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CONTENTS VOL.31(1+2), 2026 (SPECIAL ISSUE)
(Pages 1 - 160)

Page	SCIENTIFIC RESEARCH
3	Foreword: Special Issue Commemorating the 65th Anniversary of the National Institute of Medicinal Materials and Welcoming the 1st International Conference V.IMPACT-2026 - Assoc. Prof. Do Thi Ha, PhD
	REVIEW
4	Results of Scientific Activities of the National Institute of Medicinal Materials (2016–2025) and Development Orientation Toward 2030 - Tran Minh Ngoc, Do Thi Ha, Pham Tuan Anh
	SCIENTIFIC RESEARCH
10	Triterpenoid Constituents from the Leaves of <i>Walsura pinnata</i> Hassk - Bui Huu Tai, Nguyen Viet Dung, Nguyen Huy Hoang, Ngo Anh Bang, Duong Thi Dung, Duong Thi Hai Yen, Phan Thi Thanh Huong, Dan Thuy Hang, Pham Hai Yen, Phan Van Kiem, Nguyen Phuong Thao
17	Chemical Constituents from the Propolis of the Stingless Bee <i>Lepidotrigona terminata</i> (Smith, 1878) Collected in Gia Lai - Diep Thi Lan Phuong, Nguyen Thu Thao Van, Vo Thi Thanh Tuyen, Nguyen Thi Nghia, Pham Ngoc Thach, Le Thi Cam Nhung, Le Duy Thanh, Vo Manh Tien, Tran Minh Ngoc, Le Nguyen Thanh
23	Glycoside Constituents from <i>Abroma augustum</i> and their <i>in vitro</i> Antiproliferative Activity - Ninh Thi Ngoc, Pham Thi Cham, Le Thi Vien, Tran Thi Hong Hanh, Do Thi Thao, Nguyen Xuan Cuong, Nguyen Hoai Nam
29	Chemical Profiling and Anti-Inflammatory Effects of <i>Physalis angulata</i> in Lipopolysaccharide-Stimulated Bone Marrow–Derived Dendritic Cells - Dinh Thi Huong, Le Ba Vinh, Nguyen Van Chinh, Nguyen Cao Cuong
36	Phenolic Constituents from the Ethyl Ecetate Extract of <i>Camellia flava</i> Flowers (Theaceae) Collected in Phu Tho Province - Do Thi Thuy, Nguyen Duong Hong Anh, Tran The Bach, Do Van Hieu, Do Duc Nhuan, Than Kieu My, Tran Minh Ngoc, Nguyen Thanh Cong, Le Nguyen Thanh
42	Lignans and Other Compounds from the Leaves of <i>Triadica rotundifolia</i> with Inhibitory Activity on Nitric Oxide Production - Ninh Khac Ban, Tran My Linh, Ninh Khac Thanh Tung, Bui Van Thanh, Pham Hai Yen, Bui Huu Tai, Phan Van Kiem, Nguyen Phuong Thao
49	Chemical Constituents of <i>Ganoderma multipileum</i> Ding Hou, Ganodermataceae - Nguyen Thi Duyen, Tran Thi Tuyet, Phan Quynh Trang, Nguyen Trung Hieu, Nguyen Duc Hieu, Nguyen Phuong Dai Nguyen
53	Chemical Composition, Antimicrobial and Cytotoxic Activities of the Leaf Essential Oil of <i>Knema globularia</i> (Lamk.) Warb. from Thanh Hoa, Vietnam - Luong Le Uyen Trang, Pham Thi Huong Ly, Nguyen Thi Hoa Hien, Nguyen Thanh Huyen, Nguyen Thi Hien, Nguyen Thi Thu, Khong Thi Hoa

