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LIGNANS AND OTHER COMPOUNDS FROM THE LEAVES OF *TRIADICA ROTUNDIFOLIA* WITH INHIBITORY ACTIVITY ON NITRIC OXIDE PRODUCTION

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Summary

By employing chromatographic techniques, six compounds (**1**–**6**) were successfully isolated from the leaves of *Triadica rotundifolia* (Hemsl.) Esser. Their structures were elucidated to be (1*S*,2*S*)-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxy propanol (**1**), (1*S*,2*R*)-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol (**2**), syringaresinol (**3**), trimethyl-3,4-dihydrochebulate (**4**), methyl 3,4,5-trihydroxybenzoate (**5**), and 5-hydroxymethyl-2-furancarboxaldehyde (**6**), by detailed analysis of 1D, 2D NMR, and MS spectroscopic data as well as comparison with those reported. The absolute configurations of compounds **1**, **2**, and **4** were further confirmed using experimental circular dichroism (CD) spectroscopy. Several compounds were evaluated for their inhibitory activity against the production of nitric oxide (NO) in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Compounds **1**, **2**, and **6** were found to reduce NO levels in murine macrophage cells, with IC_{50} values ranging from 21.1 to 54.5 μM. This study represents the first report of compounds **1**−**6** isolated from *T. rotundifolia.*

Keywords: *Triadica rotundifolia*; Euphorbiaceae; *Lignan*; *Phenolic*; *NO inhibitory effect*.

1. Introduction

Triadica is a genus of Euphorbiaceae with approximately 120 species distributed worldwide[1]. Several species within this genus are widely employed in traditional medicine across Asia, Africa, and South America. Ethnomedicinal applications include the treatment of dermatological disorders, musculoskeletal pain, digestive and urinary ailments, hernia, wound infections, and inflammatory conditions. In many regions, preparations such as decoctions, powders, or topical applications are also used for respiratory complaints, malaria, anemia, fever, and parasitic diseases, reflecting the broad therapeutic relevance of this genus in indigenous healthcare systems [1],[2],[3],[4]. Phytochemical work on this genus has revealed the presence of a wide array of diterpenoids, triterpenoids, flavonoids, lignanoids, and coumarins [1],[2].

Triadica rotundifolia (Hemsl.) Esser (syn. *Sapium rotundifolium* Hemsl.) is one of six species reported in Vietnam [5]. This shrub is widely distributed in northern Vietnam [6] and is also found in several temperate and tropical regions of Asia, including Cambodia, China, Malaysia, and Thailand [3]. The leaves and stems of *T. rotundifolia* have traditionally been used in folk medicine to treat various conditions, such as acute skin disease, detoxification-related ailments, eczema, herpes zoster, snake bites, scabies, and ulcers [3],[4]. However, there is a lack of phytochemical and pharmacological data on this plant. To date, only one study has reported the presence of diterpenoid and triterpenoid compounds, which have attracted interest because of their potential anti-inflammatory activity [7]. In continuation of our interest in the phytochemistry of plants belonging to the genus *Triadica*, we have conducted further studies on the leaves of *T. rotundifolia* and isolated six compounds (**1**−**6**, Fig. 1). In addition, the NO production inhibitory activity of these phytochemicals was also evaluated.

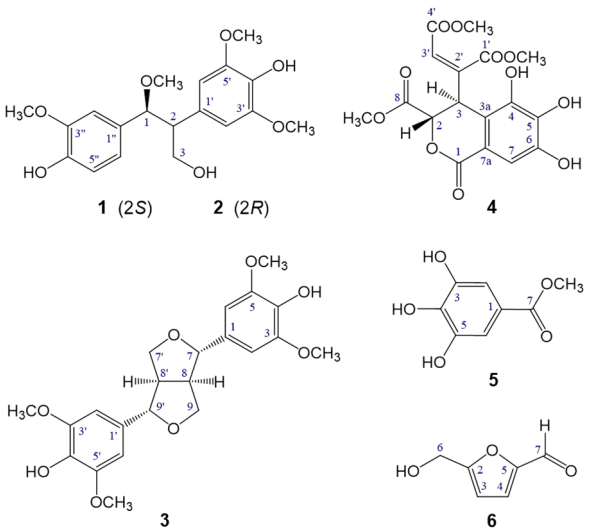

Fig. 1. Chemical structures of compounds **1**–**6**.

