated excelsione (12) [29]. The only difference between 11 and 13 was the replacement of the -CH₂OH (C-16) by a methyl group, which was supported by the chemical shifts of C-16 (δC 8.7). The structure of 11 was therefore established and named guanxidone B.

Table 2. ¹H (500 MHz) and ¹³C NMR (125 MHz) spectroscopic data for 11 in DMSO-d₆.

| Pos. | δH   | δC   | Pos. | δH   | δC   | Pos. | δH   | δC   |
|------|------|------|------|------|------|------|------|------|
| 1    | 168.9| 7    | δH  | 160.4| 13   | δH  | 113.9|
| 2    | 109.5| 8    | 6.63, s | 114.9| 14   | 143.7|
| 3    | 148.6| 9    | 140.7| 15   | 5.25, s| 68.3|
| 4    | 140.1| 10   | 111.4| 16   | 2.30, s| 8.7 |
| 5    | 160.5| 11   | 161.7| 17   | 2.30, s| 20.4|
| 6    | 114.9| 12   | 148.6| 18   | 2.12, s| 11.0|

Figure 3. Key HMBC (arrows) of 11.

2.2. Anti-Inflammatory Activity Assays

All compounds except compounds 13 and 14 were investigated for potential anti-inflammatory activity by measuring the inhibition of the nitric oxide (NO) production. As shown in Table 3, compounds 1, 11, and 12 displayed significant anti-inflammatory activity with IC₅₀ values ranging from 6.46 ± 0.23 to 9.82 ± 0.43 μM. The IC₅₀ values for the inhibition of NO production by other compounds are all > 10 μM. The anti-inflammatory activity of compound 1 is better than that of compound 2, indicating that the effect of catechin on glucose C-1 is very important for its activity. Compound 11 has better anti-inflammatory activity than compound 12, indicating that CH₃O- at C-16 of aromatic ring A has little effect on its activity.
Table 3. The anti-inflammatory activities of compounds 1–12.

| Compound | IC_{50} (µM) a |
|----------|----------------|
| 1        | 8.02 ± 0.35    |
| 2        | >50            |
| 3        | >50            |
| 4        | >50            |
| 5        | >50            |
| 6        | >50            |
| 7        | >50            |
| 8        | 21.32 ± 1.05   |
| 9        | >50            |
| 10       | >50            |
| 11       | 6.46 ± 0.23    |
| 12       | 9.82 ± 0.43    |

[a]_{25}D Dexamethasone 2.52 ± 0.26

*Values present mean ± SD of triplicate experiments.

3. Experimental

3.1. Materials

The roots of *M. malabathricum subsp. normale* were collected in Yanshan Town (Guilin, China) in September 2019, and identified by Professor Yusong Huang (Guangxi Institute of Botany, Guilin, China). A voucher specimen (registration No. 20190915) has been deposited in the Guangxi Key Laboratory of Plant Functional Phytochemicals and Sustainable Utilization Guangxi Institute of Botany, Guilin, China.

3.2. General Experimental Procedures

Optical rotations were measured at 25 °C with an ADP440+ polarimeter, Julabo, Seelbach, Germany (λ 589 nm, path length 1.0 cm). The UV spectra were recorded in MeOH on a TU-1901 spectrophotometer (Beijing Puxi General Instrument Co., Ltd., Beijing, China). The CD spectra were acquired in MeOH on a JASCO J-180 spectropolarimeter (Jasco, Tokyo, Japan). The NMR spectra were obtained on a Bruker Avance III HD-500 MHz spectrometer (Bruker Biospin AG, Fällanden, Switzerland), and the residual solvent peaks were used as references. Coupling constants and chemical shifts were given in Hz and on a δ (ppm) scale, respectively. ESI-MS and HR-ESI-MS were acquired on a Bruker Esquire 3000plus and Waters/Micromass Q-TOF-Ultima (Waters, Milford, MA, USA) mass spectrometers, respectively. Semi-preparative HPLC performed on a Shimadzu LC-20AT HPLC system at the rate of 2 mL/min. Sephadex LH-20 (GE Healthcare Bio-Science AB, Uppsala, Sweden), MCI gel CHP 20P (Mitsubishi Chemical Co., Tokyo, Japan), silica gel (Qingdao Marine Chemical Co., Ltd., Qingdao, China), and Chromatorex ODS (Merck, Darmstadt, Germany) were used for column chromatography (CC).