60	Azaphilone Derivatives Isolated from Endophytic Fungus <i>Penicillium sclerotiorum</i> - Ly Tu Loan, Vo Van Leo, Ma Chi Thanh
67	Chemical Constituents from the Aerial Parts of <i>Barleria lupulina</i> (Acanthaceae) - Ngo Thi Ngoc Yen, Do Hoang Anh, Nguyen Thi Thu, Nguyen Thi Ha Ly, Le Nguyen Thanh, Nguyen Quynh Nga, Dao Viet Quoc, Nguyen Thu Hang, Nguyen Hoang Minh, Nguyen Minh Khoi, Le Tran Nguyen Vu
74	Determination of Essential Oil Content, Composition, and Total Polyphenol Content of <i>Rhizoma homalomenae occulta</i> Collected in Vietnam - Nguyen Thi Ha Ly, Pham Thi Hien, Ngo Thi Thanh Huong, Hoang Dieu Linh, Tran Huyen Trang, Nguyen Minh Khoi
80	Establishment of an In-House Standard for <i>Prunellae spica</i> - Tran Huyen Trang, Pham Thi Hien, Nguyen Thi Ha Ly, Phan Van Truong, Vu Thi Diep, Nguyen Tra My, Nguyen Thi Hang, Nguyen Duong Hong Anh, Do Thi Ha
88	HPTLC Scanner Method for the Simultaneous Determination of Lycopene and β-Carotene in Gac Seed Aril - Pham Thi Hien, Pham Thi Khuyen, Nguyen Thi Hang, Nguyen Thi Lan Hoa, Ta Thi Thao, Do Thi Ha, Nguyen Thi Ha Ly
96	Optimization of the Extraction Process from Yellow Camellia Flowers (<i>Camellia cucphuongensis</i> Ninh & Rosmann) Collected in Phu Tho Province Using Response Surface Methodology - Nguyen Hoang Viet, Nguyen Dinh Quan, Do Duc Nhuan, Nguyen Duong Hong Anh, Tran Thi Mai Anh, Doan Van Nghia, Vu Xuan Hoa, Dang Mai Thien Ha, Nguyen Thanh Cong
105	Antioxidant, Antihyperglycemic, and Renal Protective Effects of <i>Hibiscus tiliaceus</i> L. Leaf Extract on Streptozotocin-Induced Hyperglycemia - Nguyen Thi Thu Huong, Nhieu Chinh Hoang, Nguyen Hoang Minh
113	Acute Oral Toxicity and Laxative Effects of <i>Pharbitis nil</i> (L.) Choisy Seed Extract - Nguyen Thi Kim Oanh, Tran Vien Thong, Truong Huy Hieu, Ly Hai Trieu, Pham Thi Nguyet Hang, Nguyen Thi Vinh Hue, Do Thi Ha
123	Evaluation of Memory-Enhancing Effects of <i>Morinda officinalis</i> How. Root Extracts in a Transgenic <i>hAPP Drosophila</i> Model of Alzheimer's Disease - Nguyen Van Hiep, Pham Thi Nguyet Hang, Trinh Dinh Luc, Leu Khanh Duy, Pham Anh Tung, Nguyen Thi Phuong, Nguyen Van Tai
131	Anti-Inflammatory Effects of the Bark of <i>Phellodendron amurense</i> Rupr. in Experimental Models - Do Thi Hang, Le Thi Kim Van, Do Van Dung, Bui Thanh Tung
138	Neuroprotective Effects of Tianma Gouteng Decoction in a Middle Cerebral Artery Occlusion Model of Ischemic Stroke - Le Thi Xoan, Trinh Lan Phuong, Nguyen Van Hiep, Pham Thi Nguyet Hang, Nguyen Van Tai, Nguyen Thi Thu Trang, Nguyen Thi Thanh Loan, Nguyen Hung Son
147	Investigation of the Variation of Gymnemagenin Content in <i>Gymnema R.Br</i> Under the Influence of Certain Strains of Bacteria - Tong Thi Thanh Vuong, Tran Thi Minh Thuy, Le Thi Kim Van
154	Comparative Transcriptome Profiling of <i>Momordica cochinchinensis</i> (Lour.) Spreng. Aril Using Oxford Nanopore and Illumina Sequencing Technologies - Tran Duc Trung, Nguyen Hoang, Le Thi Tu Linh, Nguyen Thi Thu Hang, Pham Thanh Huyen, Truong Nhat My, Nguyen Thi Lan Hoa

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 IC₅₀ values ranging from 21.1 to 54.5 μ M. 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.

