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SECONDARY METABOLITES FROM THE LEAVES OF *SOLANUM MELONGENA* AND THEIR NITRIC OXIDE PRODUCTION INHIBITORY ACTIVITY

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Abstract. *Solanum melongena* L. var. *esculentum* Ness. growing widely in Vietnam is a member of the plants family Solanaceae. Fruits and whole plant of *S. melongena* has been used by traditional medicine to treat various diseases, such as, toothache, turgescence, pruritus, and haemorrhoids. Previous studies indicated that this plant contain of alkaloids, anthocyanins, flavonoids, lignanamides, phenylpropanoid amides, sesquiterpenoids, steroids, steroidal saponins, and triterpenoids and exert diverse biological activities, such as anti-inflammatory, anti-cancer, anti-nociceptive, anti-oxidant, and anti-hypertensive activities. Using various chromatographic separations, four compounds were isolated from the leaves of *Solanum melongena* L growing in Thua Thien Hue province. Their chemical structures were elucidated to be quadranoside III (**1**), alangilignoside C (**2**), torvoside J (**3**), and *N-trans*-feruloyloctopamine (**4**) by 1D-, 2D-NMR spectra and compared with those reported in the literature. This study also reports the anti-inflammatory effect of isolated compounds via their inhibitory activity against the production of nitric oxide (NO) in RAW 264.7 macrophages cells stimulated by lipopolysaccharide. Compounds **1** and **4** showed NO inhibitory effects with IC_{50} values of 99.67 and 91.80 µM, respectively (posotive control L-NMMA, 32.26 µM), while compounds **2** and **3** were inactive. This is the first time that compound **1** has been reported from *Solanum* genus.

Keywords: Solanum melogena, quadranoside III, alangilignoside C, torvoside J, *N-trans*-feruloyloctopamine, and NO production.

Classification numbers: 1.1.1, 1.1.6

1. INTRODUCTION

Solanum melongena L. (called "cà tím" or "cà dái dê" in Vietnam), a herb belonging to Solanaceae family, is a Vietnamese traditional medicine distributed widely in Vietnam. Fruits and whole plant of *S. melongena* are used to treat toothache, turgescence, pruritus, and

haemorrhoids [1]. Chemical studies of the plant indicated the presence of alkaloids [2], anthocyanins [3], flavonoids [4], lignanamides [5, 6], phenylpropanoid amides [7], sesquiterpenoids [8], steroids, steroidal saponins [9-12], and triterpenoids [4]. In addition, biological activities of extracts and isolated compounds from *S. melongena* have been studied, such as anti-inflammatory [4 - 6, 11, 13], anti-cancer [8], anti-nociceptive [14], anti-oxidant [15, 16], and anti-hypertensive activities [17]. In our ongoing program of screening for anti-inflamatory agents from Vietnamese *Solanum* species, we found that a methanol extract of *S. melongena* leaves showed anti-inflammatory activity with IC_{50} value of 65.17 µg/mL. We reported herein the isolation and structure elucidation of four compounds (**1**-**4**) as well as their inhibitory activities against NO production induced by lipopolysaccharides in the RAW 264.7 macrophages.

2. MATERIALS AND METHODS

2.1. General experimental procedures

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) and Bruker Avance NEO600 spectrometer (600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR). NMR measurements, including ^{1}H-, ^{13}C-NMR, HSQC, and HMBC experiments, were carried out using 5-mm probe tubes at a temperature of 22.2 ^{0}C. Column chromatography was performed using silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (150 µm, Fuji Silysia Chemical Ltd.), thin layer chromatography (TLC) was carried out with pre-coated silica gel 60 F254 (0.25 mm, Merck) and RP-18 F254S plates (0.25 mm, Merck).

2.2. Plant materials

The leaves of *S. melongena* L. were collected in Huong Tra, Thua Thien Hue, Viet Nam in July 2019, and were identified by Dr. Tran Thi Phuong Anh (Graduated University of Science and Technology, VAST). The voucher specimens (MISR.2019-15) have been deposited at the herbarium of Mientrung Institute for Scientific Research, Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

