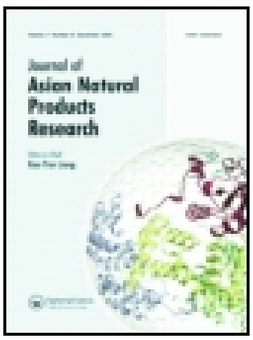


Journal of Asian Natural Products Research

ISSN: 1028-6020 (Print) 1477-2213 (Online) Journal homepage: https://www.tandfonline.com/loi/ganp20

# Flavonol glycosides from *Fissistigma maclurei*

Nguyen Van Ba, Chu Van Men, Nguyen Tien Khi, Tran Viet Tiet, Pham Gia Khanh, Le Bach Quang, Nguyen Van Chuyen, Vu Dinh Tien, Nguyen Xuan Nhiem & Phan Van Kiem

**To cite this article:** Nguyen Van Ba, Chu Van Men, Nguyen Tien Khi, Tran Viet Tiet, Pham Gia Khanh, Le Bach Quang, Nguyen Van Chuyen, Vu Dinh Tien, Nguyen Xuan Nhiem & Phan Van Kiem (2019): Flavonol glycosides from *Fissistigma maclurei*, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2019.1671374

**To link to this article:** https://doi.org/10.1080/10286020.2019.1671374

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH
https://doi.org/10.1080/10286020.2019.1671374



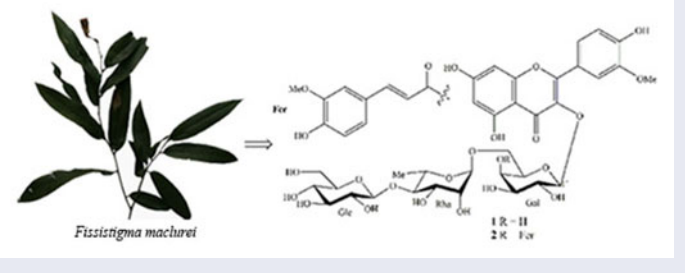
# Flavonol glycosides from *Fissistigma maclurei*

Nguyen Van Ba[a]*, Chu Van Men[a]*, Nguyen Tien Khi[b], Tran Viet Tiet[a], Pham Gia Khanh[a], Le Bach Quang[a], Nguyen Van Chuyen[a], Vu Dinh Tien[c], Nguyen Xuan Nhiem[d] and Phan Van Kiem[d]

[a]Vietnam Military Medical University, Hanoi, Vietnam; [b]Phenikaa University, Hanoi, Vietnam; [c]Vietnam National Cancer Hospital, Hanoi, Vietnam; [d]Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam

**ABSTRACT**

Two new flavonol glycosides, fissmacosides A (1) and B (2) along with two known flavonol glycosides, kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (3) and kaempferol 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-[4-(*E*)-feruloyl]-β-D-galactopyranoside (4) were isolated from the methanol extract of the leaves of *Fissistigma maclurei* Merr. Their structures were determined on the basis of extensive spectroscopic methods, including 1D-, 2D-NMR, and MS data.




**ARTICLE HISTORY**
Received 3 May 2019
Accepted 18 September 2019

**KEYWORDS**
Flavonol; Fissistigma maclurei; fissmacoside A; fissmacoside B

## 1. Introduction

*Fissistigma* is one of the important genera of Annonaceae family. There are about 80 *Fissistigma* species distributed in Australia and Asia, especially in South-East Asia such as Thailand, Malaysia, Cambodia, and Vietnam. Chemical studies of *Fissistigma* genus indicated the main components to be flavonoids [1], alkaloids [2], and sesquiterpenes [3]. These compounds exhibited antioxidant and anticancer activities. *Fissistigma maclurei* Merr. are climbers that grow up to 6 m tall. There are few reports of pharmacological potential and chemistry of this plant [4,5]. This article reports the isolation and structure elucidation of two new and two known flavonol glycosides from the leaves of *F. maclurei* Merr (Figure 1).

**CONTACT** Phan Van Kiem phankiem@yahoo.com Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
*These authors contributed equally to this work.

Supplemental data for this article can be accessed at https://doi.org/10.1080/10286020.2019.1671374.

