Flavonoid glycoside constituents of the leaves of *Solanum melongena* collected in Thua Thien Hue and their anti-inflammatory activity

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Abstract:

*Solanum melongena* is a member of *Solanum* genus that widely cultivated as fruit vegetable in Vietnam. According to Vietnamese traditional medicine, the fruits and whole plants are used to treat toothache, turgescence, pruritus, and haemorrhoids. As part of our project to study the chemical composition and bioactivity of *Solanum* genus in Vietnam, five flavonoid glycosides including kaempferol-3-O-β-D-glucopyranoside (1), isovitexin (2), kaempferol 3-O-β-D-glucoside-7-O-α-L-rhamnose (3), kaempferol 3-O-β-D-sophoroside-7-O-α-L-rhamnose (4), and kaempferol 3-O-β-D-sophoroside (5) were isolated from the methanol extract of the leaves of *Solanum melongena* growing in Thua Thien Hue province, by using various chromatography methods. Their chemical structures were determined by detailed analysis of 1D- and 2D-NMR and HR-ESI-MS data as well as comparison with the literature. Compounds (2) and (5) were isolated from *Solanum melongena* for the first time while compounds (3) and (4) were the first isolated from the *Solanum* genus. Besides, compounds 2-4 showed weak anti-inflammatory activities against NO production in RAW 264.7 macrophage line with IC<sub>50</sub> values ranging from 54.28 to 91.37 µg/ml.

Keywords: anti-inflammatory activity, flavonoid, NO production, *Solanum melongena*.

Classification number: 3.4

1. Introduction

*Solanum melongena* is widely distributed in tropical and subtropical regions [1]. As a Vietnamese traditional medicine, the fruits and whole plants are used to treat toothache, turgescence, pruritus, and haemorrhoids in Vietnam [1]. Besides, previous biological studies of isolated compounds and extracts from the plant showed various biological activities such as anti-inflammatory [2-4], cytotoxic [5, 6], anti-oxidant [7-10], anti-diabetic [11], and anti-hypertensive [11, 12] activities. Chemical investigations of *Solanum melongena* displayed the presence of lignans [2, 4], phenolic acids [4, 5], anthocyanins [13, 14], flavonols [14], flavonols [4], sesquiterpenes [5], triterpenes [4], and sterols [3, 4]. Results of our current screening program for anti-inflammatory agents from Vietnamese *Solanum* species revealed that the *Solanum melongena* leaves methanol extract showed anti-inflammatory activity with an IC<sub>50</sub> value of 65.17 µg/ml. Herein, we described the isolation and chemical structure elucidation of five flavonoids: kaempferol-3-O-β-D-glucopyranoside (1), isovitexin (2), kaempferol 3-O-β-D-glucoside-7-O-α-L-rhamnose (3), kaempferol 3-O-β-D-sophoroside-7-O-α-L-rhamnose (4), and kaempferol 3-O-β-D-sophoroside (5) from methanol extract of the leaves of *Solanum melongena*. In addition, all the compounds were tested for their inhibitory activities on LPS-induced NO in the RAW 264.7 macrophage line.

2. Experimental design

2.1. Plant materials

The leaves of *Solanum melongena* were collected in Huong Tra, Thua Thien Hue, Vietnam in July 2019. The plant sample was identified by Dr. Tran Thi Phuong Anh (Graduated University of Science and Technology, Vietnam Academy of Science and Technology). The voucher specimens (MISR.2019-15) have been deposited at Mientrung Institute for Scientific Research, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology.