The dried powder of *Solanum melongena* L. leaves (3.0 kg) was extracted with 7 L of methanol three times by sonication for 120 min. The methanol extract was concentrated under reduced pressure to obtain a residue (SM, 200.0 g). The residue was suspended in water (2.0 L) and then successively partitioned with *n*-hexane, dichloromethane, and ethyl acetate to obtain *n*-hexane (SMH, 66.5 g), dichloromethane (SMD, 14.5 g), ethyl acetate (SME, 17.0 g) residues and water layer (SMW). The SME fraction was subjected to a silica gel chromatography column and eluted with an increasing gradient of methanol (0-100 %) in dichloromethane to give seven fractions, SME1-SME7. The SME6 fraction (0.97 g) was chromatographed on an RP-18 column, which was then eluted with methanol/water (1/1, v/v) to give three fractions, SME6A-SME6C. Compound **1** (4 mg) was yielded from the SME6A fraction (110 mg) on a silica gel column, eluted with methanol/dichloromethane/water (7/1/0.05, v/v/v). The SME6C (280 mg) was further chromatographed on a Sephadex LH-20 column, eluted with methanol/water (1.5/1, v/v) to obtain compounds **2** (4.5 mg) and **3** (6 mg). The SME3 fraction (1.15 g) was separated

using an RP-18 column, eluted with methanol/water (1.5/1, v/v) to give four fractions, SEM3A-SEM3D. The SEM3B (130 mg) was continuously purified on a Sephadex LH-20 column, eluted with methanol/water (1/1, v/v) to yield compound **4** (8 mg).

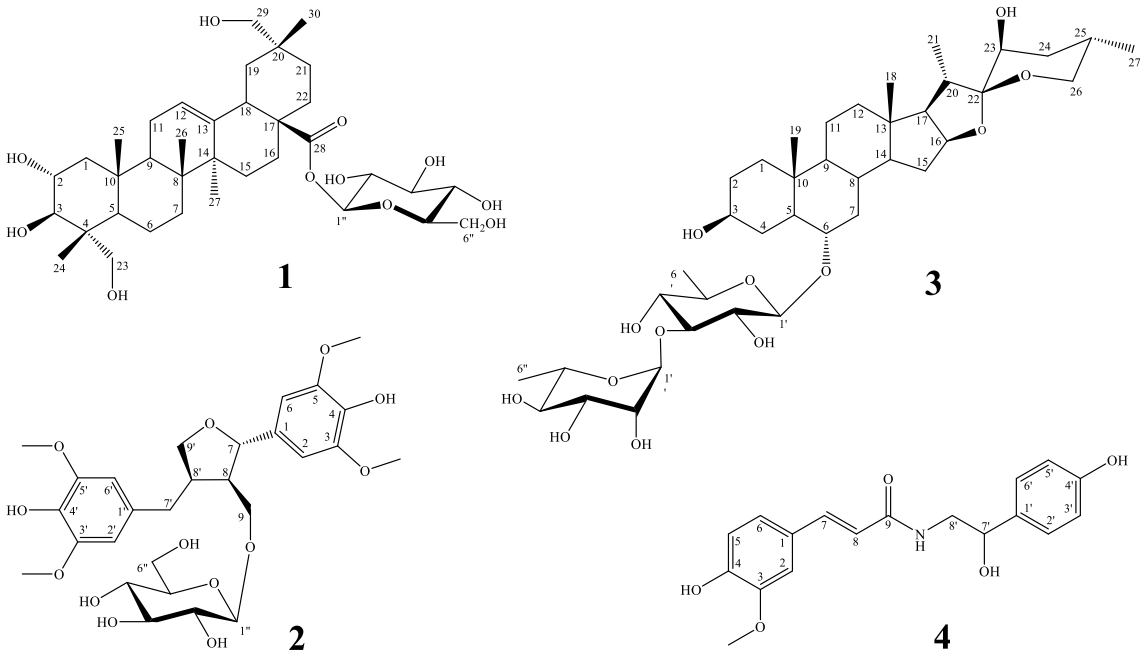

Figure 1. Chemical structures of compounds **1**-**4** from *S. melongena*.