2. Materials and methods

2.1. Chemicals and equipment

Optical rotations were determined on a JASCO P-2000 polarimeter. The CD spectra were measured on a Chirascan spectrometer (Applied Photophysics, Surrey, UK). The NMR spectra were recorded on a Bruker AVANCE NEO 600 FT-NMR spectrometer with TMS as an internal standard. HRESIMS data were acquired using an Agilent 6530 Accurate Mass Q-TOF LC/MS. Column chromatography (CC) was conducted on *silica gel*, Diaion HP-20, Sephadex LH-20, and RP-18. Preparative HPLC using an Agilent 1100, a J′ sphere ODS H-80 (4 μm, 250×20 mm) column, a flow rate of 6 mL/min, and a DAD detector at wavelengths of 205, 230, 254, and 280 nm. An isocratic mobile phase with a flow rate of 3.0 mL/min was used for pre-HPLC. Analytical thin-layer chromatography was performed on precoated *silica gel* 60 F_{254} and RP-18 $F_{254}S$ plates, and compounds were visualized by spraying with 10% H_2SO_4 in water and then heating for 1.5−2 minutes.

2.2. Plant material

The leaves of *Triadica rotundifolia* were collected at Nam Dong, Hue City, Vietnam, in December 2024. The species was taxonomically identified by Dr. Bui Van Thanh, Institute of Biology, VAST. A voucher herbarium specimen (NKB1225) has been deposited at the Herbarium of the Institute of Chemistry, VAST.

2.3. Extraction and isolation

The dried leaves of *T. rotundifolia* (2.5 kg) were extracted with 100% methanol (6 L) by maceration at room temperature, with occasional shaking for 24 hours. This process was repeated over five consecutive days (3 × 6 L). The combined methanolic extracts were filtered and concentrated under reduced pressure at 45°C using a rotary evaporator, yielding 180.6 g of a dark-brown residue. A portion (150 g) of this extract was suspended in MeOH-H_2O (10:1, 1.5 L) and partitioned with *n*-hexane (3 × 1.5 L), which was dried to give a *n*-hexane-soluble fraction (H, 25.7 g). In turn, the MeOH-H_2O (10:1) partition was completely dried and resuspended in distilled H_2O (1.5 L) and successively partitioned first with CH_2Cl_2 (3 × 1.5 L) and next with EtOAc (3 × 1.5 L). This provided 56.0 g of the CH_2Cl_2-, 15.8 g of the EtOAc-, and 82.3 g of the H_2O-soluble fractions, respectively. The EtOAc fraction was fractionated using *silica gel* CC with gradient mixtures of CH_2Cl_2-MeOH (50:1, 30:1, v/v). Fractions with similar TLC profiles were combined, yielding six pooled subfractions (E1–E6). Subfraction E4 (0.25 g) was chromatographed over a *silica gel* column and eluted with a CH_2Cl_2-EtOAc (3:1, v/v) gradient to yield four pooled subfractions (E4.1−E4.4). Of these, subfraction E4.3 (0.06 g) was purified by preparative reversed-phase HPLC, using ACN-H_2O (35% of ACN, flow rate 6 mL/min for 45 min) to yield compounds **5** (3.5 mg, t_R = 43.3 min) and **6** (t_R = 39.8 min). Compound **6** (2.6 mg) was further purified by repeated precipitation and centrifugation in MeOH. Using the same HPLC conditions, compound **3** (4.8 mg, t_R = 24.6 min) was also obtained. Subfraction E5 (0.21 g) was subjected to *silica gel* CC with CH_2Cl_2-MeOH (10:1, v/v). Altogether, three subfractions (80 mL each) were collected and combined according to their TLC similarities to give three pooled fractions (E5.1−E5.3). Among them, subfraction E5.2 (0.02 g) was purified by semipreparative reversed-phase HPLC with MeOH-H_2O (28% of MeOH, flow rate of 4 mL/min, for 40 min) to yield **4** (3.8 mg, t_R = 38.6 min). Subfraction E5.3 (0.01 g) fraction was purified by HPLC, using the reversed-phase preparative column described above, with a solvent system of ACN-H_2O (20% ACN, flow rate 6 mL/min, for 60 min). From this separation, compounds **1** (4.8 mg, t_R = 57.3 min) and **2** (36.0 mg, t_R = 58.9 min) were obtained.