2.2. General procedures

HR-ESI-MS spectra were recorded on an Agilent 6550 iFunnel Q-TOF LC/MS system (Agilent Technologies, USA).
Dried leaves of *Solanum melongena* (3.0 kg) were ultrasonic extracted with methanol for 120 min (3 times x 7 l) at 50°C. The methanol solution was concentrating under reduced pressure, then give methanol extract (SM, 200.0 g) was suspended in purified water (2.0 l) and partitioned with n-hexane, dichloromethane, and ethyl acetate (3 times, 2 l each) to give corresponding soluble extracts, n-hexane (SMH, 66.5 g), dichloromethane (SMD, 14.5 g), ethyl acetate (SME, 17.0 g), and water layer (SMW). The SME fraction was subjected to silica gel C.C. and eluted with dichloromethane/methanol (from 0% to 100% methanol in dichloromethane) to yield 5 fractions so-called SME1-SME5. Four subfractions (SME2A-SME2D) were obtained from the fraction SME2 using an RP-18 column and eluted with methanol/water (1/2, v/v). The SME2D subfraction was continuously separated on a C.C. and eluted with dichloromethane/methanol (10/1, v/v) to yield three smaller fractions, SME2D1-SME2D3. Compound (1) (3 mg) was yielded from SME2D1 on a Sephadex LH-20 column eluted with methanol/water (1/1, v/v). The SME2D3 was purified on a Sephadex LH-20 column eluted with methanol/water (1/1, v/v) to yield compound (2) (4 mg). The water layer was separated on a Diaion HP-20P column, eluted with water to remove sugars and ionomic compounds, and then with an increasing concentration of methanol in water (from 25 to 100%) to obtain four fractions, SMW1-SMW4. Fraction SMW3 was subjected to silica gel C.C. and eluted with mixtures of methanol in dichloromethane (20/1, 10/1, 5/1, 1/1, and 100% methanol) to give five subfractions, namely, SMW3A-SMW3E. Fraction SMW3C was separated on an RP-18 column and eluted with methanol/water (1/2, v/v) to give four fractions, SMW3C1-SMW3C4. Compound (3) (6 mg) was yielded from fraction SMW3C1 on a Sephadex LH-20 eluting with methanol/water (1/1, v/v). The SMW2 was subjected to silica gel C.C. and eluted with mixtures of methanol in dichloromethane (20/1, 10/1, 5/1, 1/1, and 100% methanol) to give five subfractions so-called SMW2A-SMW2E. Then, fraction SMW2C was continuously chromatographed on an RP-18 column and eluted with methanol/water (1/3, v/v) to yield four smaller fractions, SMW2C1-SMW2C4. Compound (4) (5 mg) was obtained from fraction SMW2C1 on a silica gel column eluting with dichloromethane/methanol (3/1, v/v). Finally, the fraction SWM2C4 was purified on a Sephadex LH-20 column and eluted with methanol/water (1/1, v/v) to give compound (5) (4 mg).

Kaempferol-3-O-β-D-glucopyranoside (1): Yellow powder; *C_{21}H_{20}O_{11}*; M=448; HR-ESI-MS: m/z 447.0933 [M-H] (calcd. for *C_{21}H_{20}O_{11}* 447.0927); *1^H*-NMR (DMSO-d_6, 600 MHz): δ_H (ppm) 6.22 (1H, d, J=1.8 Hz, H-6), 6.45 (1H, d, J=1.8 Hz, H-8), 8.03 (2H, d, J=9.0 Hz, H-2′ and H-6′), 6.89 (2H, d, J=9.0 Hz, H-3′ and H-5′), 5.45 (1H, d, J=7.8 Hz, H-1″), 3.20 (1H, m, H-2″), 3.22 (1H, m, H-3″), 3.10 (1H, m, H-4″), 3.08 (1H, m, H-5″), 3.34 (1H, dd, J=4.8, 11.4 Hz, H-6″), 3.55 (1H, brd, J=11.4 Hz, H-5″). *1^3^C*-NMR (DMSO-d_6, 150 MHz): δ_C (ppm) 156.3 (C-2), 133.2 (C-3), 177.5 (C-4), 161.2 (C-5), 98.8 (C-6), 164.4 (C-7), 93.7 (C-8), 156.4 (C-9), 103.9 (C-10), 120.9 (C-1′), 130.9 (C-2′ and C-6′), 115.2 (C-3′ and C-5′), 160.0 (C-4′), 100.9 (C-1″), 74.2 (C-2″), 76.4 (C-3″), 69.9 (C-4″), 77.5 (C-5″), 60.8 (C-6″).