Quadranoside III (1): Colourless amorphous solid; ^{1}H-NMR (600 MHz, CD_3OD): δ_H 0.90 (1H, dd, *J* = 11.4, 12.6 Hz, H_a-1), 1.94 (1H, m, H_b-1), 3.71 (1H, m, H-2), 3.43 (1H, m, H-3), 1.30 (1H, brd, *J* = 11.4 Hz, H-5), 1.42 (1H, m, H_a-6), 1.46 (1H, m, H_b-6), 1.30 (1H, m, H_a-7), 1.62 (1H, m, H_b-1), 3.33 (1H, overlapped, H-9), 1.96 (2H, m, H-11), 5.30 (1H, like-t, *J* = 3.0 Hz, H-12), 1.10 (1H, m, H_a-15), 1.82 (1H, m, H_b-15), 1.74 (1H, m, H_a-16), 2.07 (1H, m, H_b-16), 2.91 (1H, dd, *J* = 4.2,13.8 Hz, H-18), 1.12 (1H, m, H_a-19), 1.84 (1H, m, H_b-19), 1.18 (1H, m, H_a-21), 1.51 (1H, m, H_b-21), 1.67 (1H, m, H_a-22), 1.77 (1H, m, H_b-22), 3.28 (1H, d, *J* = 10.8 Hz, H_a-23), 3.52 (1H, d, *J* = 10.8 Hz, H_b-23), 0.72 (3H, s, H-24), 1.06 (3H, s, H-25), 0.83 (3H, s, H-26), 1.21 (3H, s, H-27), 3.21 (2H, s, H-29), 0.95 (3H, s, H-30), 5.41 (1H, d, *J* = 7.8 Hz, H-1'), 3.34 (1H, m, H-2'), 3.36 (1H, m, H-3'), 3.38 (1H, m, H-4'), 3.37 (1H, m, H-5'), 3.70 (1H, m, H_a-6'), 3.84 (1H, d, *J* = 10.8 Hz, H_b-6'); ^{13}C-NMR (150 MHz, CD_3OD): δ_C48.0 (C-1),69.7 (C-2),78.2 (C-3),44.1 (C-4),48.3 (C-5),19.1 (C-6),33.3 (C-7),40.7 (C-8),48.9 (C-9), 39.0 (C-10),24.7 (C-11),123.7 (C-12),145.0 (C-13),43.0 (C-14),28.8 (C-15),24.0 (C-16),48.3 (C-17),41.8 (C-18),41.4 (C-19),36.8 (C-20),29.3 (C-21),32.4 (C-22),66.3 (C-23),13.9 (C-24),17.6 (C-25),17.8 (C-26),26.4 (C-27),178.0 (C-28),74.4 (C-29),19.5 (C-30),95.7 (C-1'),73.9 (C-2'),78.3 (C-3'),71.1 (C-4'),78.7 (C-5'),62.4 (C-6').

Alangilignoside C (2): White amorphous powder; ^{1}H-NMR (600 MHz, CD_3OD): δ_H6.67 (2H, s, H-2, H-6), 4.89 (1H, m, H-7), 2.54 (1H, m, H-8), 3.80 (1H, m, H_a-9), 4.10 (1H, dd, *J* = 6.0, 9.6 Hz, H_b-9), 6.54 (2H, s, H-2', H-6'), 2.18 (1H, m, H_a-7'), 3.00 (1H, dd, *J* = 4.8, 13.2 Hz, H_b-7'), 2.81 (1H, m, H-8'), 3.78 (1H, m, H_a-9'), 4.03 (1H, dd, *J* = 6.0, 8.4 Hz, H_b-9'), 4.33 (1H, d, *J* = 7.8 Hz, H-1"), 3.24 (1H, m, H-2"), 3.38 (1H, m, H-3"), 3.32 (1H, m, H-4"), 3.30 (1H, m,

H-5"), 3.69 (1H, dd, *J* = 5.4, 12.0 Hz, H_a-6"), 3.88 (1H, dd, *J* = 1.8, 12.0 Hz, H_b-6"), 3.85 (6H, s, 3,5-OC<u>H</u>$_3$), 3.86 (6H, s, 3',5'-OC<u>H</u>$_3$); ^{13}C-NMR (150 MHz, CD_3OD): δ_C134.8 (C-1), 104.4 (C-2), 149.2 (C-3),135.9 (C-4),149.2 (C-5),104.4 (C-6), 84.4 (C-7), 51.8 (C-8), 68.6 (C-9), 132.9 (C-1'), 107.1 (C-2'), 149.3 (C-3'), 134.9 (C-4'),149.3 (C-5'), 107.1 (C-6'), 34.4 (C-7'), 43.9 (C-8'), 73.6 (C-9'), 104.8 (C-1"), 75.2 (C-2"), 78.2 (C-3"), 71.7 (C-4"), 78.0 (C-5"), 62.8 (C-6"), 56.8 (3,5-O<u>C</u>H$_3$), 56.8 (3',5'-O<u>C</u>H$_3$).