(1S,2S)-2-(4-Hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxy propanol (1): Yellow, amorphous powder; $[\alpha]_D^{25}$: +11.8 (*c* 0.05, MeOH); ECD (MeOH, nm) λ_{max} ($\Delta\varepsilon$): 207 (-3.48), 215 (+1.90); HRESIMS *m/z* 387.1422 $[M + Na]^+$ (calcd. for $[C_{19}H_{24}NaO_7]^+$, 387.1414); ^1H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectroscopic data, see Table 1.

(1S,2R)-2-(4-Hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxy propane-3-ol (2): Yellow, amorphous powder; $[\alpha]_D^{25}$: -14.1 (*c* 0.05, MeOH); ECD (MeOH, nm) λ_{max} ($\Delta\varepsilon$): 205 (+5.65), 220 (-2.28); ESIMS *m/z* 363.1 $[M - H]^-$; ^1H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectroscopic data, see Table 1.

Syringaresinol (3): White, amorphous powder; ^1H NMR (CD_3OD, 600 MHz) δ (ppm): 6.73 (4H, s, H-2, H-6, H-2', H-6'), 4.73 (2H, d, *J* = 4.5 Hz, H-7, H-7'), 3.14 (2H, m, H-8, H-8'), 4.27 (2H, dd, *J* = 9.0, 6.5 Hz, H_a-9, H_a-9'), 3.89 (2H, dd, *J* = 9.0, 3.5 Hz, H_b-9, H_b-9'), 3.86 (12H, s, 4 × OCH_3); ^{13}C NMR (CD_3OD, 150 MHz) δ

(ppm): 133.2 (C-1, C-1'), 104.6 (C-2, C-6, C-2', C-6'), 149.4 (C-3, C-5, C-3', C-5'), 136.3 (C-4, C-4'), 87.6 (C-7, C-7'), 55.5 (C-8, C-8'), 72.8 (C-9, C-9'), and 56.8 (4 × OCH_3).

Trimethyl-3,4-dihydrochebulate (4): Brown, amorphous powder; $[\alpha]_D^{25}$: +121.6 (*c* 0.05, MeOH-H_2O); ECD (MeOH, nm) λ_{max} ($\Delta\varepsilon$): 315 (+1.56), 279 (+4.46), 250 (+1.84), 233 (+12.2), 210 (-6.97); HRESIMS ion at *m/z* 405.0464 $[M + Na]^+$ (calcd. for $[C_{16}H_{14}NaO_{11}]^+$, 405.0428); ^1H (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectroscopic data, see Table 2.

Methyl 3,4,5-trihydroxybenzoate (5): White, amorphous powder; ^1H NMR (CD_3OD, 600 MHz) δ (ppm): 7.07 (2H, s, H-2, H-6), 3.83 (3H, s, OCH_3); ^{13}C NMR (CD_3OD, 150 MHz) δ (ppm): 122.0 (C-1), 110.5 (C-2, C-6), 147.0 (C-3, C-5), 140.2 (C-4), 169.5 (C-7), and 52.7 (OCH_3).

5-Hydroxymethyl-2-furancarboxaldehyde (6): Yellow needle; ^1H NMR (CD_3OD, 600 MHz) δ (ppm): 7.39 (1H, d, J = 3.6 Hz, H-3), 6.60 (1H, d, J = 3.6 Hz, H-4), 4.63 (2H, s, H-6), 9.55 (1H, s, H-7); ^{13}C NMR (CD_3OD, 150 MHz) δ (ppm): 153.9 (C-2), 124.8 (C-3), 110.9 (C-4), 163.2 (C-5), 57.6 (C-6), and 179.4 (C-7).