Isovitexin (2): Yellow powder; *C_{19}H_{18}O_{9}*; M=342; HR-ESI-MS: m/z 433.1129 [M+H] (calcd. for *C_{19}H_{18}O_{9}* 433.1135); *1^H*-NMR (DMSO-d_6, 600 MHz): δ_H (ppm) 6.78 (1H, s, H-3), 6.52 (1H, s, H-8), 7.93 (2H, d, J=8.4 Hz, H-2’ and H-6′), 6.93 (2H, d, J=8.4 Hz, H-3′ and H-5′), 4.58 (1H, dd, J=10.2 Hz, H-1″), 4.04 (1H, t, J=9.0 Hz, H-2″), 3.20 (1H, m, H-3″), 3.13 (1H, m, H-4″), 3.17 (1H, m, H-5″), 3.41 (1H, dd, J=6.0, 11.4 Hz, H-6″), 3.68 (1H, brd, J=10.8 Hz, H-5″). *1^3^C*-NMR (DMSO-d_6, 150 MHz): δ_C (ppm) 163.5 (C-2), 102.7 (C-3), 181.9 (C-4), 161.1 (C-5), 108.8 (C-6), 163.3 (C-7), 93.6 (C-8), 156.2 (C-9), 103.4 (C-10), 121.1 (C-1′), 128.4 (C-2′ and C-6′), 115.9 (C-3′ and C-5′), 160.5 (C-4′), 73.0 (C-1″), 70.5 (C-2″), 78.9 (C-3″), 70.2 (C-4″), 81.5 (C-5″), 61.4 (C-6″).

Kaempferol 3-O-β-D-glucoside-7-O-α-L-rhamnoside (3): Yellow powder; *C_{22}H_{22}O_{14}*; M=594; HR-ESI-MS: m/z 595.1657 [M+H]^+ (calcd. for *C_{22}H_{22}O_{14}* 595.1663); *1^H*-NMR (DMSO-d_6, 600 MHz): δ_H (ppm) 6.44 (1H, s, H-6), 6.82 (1H, s, H-8), 8.07 (2H, d, J=9.0 Hz, H-2′ and H-6′), 6.89 (2H, d, J=9.0 Hz, H-3′ and H-5′), 5.48 (1H, d, J=7.2 Hz, H-1″), 3.19 (1H, m, H-2″), 3.08 (1H, m, H-3″), 3.84 (1H, brs, H-4″), 3.22 (1H, m, H-5″), 3.32 (1H, brd, J=10.8 Hz, H-6″), 3.56 (1H, brd, J=10.8 Hz, H-6″), 5.55 (1H, s, H-1″), 3.63 (1H, dd, J=3.0, 9.0 Hz, H-2″), 3.42 (1H, m, H-3″), 3.30 (1H, m, H-4″), 3.09 (1H, m, H-5″), 1.12 (3H, d, J=6.0 Hz, H-6″). *1^3^C*-NMR (DMSO-d_6, 150 MHz): δ_C (ppm) 156.7 (C-2), 133.5 (C-3), 177.6 (C-4), 160.9 (C-5), 99.4 (C-6), 161.7 (C-7), 94.5 (C-8), 156.0 (C-9), 105.6 (C-10), 120.7 (C-1′), 131.0 (C-2′ and C-6′), 115.1 (C-3′ and C-5′), 160.1 (C-4′), 100.8 (C-1″), 74.2 (C-2″), 77.5 (C-3″), 69.8 (C-4″), 76.4 (C-5″), 60.8 (C-6″), 98.4 (C-1″), 70.2 (C-2″), 70.0 (C-3″), 71.6 (C-4″), 69.9 (C-5″), 17.9 (C-6″).
Kaempferol 3-O-β-D-sophoroside-7-O-α-L-rhamnioside (4): Yellow powder; C_{21}H_{20}O_{11}, M=447; HR-ESI-MS: m/z 447.0933 [M+H]^+ (calcd. for [C_{21}H_{19}O_{11}]^- 447.0927). The 1H-NMR spectrum of (I) showed signals of four aromatic protons of an AA’BB’ system at δ_H 6.89 (2H, d, J=9.0 Hz) and 8.03 (2H, d, J=9.0 Hz); two aromatic protons at δ_H 6.22 (1H, d, J=1.8 Hz) and 6.45 (1H, d, J=1.8 Hz); one anomeric proton at 5.45 (1H, d, J=7.8 Hz); and two oxymethylene protons at δ_H 3.34 (1H, d, J=4.8, 11.4 Hz) and 3.55 (1H, brd, J=11.4 Hz). The 13C-NMR, HSQC spectra of (I) displayed 15 carbon signals of a flavonoid aglycone, which corresponded to