Torvoside J (3): White amorphous powder; ^{1}H-NMR (600 MHz, CD_3OD): δ_H1.05 (1H, m, H_a-1), 1.71 (1H, m, H_b-1), 1.42 (1H, m, H_a-2), 1.77 (1H, m, H_b-2), 3.49 (1H, m, H-3), 1.18 (1H, m, H_a-4), 2.39 (1H, m, H_b-4), 1.20 (1H, m, H-5), 3.39 (1H, m, H-6), 0.97 (1H, m, H_a-7), 2.20 (1H, dt, *J* = 2.4, 36.0 Hz, H_b-7), 1.64 (1H, m, H-8), 0.71 (1H, m, H-9), 1.33 (1H, m, H_a-11), 1.57 (1H, m, H_b-11), 1.15 (1H, m, H_a-12), 1.77 (1H, m, H_b-12), 1.18 (1H, m, H-14), 1.19 (1H, m, H_a-15), 2.00 (1H, m, H_b-15), 4.48 (1H, m, H-16), 1.72 (1H, m, H-17), 0.84 (3H, s, H-18), 0.89 (3H, s, H-19), 2.27 (1H, m, H-20), 1.12 (1h, d, *J* = 7.2 Hz, H-21), 3.55 (1H, t, *J* = 3.0 Hz, H-23), 1.65 (1H, m, H_a-24), 1.68 (1H, m, H_b-24), 2.07 (1H, m, H-25), 3.38 (1H, m, H_a-26), 3.49 (1H, m, H_b-26), 0.79 (3H, d, *J* = 6.0 Hz, H-27), 4.29 (1H, d, *J* = 7.8 Hz, H-1'), 3.31 (1H, m, H-2'), 3.45 (1H, m, H-3'), 3.04 (1H, m, H-4'), 3.96 (1H, m, H-5'), 1.30 (3H, d, *J* = 6.0 Hz, H-6'), 5.16 (1H, d, *J* = 1.2 Hz, H-1"), 3.72 (1H, dd, *J* = 3.0, 9.6 Hz, H-2"), 3.34 (1H, m, H-3"), 3.43 (1H, m, H-4"), 4.02 (1H, m, H-5"), 1.27 (3H, d, *J* = 6.6 Hz, H-6"); ^{13}C-NMR (150 MHz, CD_3OD): δ_C38.5 (C-1), 31.9 (C-2), 71.8 (C-3), 32.7 (C-4), 51.8 (C-5), 80.4 (C-6), 41.6 (C-7), 35.3 (C-8), 55.1 (C-9), 37.5 (C-10), 22.0 (C-11), 40.7 (C-12), 42.1 (C-13), 57.5 (C-14), 32.9 (C-15), 82.4 (C-16), 65.7 (C-17), 16.8 (C-18), 13.9 (C-19), 41.7 (C-20), 17.1 (C-21), 110.0 (C-22), 71.1 (C-23), 37.6 (C-24), 25.0 (C-25), 67.5 (C-26), 17.4 (C-27), 105.1 (C-1'), 76.4 (C-2'), 84.3 (C-3'), 75.7 (C-4'), 72.3 (C-5'), 18.4 (C-6'), 102.8 (C-1"), 72.3 (C-2"), 72.9 (C-3"), 74.0 (C-4"), 70.0 (C-5"), 17.9 (C-6").

***N-trans*-feruloyloctopamine (4):** Colourless oil; ESI-MS: m/z 330.2 $[M+H]^+$;^{1}H-NMR (500 MHz, CD_3OD): δ_H7.14 (1H, d, *J* = 2.0 Hz, H-2), 6.81 (1H, d, *J* = 8.5 Hz, H-5), 7.04 (1H, dd, *J* = 2.0, 8.5 Hz, H-6), 7.46 (1H, d, *J* = 16.0 Hz, H-7), 6.48 (1H, d, *J* = 16.0 Hz, H-8), 7.24 (2H, d, *J* = 8.5 Hz, H-2′, H-5′), 6.79 (2H, d, *J* = 8.5 Hz, H-2′, H-5′), 4.74 (1H, m, H-7′), 3.46 (1H, dd, *J* = 8.0, 13.5 Hz, H_a-8′), 3.56 (1H, dd, *J* = 5.0, 13.5 Hz, H_b-8′), 3.90 (3H, s, 3-OC<u>H</u>$_3$); ^{13}C-NMR (125 MHz, CD_3OD): δ_C128.3 (C-1), 111.6 (C-2), 149.3 (C-3), 149.9 (C-4), 116.5 (C-5), 123.3 (C-6), 142.2 (C-7), 118.7 (C-8), 169.5 (C-9), 134.7 (C-1′), 128.5 (C-2′), 116.1 (C-3′), 158.1 (C-4′), 116.1 (C-5′), 128.5 (C-6′), 73.5 (C-7′), 48.3 (C-8′), 56.4 (3-O<u>C</u>H$_3$).