2.4. NO production inhibitory assay

Nitric oxide assay was performed as previously described [8],[9]. In brief, RAW264.7 cells, originally sourced from the American Type Culture Collection (ATCC, Manassas, VA, USA), were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco-BRL, St. Louis, MO, USA) supplemented with 5% heat-inactivated fetal bovine serum, along with 100 U/mL penicillin and 100 μg/mL streptomycin. The cultures were incubated under controlled conditions at 37°C under a humidified atmosphere containing 5% CO_2. After 24 hours of incubation, compounds were added to each well (0.4−100 μM) or vehicle and followed by LPS (1 μg/mL) after 2h. Cells were seeded at a density of 2 × 10^5 cells per well in a 96-well plate and allowed to adhere for 6h before treatment. To assess cell viability, the remaining adherent cells were subjected to the MTT assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Sigma Chemical Co., St. Louis, MO, USA), and absorbance was measured at 570 nm. Cultural medium (100 μL) was mixed with an equal volume of Griess reagent and incubated at room temperature for 10 minutes. Absorbance was measured at 540 nm on a microplate reader. Nitrite concentration as an indicator of NO production was determined using a standard curve that was built by serially diluting $NaNO_2$ solutions. Experiments were performed in triplicate. The IC_{50} values were generated by TableCurve 2Dv4 software.

3. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula, $C_{19}H_{24}O_7$, was established from a sodium adduct ion at *m/z* 387.1422 $[M + Na]^+$ (calcd. for $[C_{19}H_{24}NaO_7]^+$, 387.1414) observed in the HRESIMS data. Analysis of the ^1H, ^{13}C NMR, and HSQC data of **1** (Table 1) revealed the occurrence of five aromatic methines, an oxymethine, an oxymethylene, a methine, five oxygenated aromatic carbons, seven quaternary aromatic carbons, and four methoxy groups. In the ^1H NMR spectrum, two different sets of aromatic protons were discernible. One of these at δ_H 6.40 (2H, s, H-2', H-6') integrated for two protons, suggesting symmetry consistent with a 1,3,4,5-tetrasubstituted aromatic ring. The remaining set of aromatic resonances showed the typical splitting patterns and coupling constants of a 1,3,4-trisubstituted aromatic ring [δ_H 6.53 (1H, d, J = 1.2 Hz, H-2"), 6.74 (1H, d, J = 8.4 Hz, H-5"), and 6.65 (1H, dd, J = 8.4, 1.2 Hz, H-6")]. In addition, an oxymethine [δ_H 4.50 (1H, d, J = 5.4 Hz, H-1)], a methine [δ_H 2.88 (1H, m, H-2], an oxymethylene [δ_H 3.88 (1H, dd, J = 10.8, 4.2 Hz, H_a-3) and 3.70 (1H, dd, J = 10.8, 8.4 Hz, H_b-3)], as well as four methoxy groups [δ_H 3.19 (3H, s, 1-OCH_3), 3.78 (6H, s, 3',5'-OCH_3), and 3.69 (3H, s, 3"-OCH_3)], were also determined in the ^1H NMR spectrum. Analysis of the additional aromatic proton resonances led to the proposal of a further 1,3,4-trisubstituted ring, with one of the substituents corresponding to a methoxy group, a second to a hydroxy group, and a quaternary carbon. The attachment of the methoxy group at C-3" (δ_C 148.7) was confirmed by a correlation with the protons of the methyl group (δ_H 3.69) and this carbon, in the HMBC spectrum. The hydroxy group was attached to C-4" (δ_C 147.0), consistent with its characteristic downfield chemical shift. Furthermore, the structure of **1** was identified as 1,2-diarylpropan-1-alkoxy-3-ol [10],[11] based on HMBC correlations of H-1 (δ_H 4.50) with C-2 (δ_C 56.9), 1-OCH_3 (δ_C 57.1), C-1" (δ_C 133.1), C-2" (δ_C 111.8), C-6" (δ_C 121.3), and H-2 (δ_H 2.88) with C-1 (δ_C 85.0), C-3 (δ_C 64.3), C-1' (δ_C 131.5), and C-2'/C-6' (δ_C 108.2) (Fig. 2). The relative configuration of 1,2-diarylpropan-1-alkoxy-3-ol could be readily determined by the chemical shift differences between H-1 and H-2

(in methanol-d_4) [10],[11]. Comparing the chemical shift differences between H-1 and H-2 ($\Delta\delta_{1,2}$ = 1.62 ppm) with published data confirmed the *erythro* configuration of substituents at C-1 and C-2 in compound **1** [10],[11]. This assignment was further supported by the experimental CD spectrum, which displayed a negative Cotton effect (CE) at 207 nm and a positive CE at 215 nm. These spectral features closely matched the calculated ECD spectrum of (1*S*,2*S*)-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol [10]. Therefore, the structure of compound **1** was elucidated as (1*S*,2*S*)-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol. This compound was previously isolated from the seed shells of *Cerasus humilis* [10]. Its biological activities were evaluated through *in vitro* experiments, which revealed only weak cytotoxicity against HepG2 and A549 cell lines (IC_{50} ~ 50 μM). **1** demonstrated significant antioxidant activity as revealed by the DPPH (IC_{50} = 9.8 μg/mL) and ATBS (IC_{50} = 15.3 μg/mL) assays [10].