3.3. Extraction and Separation

Air-dried, powdered roots of *M. malabathricum subsp. normale* (9.0 kg) were extracted with the 80% aqueous acetone for three times (each for 7 days at room temperature to afford a residue (0.6 kg). Then, the residue was suspended in H_{2}O (1 L) and successively partitioned with petroleum ether, EtOAc into petroleum ether (Fraction A, 175.0 g), EtOAc (Fraction B, 80.0 g), and water (Fraction C, 345.0 g) fractions. Fraction B (80.0 g) was chromatographed on silica gel column (10 × 30 cm) and eluted with a gradient of MeOH-CH_{2}Cl_{2} (0:100–100:0, v/v) to afford ten fractions (Fr.B1–Fr.B10). Fr.B4 (15.0 g) was purified by ODS C18 CC (6 × 50 cm) eluting with a gradient of MeOH-H_{2}O (0:100–100:0, v/v) to obtained eighteen subfractions (Fr.B4-1–Fr.B4-18). Further separation of Fr.B4-6 (5.6 g) using silica gel column (4 × 20 cm) in a gradient of MeOH-CH_{2}Cl_{2} (10:100–40:60, v/v) and then Sephadex LH-20 CC (1.5 × 40 cm) in a gradient of MeOH-H_{2}O (0:100–100:0, v/v, 10% stepwise, each 200 mL) to give compounds 2 (34.0 mg) and 3 (8.1 mg). Separation of Fr.B4-7 (7.2 g) was done by silica gel column (5 × 20 cm) eluting with a gradient of
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MeOH–CH₂Cl₂ (0:100–20:80, v/v) and then Sephadex LH-20 CC eluting with MeOH-
CH₂Cl₂ (1:1, v/v) to yield 1 (28.2 mg), 8 (5.3 mg), 9 (14.3 mg), 11 (8.8 mg), and 12 (7.6 mg). Compounds 13 (1.1 mg) and 14 (1.3 mg) were obtained from Fr 4–12 (0.6 g) successively via semi-preparative HPLC eluted with a gradient of MeOH-H₂O (50:50–90:10, v/v, 0–40 min) and Sephadex LH-20 CC eluted with CH₂Cl₂–MeOH (1:1, v/v).

Fraction C (345.0 g) was divided into twenty fractions (Fr.C1–Fr.C20) by Sephadex LH-20 CC (10 × 45 cm) eluting with a gradient of MeOH-H₂O gradients (0:100–100:0, v/v, 20% stepwise, each 5000 mL). Fr.C1 (40.0 g) was subjected to HP20SS column (6 × 60 cm) with a gradient of MeOH-H₂O (0:100–100:0, v/v) to give nine subfractions (Fr.C1–1–Fr.C1-9). Fr.C1-5 (12.0 g) was further chromatographed on ODS C18 column (4 × 30 cm) using MeOH-H₂O step gradient (0:100–100:0, v/v), then purified by Sephadex LH-20 eluting with MeOH to yield compounds 4 (3.8 mg), 5 (2.2 mg), 6 (2.5 mg), and 7 (5.4 mg). Fr.C3 (10.9 g) was loaded onto a MCI gel CHP 20P (4 × 20% stepwise, each 5000 mL) in a gradient of MeOH-H₂O (0:100–100:0, v/v) to afford 10 fractions (Fr.C3-1–Fr.C3-10). Compound 10 (2.3 mg) was obtained from Fr.C3-3 (2.8 g) using Sephadex LH-20 column (2 × 40 cm) in a gradient of MeOH-H₂O (0:100–50:50, v/v, 10% stepwise, each 300 mL).