2.4. Anti-inflammatory assay

The anti-inflammatory activities of compounds (**1-4**) towards NO overproduction in LPS-stimulated RAW 264.7 macrophage line were evaluated using the Griess assay as previously described [18]. Macrophages were donated by Prof. Chi-Ying Huang from National Yang-Ming University, Taiwan. N^G-methyl-L-arginine acetate salt (L-NMMA) was used as a positive control for the NO assay (IC_{50}= 32.26 ± 3.42 µM).

3. RESULTS AND DISCUSSION

3.1. The NMR-structural elucidation

Compound **1** was isolated as a colourless amorphous solid, and its molecular formula was determined to be $C_{36}H_{58}O_{11}$ by ESI-MS at *m/z* 667.4 $[M+H]^+$ with a combination of ^{1}H- and ^{13}C-NMR data. The ^{1}H-NMR spectrum of **1** showed five tertiary methyl groups at δ_H0.72 (3H, s),

0.83 (3H, s), 0.95 (3H, s), 1.06 (3H, s), and 1.21 (3H, s); one olefinic proton at δ_H5.30 (1H, t, *J* = 3.0 Hz), and one anomeric proton at δ_H5.41 (1H, d, *J* = 7.8 Hz). In addition, the ^{13}C-NMR spectrum with the aid of the HSQC of **1** revealed signals of 36 carbons, including five methyl carbons at δ_C13.9, 17.6, 17.8, 19.5, and 26.4; 12 methylene carbons at δ_C19.1, 24.0, 24.7, 28.8, 29.3, 32.4, 33.3, 41.4, 48.0, 62.4, 66.3, and 74.4; 11 methine carbons at δ_C41.8, 48.3, 48.9, 69.7, 71.1, 73.9, 78.2, 78.3, 78.7, 95.7, and 123.7; and seven non-protonated carbons at δ_C 36.8, 39.0, 40.7, 43.0, 48.3, 145.0, and 178.0. The ^{13}C data of sugar unit (δ_C 95.7, 73.9, 78.3, 71.1, 78.7, and 62.4) and the coupling constant of H-1′ and H-2′, $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 7.8 Hz showed the presence of *O-β*-glucopyranosyl. The above-mentioned data suggested that **1** was an oleanane-*type* triterpene glycoside. The carbon signals corresponding to ring A, including two oxymethine carbons and an oxymethylene carbon, indicated the presence of three hydroxy groups at C-2, C-3, and C-23. These were supported by the HMBC correlations from H-23 (δ_H3.28 and 3.52)/H-24 (δ_H 0.72) to C-3 (δ_C 78.2)/C-4 (δ_C 44.1)/C-5 (δ_C 48.3) and from H-25 (δ_H 1.06) to C-1 (δ_C 48.0)/C-5 (δ_C 48.3)/C-9 (δ_C 48.9)/C-10 (δ_C 39.0). The HMBC correlations from H-27 (δ_H1.21) to C-8 (δ_C 40.7)/C-13 (δ_C 145.0)/C-14 (δ_C 43.0)/C-15 (δ_C 28.8), from H-12 (δ_H 5.30) to C-9 (δ_C 48.9)/C-18 (δ_C 41.8) determined the position of the double bond at C-12/C-13. The HMBC correlations between H-29 (δ_H 3.21)/H-30 (δ_H 0.95) and C-19 (δ_C 41.4)/C-20 (δ_C 36.8)/C-21 (δ_C 29.3) and the chemical shift of C-29 (δ_C74.4) determined the presence of a hydroxy group at C-29. Finally, the sugar moiety at C-28 was confirmed by a long-range correlation from H-1′ (δ_C 5.41) to C-28 (δ_C 178.0). On the basis of the evidence and when comparing the NMR data of **1** with those reported in the literature [19], compound **1** was elucidated as quadranoside III. To the best of our knowledge, this compound was reported from *Solanum* genus for the first time.