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of compounds **1** and **2** and the reference compounds

No.	$^{\#}\delta_C$ [a]	**1**		$^{\$}\delta_C$ [b]	**2**	
		δ_C [a,c]	δ_H [a,d] mult. (*J* in Hz)		δ_C [a,c]	δ_H [a,d] mult. (*J* in Hz)
1	85.0	85.0	4.50 d (5.4)	84.3	87.2	4.33 d (8.4)
2	56.9	56.9	2.88 m	54.1	56.5	3.00 m
3	64.3	64.3	3.88 dd (10.8, 4.2) 3.70 dd (10.8, 8.4)	62.4	64.8	4.08 dd (10.8, 5.4) 3.39 dd (10.8, 7.2)
1'	131.5	131.5	-	130.9	131.8	-
2', 6'	108.2	108.2	6.40 s	106.9	107.8	6.26 s
3', 5'	148.7	148.7	-	147.2	148.7	-
4'	135.3	135.3	-	133.7	135.5	-
1"	133.1	133.1	-	130.8	132.7	-
2"	111.8	111.8	6.53 d (1.2)	111.5	112.4	6.55 d (1.8)
3"	148.7	148.7	-	147.0	148.6	-
4"	147.0	147.0	-	145.5	146.9	-
5"	115.6	115.6	6.74 d (8.4)	114.6	115.5	6.66 d (7.8)
6"	121.3	121.3	6.65 dd (8.4, 1.2)	120.2	121.5	6.58 dd (7.8, 1.8)
1-OCH_3	57.1	57.1	3.19 s	56.2	56.7	3.24 s
3',5'-OCH_3	56.7	56.8	3.78 s	55.9	56.8	3.72 s
3"-OCH_3	56.2	56.2	3.69 s	55.4	56.3	3.70 s

Measured in [a] CD_3OD, [b] DMSO-d_6, [c]125 MHz, and [d]600 MHz. [#]δ_C of (1S,2S)-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol [10] and [$]$\delta_C$ of (1S,2R)-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol [10].

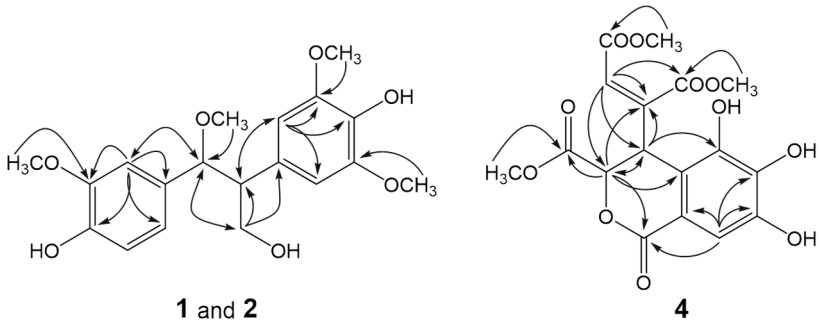

Fig. 2. Key HMBC (⟶) correlations of compounds **1**, **2**, and **4**

Compound **2** was obtained as a yellow amorphous powder. Its molecular formula, $C_{19}H_{27}O_7$, was supported by the observation of a deprotonated molecular ion at *m/z* 363.1 $[M - H]^-$ present in the ESIMS data. The ^{1}H and ^{13}C NMR data of compound **2** were closely comparable to those of compound **1** (Table 1), indicating that they share a similar structural scaffold. However, distinct differences in the chemical shifts and splitting patterns of the protons in the propanyl