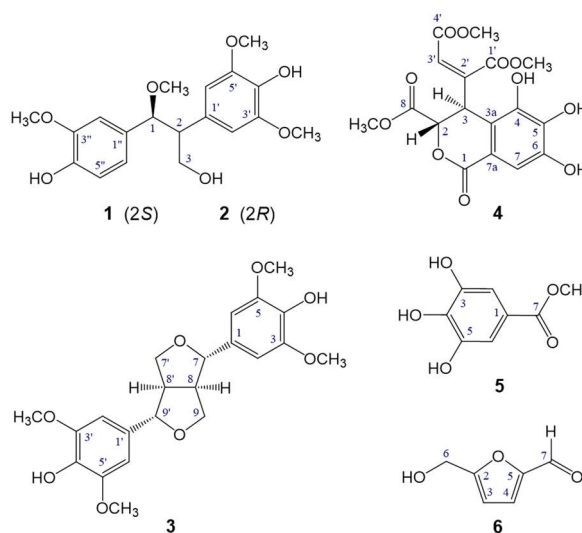


Fig. 1. Chemical structures of compounds **1–6**.

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 μ m, 250 $\times$ 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 F₂₅₄ and RP-18 F₂₅₄S plates, and compounds were visualized by spraying with 10% H₂SO₄ 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 $\times$ 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-H₂O (10:1, 1.5 L) and partitioned with *n*-hexane (3 $\times$ 1.5 L), which was dried to give a *n*-hexane-soluble fraction (H, 25.7 g). In turn, the MeOH-H₂O (10:1) partition was completely dried and resuspended in distilled H₂O (1.5 L) and successively partitioned first with CH₂Cl₂ (3 $\times$ 1.5 L) and next with EtOAc (3 $\times$ 1.5 L). This provided 56.0 g of the CH₂Cl₂-, 15.8 g of the EtOAc-, and 82.3 g of the H₂O-soluble fractions, respectively. The EtOAc fraction was fractionated using *silica gel* CC with gradient mixtures of CH₂Cl₂-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 CH₂Cl₂-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-H₂O (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 CH₂Cl₂-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-H₂O (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-H₂O (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.

(1*S*,2*S*)-2-(4-Hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropanol (1): Yellow, amorphous powder; $[\alpha]_D^{25}$: +11.8 (*c* 0.05, MeOH); ECD (MeOH, nm) $\lambda_{\max}$ ($\Delta\epsilon$): 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, CD₃OD) and ¹³C NMR (150 MHz, CD₃OD) spectroscopic data, see Table 1.

(1*S*,2*R*)-2-(4-Hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol (2): Yellow, amorphous powder; $[\alpha]_D^{25}$: -14.1 (*c* 0.05, MeOH); ECD (MeOH, nm) $\lambda_{\max}$ ($\Delta\epsilon$): 205 (+5.65), 220 (-2.28); ESIMS *m/z* 363.1 $[M - H]^-$; ¹H (600 MHz, CD₃OD) and ¹³C NMR (150 MHz, CD₃OD) spectroscopic data, see Table 1.

Syringaresinol (3): White, amorphous powder; ¹H NMR (CD₃OD, 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 $\times$ OCH₃); ¹³C NMR (CD₃OD, 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 ($4 \times \text{OCH}_3$).

Trimethyl-3,4-dihydrochebulate (4): Brown, amorphous powder; $[\alpha]_D^{25}$: +121.6 (c 0.05, MeOH-H₂O); ECD (MeOH, nm) λ_{max} ($\Delta\epsilon$): 315 (+1.56), 279 (+4.46), 250 (+1.84), 233 (+12.2), 210 (-6.97); HRESIMS ion at m/z 405.0464 $[\text{M} + \text{Na}]^+$ (calcd. for $[\text{C}_{16}\text{H}_{14}\text{NaO}_{11}]^+$, 405.0428); ^1H (600 MHz, CD₃OD) and ^{13}C NMR (150 MHz, CD₃OD) spectroscopic data, see Table 2.

Methyl 3,4,5-trihydroxybenzoate (5): White, amorphous powder; ^1H NMR (CD₃OD, 600 MHz) δ (ppm): 7.07 (2H, s, H-2, H-6), 3.83 (3H, s, OCH₃); ^{13}C NMR (CD₃OD, 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 (OCH₃).

5-Hydroxymethyl-2-furancarboxaldehyde (6): Yellow needle; ^1H NMR (CD₃OD, 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 (CD₃OD, 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 $\mu\text{g/mL}$ streptomycin. The cultures were incubated under controlled conditions at 37°C under a humidified atmosphere containing 5% CO₂. After 24 hours of incubation, compounds were added to each well (0.4–100 μM) or vehicle and followed by LPS (1 $\mu\text{g/mL}$) 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 NaNO₂ solutions. Experiments were performed in triplicate. The IC₅₀ values were generated by TableCurve 2Dv4 software.

3. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula, C₁₉H₂₄O₇, was established from a sodium adduct ion at m/z 387.1422 $[\text{M} + \text{Na}]^+$ (calcd. for $[\text{C}_{19}\text{H}_{24}\text{NaO}_7]^+$, 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-OCH₃), 3.78 (6H, s, 3',5'-OCH₃), and 3.69 (3H, s, 3''-OCH₃)], 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-OCH₃ (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 ($IC_{50} \sim 50 \mu M$). **1** demonstrated significant antioxidant activity as revealed by the DPPH ($IC_{50} = 9.8 \mu g/mL$) and ATBS ($IC_{50} = 15.3 \mu g/mL$) assays [10].

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

No.	# δ_C^a	1		δ_C^b	2	
		$\delta_C^{a,c}$	$\delta_H^{a,d}$ mult. (J in Hz)		$\delta_C^{a,c}$	$\delta_H^{a,d}$ 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 CD₃OD, ^b DMSO-*d*₆, ^c 125 MHz, and ^d 600 MHz. # δ_C of (1*S*,2*S*)-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol [10] and δ_C of (1*S*,2*R*)-2-(4-hydroxy-3,5-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol [10].

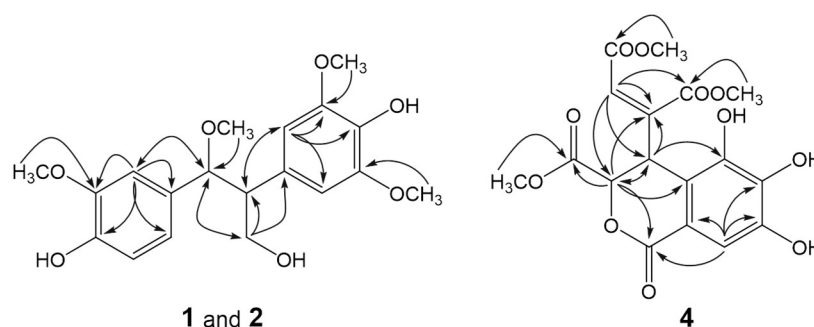


Fig. 2. Key HMBC (→) correlations of compounds **1**, **2**, and **4**

Compound **2** was obtained as a yellow amorphous powder. Its molecular formula, C₁₉H₂₇O₇, was supported by the observation of a deprotonated molecular ion at m/z 363.1 [M - H]⁻ present in the ESIMS data. The 1H 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 propenyl

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-dimethoxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-1-methoxypropane-3-ol. Previously, compound **2** exhibited only weak cytotoxicity against the HepG2 and A549 cell lines, with IC₅₀ values exceeding 50 μ M. In contrast, it demonstrated strong antioxidant activity, as evidenced by the DPPH (IC₅₀ = 8.6 μ g/mL) and ABTS (IC₅₀ = 9.7 μ g/mL) assays [10].

Compound **4** was obtained as colorless crystals and determined to have the molecular formula of C₁₉H₂₀O₁₁ with 10 indices of hydrogen deficiency, as established on the basis of its ¹³C NMR data and a sodium adduct ion observed in the HRESIMS at *m/z* 405.0464 [M + Na]⁺ (calcd. for [C₁₆H₁₄O₁₁]⁺, 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 $\times$ 2 (each 3H, s)]. Its ¹³C 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-OCH₃ to C-8, H-3' (δ_{H} 6.38)/1'-OCH₃ to C-1' (δ_{C} 167.6), and 4'-OCH₃ 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 (³*J*_{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 IC₅₀ value of 9.4 μ M. Moreover, it markedly inhibited NO production in a dose-dependent manner, and the inhibition reached 84.2% at a concentration of 30 μ M [15].

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

No.	@ δ_{C}	4	
		$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ mult. (<i>J</i> 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.	$^a\delta_C$	4	
		$\delta_C^{a,b}$	$\delta_H^{a,c}$ 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 aCD_3OD , b125 MHz, and c600 MHz. $^a\delta_C$ 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 IC₅₀ values of 26.9 and 21.1 μM, respectively, compared with the positive control, dexamethasone (IC₅₀ = 13.6 μM). Compound **6** demonstrated only moderate inhibition, with an IC₅₀ 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

IC₅₀ values ranging from 21.1 to 54.5 μ M. 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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