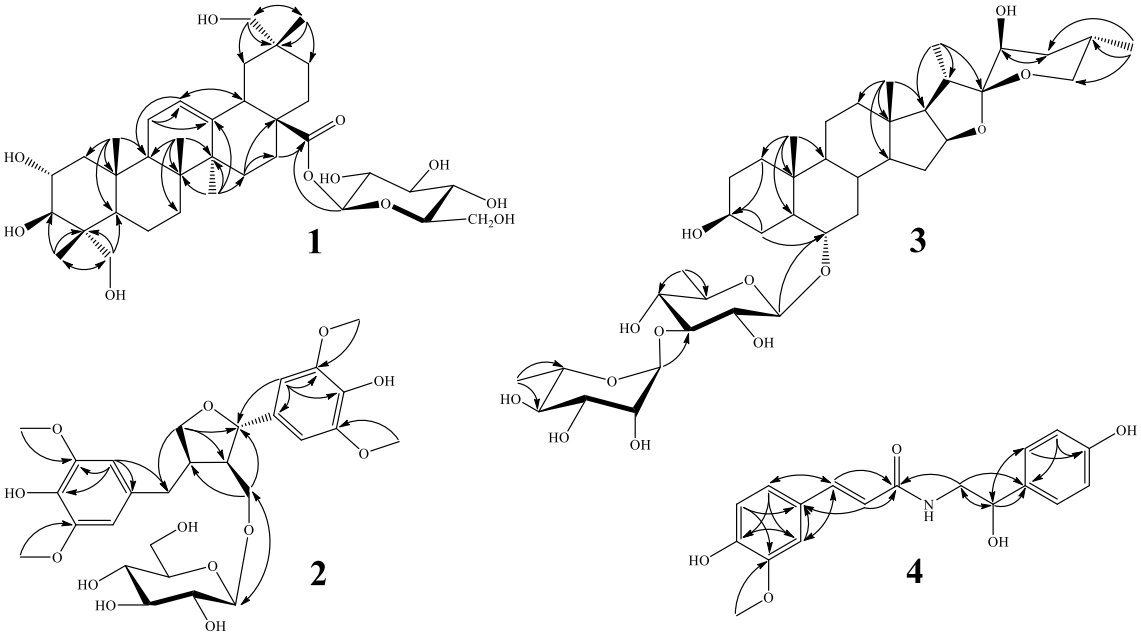

Figure 2. The key HMBC correlations of compounds **1**-**4**.

Compound **2** was isolated as a white amorphous powder. The ^{1}H-NMR spectrum of **2** showed the presence of four aromantic protons at δ_H6.54 (2H, s) and 6.67 (2H, s); four methoxy groups at δ_H 3.85 (6H, s), and 3.86 (6H, s); and one anomeric proton at δ_H 4.33 (1H, d, *J* = 7.8

Hz). The ^{13}C-NMR and HSQC spectra displayed signals of 28 carbons, including eight non-protonated carbons at δ_C 132.9, 134.8, 134.9, 135.9, 149.2 (2xC), and 149.3 (2xC); 12 methine carbons at δ_C 43.9, 51.8, 71.7, 75.2, 78.0, 78.2, 84.4, 104.4 (2xCH), and 107.1 (2xCH); four methylene carbons at δ_C 34.4, 62.8, 68.6, and 73.6; and four methoxy carbons at δ_C 56.8 (4xOCH_3). The coupling $J_{H\text{-}1''/H\text{-}2''}$ = 7.8 Hz of anomeric proton and the ^{13}C chemical shifts of the sugar moiety (δ_C 104.8, 75.2, 78.2, 71.7, 78.0, and 62.8) suggested the presence of *β*-D-glucopyranosyl moiety. The HMBC spectrum of **2** showing correlations from H-2/H-6 (δ_H 6.67) to C-1 (δ_C 134.8)/C-3/C-5 (δ_C 149.2)/C-4 (δ_C 135.9)/C-7 (δ_C 84.4) and the methoxy protons (δ_H 3.85) to C-3, C-5 (δ_C 149.2) indicated the presence of the position of one hydroxy group at C-4 and two methoxy groups at C-3, C-5 of a benzene ring, and this ring was connected to C-7. Besides, the HMBC correlations from H-2', H-6' (δ_H 6.54) to C-1' (δ_C 132.9)/C-3', C-5' (δ_C 149.3)/C-4' (δ_C 134.9)/C-7' (δ_C 34.4) and the methoxy protons (δ_H 3.86) to C-3 ', C-5' (δ_C 149.3) suggested the presence of one hydroxy and two methoxy groups at C-4', C-3' and C-5' of a benzene ring, respectively, and this ring was connected to C-7'. In addition, the HMBC correlations from H-1″ (δ_H4.33) to C-9 (δ_C 68.6) and H-9 (δ_H 3.80 and 4.10) to C-1″ (δ_C 104.8) determined the sugar moiety at C-9 of aglycone. According to the analysis and comparison with the literature data [20], compound **2** was determined to be alangilignoside C. This compound was isolated from *S. buddleifolium* [21], however, this is the first time the compound has been reported from *Solanum melongena*.