moiety (C/H-1 to C/H-3) were observed (Table 1), suggesting that compound **2** has subtle configurational differences compared to **1**. The relative configuration at C-1 and C-2 in compound **2** was confirmed as *threo* configuration based on $\Delta\delta_{1,2} \leq 1.33$ ppm [10],[11]. Moreover, the experimental CD spectrum of **2** exhibited a positive CE at 205 nm and a negative CE at 220 nm, consistent with the absolute configuration of (1*S*,2*R*). These CEs closely matched those reported for (1*S*,2*R*)-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol [(1*S*,2*R*): positive CE at 204 nm and a negative CE at 221 nm] as previously reported [10]. Accordingly, the structure of compound **2** was proposed as (1*S*,2*R*)-2-(4-hydroxy-3,5-dimethoxy phenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxy propane-3-ol. Previously, compound **2** exhibited only weak cytotoxicity against the HepG2 and A549 cell lines, with IC_{50} values exceeding 50 μM. In contrast, it demonstrated strong antioxidant activity, as evidenced by the DPPH (IC_{50} = 8.6 μg/mL) and ABTS (IC_{50} = 9.7 μg/mL) assays [10].

Compound **4** was obtained as colorless crystals and determined to have the molecular formula of $C_{19}H_{20}O_{11}$ with 10 indices of hydrogen deficiency, as established on the basis of its ^{13}C NMR data and a sodium adduct ion observed in the HRESIMS at *m/z* 405.0464 $[M + Na]^+$ (calcd. for $[C_{16}H_{14}O_{11}]^+$, 405.0428). The ^1H NMR data (Table 2) indicated characteristic signals for an aromatic proton [δ_H 7.08 (1H, s, H-7)], an olefinic proton [δ_H 6.83 (1H, s, H-3')], two methine protons [δ_H 5.31 (1H, d, J = 1.2 Hz, H-2) and 5.38 (1H, br s, H-3], and three methoxy groups [δ_H 3.70, 3.65 × 2 (each 3H, s)]. Its ^{13}C NMR data (Table 2), in conjunction with the HSQC experiment, displayed 17 carbon signals. These comprised four carbonyl carbons [δ_C 171.1, 167.6, 166.9, and 166.0], eight sp^2-hybridized carbons [δ_C 108.7−146.7], two methines (δ_C 79.9 and 35.5), and three methoxy groups (δ_C 53.5, 53.0, and 52.6). The above information implied that compound **4** should be a dehydrochebulic acid derivative [12]. Detailed analysis of its 2D NMR data not only supported the above inference but also verified the planar structure as shown in Fig. 1. In the HMBC spectrum, the proton H-2 (δ_H 5.31) showed long-range correlations with C-1 (δ_C 166.0), C-3 (δ_C 35.5), C-3a (δ_C 116.2), C-8 (δ_C 171.1), and C-2' (δ_C 142.9). Similarly, the proton H-3 (δ_H 5.38) exhibited correlations with C-2 (δ_C 79.9), C-4 (δ_C 144.1), and C-2'. Additional HMBC correlations from H-2/8-OCH_3 to C-8, H-3' (δ_H 6.38)/1'-OCH_3 to C-1' (δ_C 167.6), and 4'-OCH_3 to C-4' confirmed the positions of the three methoxy substituents at C-8, C-1', and C-4', respectively (Fig. 2). The small coupling constant ($^3J_{2,3}$ = 1.2 Hz) [12],[13],[14], along with the NOESY correlation of H-2/H-3 indicated that these protons were a *trans*-diequatorial relative configuration with both hydrogens in an equatorial orientation. Based on the putative biosynthetic pathway to the formation of the dehydrochebulic acid derivatives and their similar NMR patterns (Table 2), the *E*-configuration of the $\Delta^{2(3')}$ olefinic bond and the absolute configurations of C-2 and C-3 in **4** were proposed as depicted and confirmed by their virtually identical ECD spectra compared to those of **4**. The ECD spectra of **4** showed a positive n−π* Cotton effect near 279 nm, which defined the (2*S*,3*R*) absolute configurations based on the dihydroisocoumarin P-helicity rule [12],[13],[14]. Based on the above evidence and comparison with previously reported literature [12], compound **4** was finally identified as dehydrochebulic acid trimethyl ester. This compound was previously obtained from *Phyllanthus urinaria* (Euphorbiaceae) [15], *Geranium bellum* (Geraniaceae) [12], and *Sapium insigne* (Euphorbiaceae) [16]. In the antioxidant assay, compound 4 exhibited significant DPPH radical scavenging activity with an IC_{50} value of 9.4 μM. Moreover, it markedly inhibited NO production in a dose-dependent manner, and the inhibition reached 84.2% at a concentration of 30 μM [15].