3.4. Spectroscopic Data

Whiskey tannin D (1): A pale brown amorphous powder; [α]D25 –15.6° (c = 0.12, MeOH); UV (MeOH) λmax nm (log ε): 204 (2.12), 272 (1.20); CD (MeOH) λmax (Δε) 263 (–6.6), 240 (+9.5) (Supplementary Materials, Figure S8). 1H and 13C NMR data, see Table 1; 1D and 2D NMR spectra of 1 (Supplementary Materials, Figure S1–S6); HR-ESI-MS m/z: 1249.1580 [M – H]– (calcd, 1249.1586) (Supplementary Materials, Figure S7).

Guanxidone B (11): a white powder; UV (MeOH) λmax nm (log ε): 206 (1.60). 1H and 13C NMR data, see Table 2; 1D and 2D NMR spectra of 11 (Supplementary Materials, Figures S9–S12); HR-ESI-MS m/z: 341.0669 [M – H]– (calcd, 341.0661) (Supplementary Materials, Figure S13).

3.5. Acid-Catalyzed Degradation of 1

Compound 1 (10 mg) was dissolved in 20% acetic acid ethanol (3 mL) and reacted under reflux for 5 d. The solvent was concentrated under reduced pressure, and the residue was chromatographed on Sephadex LH-20 column eluting with ethanol to yield a colorless needles (+)-catechin (3.5 mg) [m.p. 170–171 °C; [α]D25 +14° (acetone)].

3.6. Methylation of 1

Compound 1 (10 mg) was dissolved in dimethyl sulfate (1 mL), then anhydrous potassium carbonate (0.5 g) in acetone was added and heated under reflux for 3 h. After removing inorganic salts by filtration, the filtrate was evaporated off under reduced pressure, and loaded onto a silica gel CC gradually eluting with benzene with increased proportion of acetone to yield compound 1a (2.1 mg), [α]D25 –123.0° (c = 0.42, CHCl₃). HR-ESI-MS m/z: 1473.1095 [M – H]– (Calcd. 1473.1090 for C₇₄H₇₇O₉³⁻). The flavan part of 1a: 1H-NMR (CDCl₃) δ 5.71 (1H, d, J = 8.9 Hz, 5”-H), 6.27 (1H, s, 6-H), 6.61–6.93 (6H, m, aromatic H). 13C-NMR (CDCl₃): δ 27.8 (C-4), 38.1 (C-1”), 65.7 (C-6”), 68.1 (C-3), 69.5, 70.2, 71.5, 76.5 (C-2”, C-3”, C-4” and C-5”), 82.5 (C-2), 89.6 (C-6) [34].

3.7. Anti-Inflammatory Activity

The anti-inflammatory activities of compounds 1–12 were investigated on the production of the NO in LPS-stimulated cells according to our previously described method [17]. That is, the RAW 264.7 cells were cultivated in DMEM supplemented with 10% FBS at 37 °C for 24 h. Cells in 24-well plate were treated with 200 ng/mL LPS and the test compounds. After 22 h, the media were collected, and the level of nitrite was measured using the Griess Reagent reagent System (Promega), Madison, WI, USA. The results are expressed as the mean ± SD, n = 3.
4. Conclusions

The study aimed to discover new anti-inflammatory drugs from the roots of *M. malabathricum subsp. normale* based on our previous works [18,37]. As expected, fourteen compounds were obtained from the tile plant for the first time, and compounds 1 and 11 are new compounds. In addition, this is the first report of compounds 1–4, 8, and 10–14 from the genus *Melastoma*, and compounds 1, 2, and 11–14 from the family Melastomataceae. Compounds 1, 11, and 12 showed anti-inflammatory activities, which make them potential anti-inflammatory drugs. The study of structure–activity relationship is helpful to quickly find out new anti-inflammatory drugs. The successful isolation and structure identification of compounds 1–14 not only provide materials for this experiment, but also contribute to the chemotaxonomic studies of the species of the genus *Melastoma*.

**Supplementary Materials:** The following supporting information can be downloaded, Figures S1–S6: 1D and 2D NMR spectra of 1, Figure S7: HRESIMS spectrum of 1; Figure S8: CD spectra of 1. Figures S9–S12: 1D and 2D NMR spectra of 11, Figure S13: HR-ESI-MS spectrum of 11.

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