![Chemical structure of compounds (1-5).](image-url)
nine quaternary carbons at δC 103.9, 120.9, 133.2, 156.3, 156.4, 160.0, 161.2, 164.4, and 177.5; six methine carbons at δC 93.7, 98.8, 130.9 (2xCH), and 115.2 (2xCH); and six carbon signals of a sugar unit, which corresponded to five methine carbons at δC 69.9, 74.2, 76.4, 77.5, and 100.9 and one oxymethylene carbon at δC 60.8. Analysis of 1H- and 13 C-NMR data suggested the chemical structure of (1) is a kaempferol glycoside [16]. The anomeric proton (JH-1'H=7.8 Hz) and the 13C-NMR data of the sugar unit demonstrated the presence of a β-D-glucopyranosyl moiety in (1). Besides, the HMBC correlation from H-1” (δH 5.45) to C-3 (δC 133.2) determines a linkage between the sugar moiety and C-3 of the aglycone (Fig. 2). Based on the spectra evidence and comparison with those of kaempferol-3-O-β-D-glucopyranoside in the literature [16], compound (1) was identified as kaempferol-3-O-β-D-glucopyranoside. This compound was reported from Solanum melongena [17].

Compound (3) was obtained as a yellow powder. The molecular formula of (3) was elucidated as C27H31O15 based on the HR-ESI-MS ion at m/z 595.1657 [M+H]+ (calcd. for C27H31O15 595.1663). The 1H-NMR of (3) showed signals of six aromatic protons of an AX system and an AA’BB’ system at δH 6.44 (1H, s, H-6), 6.82 (1H, s, H-8), 8.07 (2H, d, J=9.0 Hz, H-2’, H-6’), and 6.89 (2H, d, J=9.0 Hz, H-3’, H-5’); two anomeric protons at δH 5.48 (1H, d, J=7.2 Hz, H-1’); and 5.55 (1H, s, H-1’’); and a CH2 group at δH 1.12 (3H, d, J=6.0 Hz, H-6”). The 13C-NMR and HSQC spectra of (3) displayed the presence of 27 carbon signals including nine quaternary carbons, 16 methine groups, one methylene group, and one methyl group. The comparison of the 1H- and 13C-NMR data of (3) with those of (1) displayed close similarity except for the additional presence of a rhamnopyranosyl moiety at δH 98.4 (C-1’”)/5.55 (H-1’’), 70.2 (C-2’”)/3.63 (H-2’’), 70.0 (C-3’”)/3.42 (H-3’’), 71.6 (C-4’”)/3.30 (H-4’’), 69.9 (C-5’”)/3.09 (H-5’’), and 17.9 (C-6”)/1.12 (H-6”) in (3). The linkage between C-7 of the aglycone and the glucopyranosyl moiety was confirmed by HMBC correlation from H-1” (δH 5.48) to C-3 (δC 133.5). Besides, the HMBC correlation from H-1” (δH 5.55) to C-7 (δC 161.7) determines the position of the rhamnopyranosyl moiety at C-7. The structure of (3) was confirmed by its good agreement with the 13C-NMR data of those reported in the literature [18]. Therefore, compound (3) was determined to be kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnoside. To our best knowledge, this compound was reported from the genus Solanum for the first time.