Table 2. NMR spectroscopic data of **4** and the reference compound

No.	@δ_C	**4**	
		δ_C[a,b]	δ_H[a,c] mult. (J in Hz)
1	163.0	166.0	-
2	79.2	79.9	5.31 d (1.2)
3	35.3	35.5	5.38 br s
3a	116.1	116.2	-
4	145.0	144.1	-

No.	$^{@}\delta_C$	4	
		δ_C [a,b]	δ_H [a,c] mult. (*J* in Hz)
5	141.2	140.4	-
6	147.4	146.7	-
7	108.6	108.7	7.08 s
7a	117.5	117.7	-
8	170.5	171.1	-
1'	166.7	167.6	-
2'	142.8	142.9	-
3'	129.6	130.2	6.83 s
4'	165.7	166.9	-
8-OCH_3	52.2	53.5	3.70 s
1'-OCH_3	52.7	53.0	3.65 s
4'-OCH_3	51.9	52.6	3.65 s

Measured in [a]CD_3OD, [b]125 MHz, and [c]600 MHz. $^{@}\delta_C$ of dehydrochebulic acid trimethyl ester in pyridine-d_5 [12].

Table 3. Inhibitory effects of isolated compounds on NO production in LPS-induced RAW264.7 cells

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
1	26.9 ± 1.5	**6**	54.5 ± 2.3
2	21.1 ± 1.2	Dexamethasone	13.6 ± 1.1

Results were indicated as the mean IC50 ± SD of three independent experiments in triplicate. Dexamethasone was used as a reference drug.

The remaining compounds were identified as syringaresinol (**3**) [17], methyl 3,4,5-trihydroxybenzoate (**5**) [18], and 5-hydroxymethyl-2-furancarboxaldehyde (**6**) [19], based on our spectroscopic data and by comparison with those of reported data.

NO formation and secretion were closely associated with a range of inflammatory pathophysiological processes. As a key signaling molecule in living systems, NO plays essential biological roles; however, excessive production of NO has been implicated in the development of inflammatory diseases [20]. Therefore, it is highly significant to suppress its excessive production. To evaluate whether the compounds isolated from *T. rotundifolia* possess anti-inflammatory activity, we assessed their inhibitory effects on LPS-induced NO production in RAW264.7 cells. Only compounds that were both stable and obtainable in sufficient amounts (**1**, **2**, and **6**) were selected for evaluation of their inhibitory activity against NO production in LPS-induced RAW264.7 cells. At their effective concentrations, none of the tested compounds exhibited cytotoxicity toward RAW264.7 cells. Among them, compounds **1** and **2** exhibited significant inhibitory effects, with the IC_{50} values of 26.9 and 21.1 μM, respectively, compared with the positive control, dexamethasone (IC_{50} = 13.6 μM). Compound **6** demonstrated only moderate inhibition, with an IC_{50} value of 54.5 μM (Table 3). Building on previous studies, syringaresinol (**3**) [21], dehydrochebulic acid trimethyl ester (**4**) [12],[13],[14], and methyl galate (**5**) [22] derived from many plants have been found to possess anti-inflammatory activities. Structural features such as the number of methoxy groups and geometric symmetry of its benzene ring appears to significantly contribute to its anti-inflammatory effects.

4. Conclusions

Phytochemical study of the methanol extract from *T. rotundifolia* leaves resulted in the isolation of six compounds (**1**−**6**). Their chemical structures were determined by comprehensive analysis of MS and NMR spectral data, as well as by comparison of their spectral data with those reported in the literature. Lignans were identified as the major constituents, marking the first report of lignan derivatives in *T. rotundifolia*. Compounds **1**, **2**, and **6** showed significant inhibitory effect against NO production, with

IC_{50} values ranging from 21.1 to 54.5 μM. These findings highlight the bioactive metabolites of *T. rotundifolia* and provide a scientific basis for further exploration of natural products within the genus *Triadica*.

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