Compound **3** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **3** displayed two tertiary methyl groups at δ_H 0.84 (3H, s) and 0.89 (3H, s); four secondary methyl groups at δ_H 0.79 (3H, d, *J* = 6.0 Hz), 1.12 (3H, d, *J* = 7.2 Hz), 1.27 (1H, d, *J* = 6.8 Hz), and 1.30 (1H, d, *J* = 6.0 Hz); and two anomeric protons at δ_H 4.29 (1H, d, *J* = 7.8 Hz) and 5.16 (1H, d, *J* = 1.2 Hz). In addition, the ^{13}C-NMR and HSQC spectral of **3** displayed the signals of 39 carbons, including 27 carbons assigned to an aglycone and 12 carbons assigned to two sugar units. The aglycone was deduced to be a spirostane-*type* steroid with four methyl carbons at δ_C13.9, 16.8, 17.1, and 17.4; nine methylene carbons at δ_C22.0, 31.9, 32.7, 32.9, 37.6, 38.5, 40.7, 41.6, and 67.5; 11 methine carbons at δ_C25.0, 35.3, 41.7, 51.8, 55.1, 57.5, 65.7, 71.1, 71.8, 80.4, and 82.4; and three non-protonated carbons at δ_C37.5, 42.1, and 110.0 [22]. Besides, the HMBC correlations from H-18 (δ_H 0.84) to C-12 (δ_C 40.7)/C-13 (δ_C 42.1)/C-14 (δ_C 57.5); H-19 (δ_H 0.89) to C-1 (δ_C 38.5)/C-2 (δ_C 31.9)/C-5 (δ_C 51.8)/C-10 (δ_C 37.5); H-21 (δ_H 1.12) to C-17 (δ_C 65.7)/C-20 (δ_C 41.7); and H-27 (δ_H 0.79) to C-24 (δ_C 37.6)/C-25 (δ_C 25.0)/C-26 (δ_C 67.5) as well as other HMBC correlations on the aglycone moiety confirmed the spirostane skeleton of **3** (Figure 2). The HMBC correlations from H-2 (δ_H1.42 and 1.77) and H-4 (δ_H 1.18 and 2.39) to C-3 (δ_C 71.8) led to the assigment of a hydroxy group at C-3. Similarly, the HMBC correlations from H-4 (δ_H1.18 and 2.39) to C-6 (δ_C 80.4) and H-24 (δ_H 1.65 and 1.68) to C-23 (δ_C 71.1) showed the attachment of two hydroxy groups at C-6 and C-23, respectively. The absolute configuration of C-22 chiral center was deduced by comparing δ_H (H_3-21)/δ_C (C-20) value of **3** (1.12/41.7) with that of 22*S* and 22*R* forms of 23-hydroxyspirostane glycosides. In the 22*S* form such as paniculonins A-B, δ_H (H_3-21)/δ_C (C-20) values were 0.96/37.0 and 0.96/36.6, respectively. While the strong downfield shifts of δ_H (H_3-21)/δ_C (C-20) were observed in the 22*R* form such as torvosides J-K [δ_H (H_3-21)/δ_C (C-20): 1.10/41.6 and 1.12/40.8, respectively] [22]. The δ_H (H_3-21)/δ_C (C-20) of **3** was 1.12/41.7; consequently, the 22*R* configuration was suggested for **3**. The 25*S* configuration was confirmed by the differences in chemical shift values between H_2-24 ($\Delta_{a\text{-}b}$ = 0.03 ppm) and H_2-26 ($\Delta_{a\text{-}b}$ = 0.11 ppm) geminal protons [in 25*R* compounds ($\Delta_{a\text{-}b}$ < 0.2 ppm); and in 25*S* compounds ($\Delta_{a\text{-}b}$ > 0.35 ppm)] [23]. The sugar chain was assigned to *α*-L-rhamnopyranosyl-(1→3)-*β*-D-quinovopyranosyl moiety by comparing their NMR data with those reported in the literature

[22]. Futhermore, the linkage of the sugar chain at C-6 was confirmed by the HMBC correlations from H-1′ (δ_H4.29) to C-6 (δ_C 80.4). The above evidence and comparison of the NMR data with those reported in [22] led to the elucidation of **3** as torvoside J.