Compound (4) was yielded as a yellow powder. The molecular formula of (4) was determined to be C27H30O15 from HR-ESI-MS ion at m/z 791.1807 [M+Cl]+ (calcd. for C27H30O15 791.1801). The 1H-NMR of (4) showed signals of six aromatic protons at δH 6.43 (1H, d, J=1.8 Hz), 6.82 (1H, d, J=1.8 Hz), 6.92 (2H, d, J=9.0 Hz), and 8.09 (2H, d, J=9.0 Hz); three anomeric protons at δH 4.42 (1H, d, J=7.8 Hz), 5.55 (1H, d, J=1.2 Hz), and 5.70 (1H, d, J=7.2 Hz); and three protons of a methyl group at δH 1.13 (3H, d, J=6.6 Hz). The 13C-NMR and HSQC spectra of (4) displayed the presence of 33 carbon signals including nine quaternary carbons, 21 methine groups, two methylene groups, and one methyl group. The coupling constant of the anomeric protons, including JH-1’”/H-2’”=7.2 Hz, JH-1’”/H-3’”=7.8 Hz, and JH-1’”/H-4’”=1.2 Hz, and 13C-NMR data of the sugar units suggest the presence of one rhamnopyranosyl and two glucopyranosyl moieties in (4). The comparison of NMR data of (3) and (4) revealed that compound (4) was also a kaempferol glycoside, but it contained one more glucopyranosyl moiety than (3). The HMBC correlations from H-1” (δH 5.70) to C-3 (δC 133.3) and H-1” (δH 4.42) to C-2” (δC 82.4) determined the position of a sophorosyl moiety at C-3. Besides, the linkage between C-7 of the aglycone and the rhamnopyranosyl moiety was determined by the HMBC correlation from H-1” (δH 5.55) to C-7 (δC 161.6). Based on the spectra evidence and comparison with those in the literature [19], compound (4) was identified as kaempferol 3-O-β-D-sophorosyl-7-O-α-L-rhamnoside. This is the first time the compound was isolated from the genus Solanum.

The remaining compounds were characterized as isovitexin (2) and kaempferol-3-O-sophoroside (5) by comparison of their NMR data with those previously reported [16, 20].
All isolated compounds were assayed for their inhibitory activities on LPS-induced NO in the RAW 264.7 macrophage line. The results showed that compounds (2-4) had a weak anti-inflammatory effect with IC_{50} values of 91.37, 54.28, and 67.45 μg/ml, respectively, relative to the positive control L-NMMA with IC_{50} values of 8.01±0.85 μg/ml. Compounds (1) and (5) were considered to be inactive (IC_{50}>100 μg/ml).

4. Conclusions

A phytochemical study on the methanol extract of the leaves of Solanum melongena led to the isolation of five flavonoid glycosides including kaempferol-3-O-β-D-glucopyranoside (1), isovitexin (2), kaempferol 3-O-β-D-glucoside-7-O-α-L-rhamnoside (3), kaempferol 3-O-β-D-sophoroside-7-O-α-L-rhamnoside (4), and kaempferol 3-O-β-D-sophoroside (5). Among them, compounds (2-4) showed weak anti-inflammatory activities against NO production in RAW 264.7 macrophage line with IC_{50} values ranging from 54.28 to 91.37 μg/ml. Compounds (2) and (5) were isolated from this plant for the first time, while compounds (3) and (4) were first reported from the genus Solanum.

CRediT author statement

Phuong Ha Tran, Thi Loi Nguyen, and Thi Kim Thu Vo: Isolated the compounds and elucidated the structures; That Huu Dat Ton and Tuan Anh Le: Evaluated anti-inflammatory activity of isolated compounds; Thi Quynh Chi Vu and Canh Viet Cuong Le: Wrote the manuscript and carried out the data analysis and structure elucidation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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