Compound **4** was obtained as a colourless oil, and its molecular formula was determined to be $C_{18}H_{19}NO_5$ by ESI-MS at m/z 330.2 $[M+H]^+$ with a combination of NMR data. The ^{1}H-NMR spectrum of **4** showed signals for aromantic protons of an ABX system at δ_H 7.14 (1H, d, *J* = 2.0 Hz), 6.81 (1H, d, *J* = 8.5 Hz), and 7.04 (1H, dd, 2.0, *J* = 8.5 Hz); four aromantic protons of AA′BB′ system at δ_H7.24 (2H, d, *J* = 8.5 Hz) and 6.79 (2H, d, *J* = 8.5 Hz); two olefinic protons of one *trans* double bond at δ_H7.46 (1H, d, *J* = 16.0 Hz) and 6.48 (1H, d, *J* = 16.0 Hz); one oxymethine proton at δ_H 4.74 (1H, m); two methylene protons at δ_H 3.46 (1H, dd, *J* = 8.0, 13.5 Hz) and 3.56 (1H, dd, *J* = 5.0, 13.5 Hz); and three protons of a methoxy group at δ_H 3.90 (3H, s). The analysis of the ^{13}C-NMR spectrum with the aid of the HSQC of **4** showed signals of 18 carbons, including six non-protonated carbons at δ_H 128.3, 134.7, 149.3, 149.9, 158.1, and 169.5; nine methine carbons at δ_C 111.6, 116.1 (2xCH), 116.5, 118.7, 123.3, 128.5 (2xCH), and 142.2; one oxymethine carbon at δ_C 73.5; one methylene carbon at δ_C 48.3; and one methoxy carbon at δ_C 56.4. The HMBC correlations from H-7 (δ_H 7.46) to C-2 (δ_C 111.6)/C-6 (δ_C 123.3); from H-8 (δ_H 6.48) to C-1 (δ_C 128.3)/C-9 (δ_C 169.5); from H-2 (δ_H 7.14) to C-4 (δ_C 149.9)/C-7 (δ_C 142.2); from H-5 (δ_H 6.81) to C-1 (δ_C 128.3)/C-3 (δ_C 149.3); and from the protons of methoxy group (δ_H 3.90) to C-3 (δ_C 149.3) showed the presence of a furuloyl moiety. Besides, the HMBC correlations from H-7′ (δ_H4.74) to C-1′ (δ_C 134.7)/C-2′/C-6′ (δ_C 128.5)/C-8′ (δ_C 48.3); and H-2′/H-6′(δ_H 7.24) to C-4′ (δ_C 158.1)/C-7′(δ_C 73.5) indicated the presence of an octopamine moiety. Furthermore, the HMBC correlation between H-8′ (δ_H 3.46, 3.56) and C-9 (δ_C 169.5) confirmed that the feruloyl moiety was linked to the octopamine moiety at C-8′/C-9 through an amide bond. Based on the spectroscopic evidence, compound **4** was identified to be *N-trans*-feruloyloctopamine [24].

3.2. NO production inhibitory assay

All isolated compounds (**1-4)** were evaluated for their inhibitory activity on LPS-induced NO production in RAW264.7 macrophages. The results (Table 1) showed that compounds **1** and **4** had modest effects with IC_{50} values of 99.67 and 91.80 µM, respectively (posotive control L-NMMA, 32.26 µM), while compounds **2** and **3** were inactive. Previous studies have reported that lignanamides and steroidal saponins from *Solanum melongena* showed significant dose-dependent inhibitory effects against NO production in RAW264.7 macrophages with IC_{50} values ranging from 10.6 to 59.5 µM [5, 6, 11]. To our knowledge, this is the first time that compounds (**1)** and (**4)** from *S. melongena* have been reported for NO production inhibitory activity.

Table 1. Nitric oxide inhibitory effects of compounds **1-4**.

Compound	IC_{50} (µM)[b]	Cell viability (%)[c]
1	99.67 ± 3.86	100.15
2	> 100	92.99
3	> 100	99.74
4	91.80 ± 4.62	96.66
L-NMMA[a]	32.26 ± 3.42	87.20

[a]Positive control; [b]The values are mean ± SD (n = 3); [c]At the concentration of 100 µM.

4. CONCLUSIONS

In conclusion, four known compounds, quadranoside III (**1**), alangilignoside C (**2**), torvoside J (**3**), and *N-trans*-feruloyloctopamine (**4**) were isolated from the leaves of *Solanum melongena* using combined chromatographic methods. Among them, compounds **1** and **4** showed weak inhibitory effects on LPS-induced NO production in RAW264.7 macrophages with IC_{50} values of 99.67 and 91.80 μM, respectively. This is the first time that compound **1** has been reported from *Solanum* genus.

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CRediT authorship contribution statement. Nhi NPK, Ha TP, and Anh LT performed and experiments and analysed the data. Chi VTQ and Cuong LCV designed the experiments and wrote the article.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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