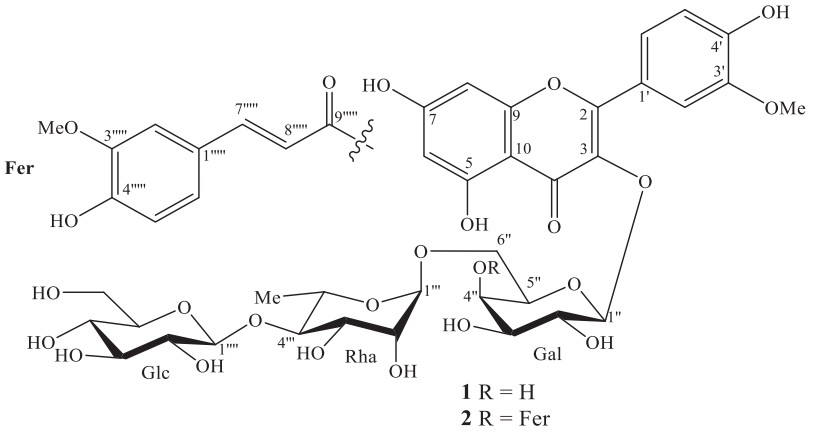



**Figure 1.** Chemical structures of compounds **1** and **2**.

## 2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder and its molecular formula was determined to be $C_{34}H_{42}O_{21}$ from HR-ESI-MS ion peak at *m/z* 809.2110 $[M+Na]^+$. The $^1$H-NMR spectrum of **1** (in methanol-$d_4$) showed the specific signals: two aromatic protons of A ring at $\delta_H$ 6.19 (1 H, br s) and 6.41 (1 H, br s); three aromatic protons of ABX coupling system of B ring at $\delta_H$ 6.89 (1 H, d, $J = 8.4$ Hz), 7.59 (1 H, d, $J = 8.4$ Hz), and 8.02 (1 H, s); one methoxy group at $\delta_H$ 3.94 (3 H, s); three anomeric protons at $\delta_H$ 4.48 (1 H, d, $J = 7.6$ Hz), 4.50 (1 H, br s), and 5.20 (d, $J = 7.8$ Hz) and one secondary methyl group at $\delta_H$ 1.20 (3 H, d, $J = 6.0$ Hz), suggesting the presence of three sugar moieties. The $^{13}$C-NMR and HSQC spectra of **1** revealed the signals of 34 carbons, of which 15 carbons were assigned to a flavonol and 18 carbons to three monosaccharide moieties (Table 1). The HMBC correlations between H-2′ ($\delta_H$ 8.02)/H-6′ ($\delta_H$ 7.59) and C-2 ($\delta_C$ 158.9)/C-1′ ($\delta_C$ 122.9)/C-4′ ($\delta_C$ 150.9); between H-5′ ($\delta_H$ 6.89) and C-3′ ($\delta_C$ 148.4); and between the methoxy ($\delta_H$ 3.94) and C-3′ ($\delta_C$ 148.4) suggested that the methoxy and the hydroxy were at C-3′ and C-4′, respectively (Figure 2). Thus, the flavonol aglycone was identified as isorhamnetin [6]. Acid hydrolysis of **1** revealed D-glucose, D-galactose, and L-rhamnose (identified as trimethylsilyl (TMS) derivatives by a gas chromatography method). In addition, the multiplicity of gal H-1″ [5.20 (d, $J = 7.8$ Hz], rha H-1‴ [4.50 (br s)], and glc H-1″″ [4.48 (d, $J = 7.6$ Hz)] in the $^1$H-NMR spectrum of **1** proved configurations of sugar moieties as *β*-D-galactopyranosyl, *β*-D-glucopyranosyl, and α-L-rhamnopyranosyl. The HMBC correlations between rha H-1‴ ($\delta_H$ 4.50) and gal C-6″ ($\delta_C$ 68.0); glc H-1″″ ($\delta_H$ 4.48) and rha C-4‴ ($\delta_C$ 83.4); and between gal H-1″ ($\delta_H$ 5.20) and C-3 ($\delta_C$ 135.5) suggested the sugar linkages as *O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranosyl and at C-3 of flavonol [7]. Consequently, the new compound **1** was determined to be isorhamnetin 3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside and named as fissmacoside A.

The HR-ESI-MS experiment of **2** resulted to the molecular formula of $C_{44}H_{48}O_{23}$. The $^1$H-NMR spectrum suggested that compound **2** was a flavonol glycoside with the following signals: two *meta*-protons of A ring at $\delta_H$ 6.20 (1 H, s) and 6.42 (1 H, s); an

**Table 1.** $^{1}$H- and $^{13}$C-NMR spectroscopic data for compounds **1** and **2** in methanol-$d_4$.

| | 1 | | 2 | |
|---|---|---|---|---|
| C | $\delta_C$ | $\delta_H$ (mult., $J$ = Hz) | $\delta_C$ | $\delta_H$ (mult., $J$ = Hz) |
| Flavonol | | | | |
| 2 | 158.9 | – | 159.0 | – |
| 3 | 135.5 | – | 135.4 | – |
| 4 | 179.5 | – | 179.3 | – |
| 5 | 163.0 | – | 163.0 | – |
| 6 | 100.0 | 6.19 (s) | 100.0 | 6.20 (s) |
| 7 | 166.0 | – | 166.0 | – |
| 8 | 94.9 | 6.41 (s) | 95.0 | 6.42 (s) |
| 9 | 158.5 | – | 158.5 | – |
| 10 | 105.7 | – | 105.7 | – |
| 1′ | 122.9 | – | 123.0 | – |
| 2′ | 114.6 | 8.02 (s) | 114.9 | 8.05 (s) |
| 3′ | 148.4 | – | 148.3 | – |
| 4′ | 150.9 | – | 151.1 | – |
| 5′ | 116.0 | 6.89 (d, 8.4) | 116.0 | 6.91 (d, 8.4) |
| 6′ | 123.8 | 7.59 (d, 8.4) | 123.9 | 7.63 (d, 8.4) |
| 3′-OMe | 57.0 | 3.94 (s) | 57.0 | 3.96 (s) |
| 3-*O*Gal | | | | |
| 1″ | 104.9 | 5.20 (d, 7.8) | 105.0 | 5.29 (d, 6.0) |
| 2″ | 73.0 | 3.78–3.82 (m) | 73.5 | 3.81–3.85 (m) |
| 3″ | 75.0 | 3.53–3.55 (m) | 73.5 | 3.83–3.85 (m) |
| 4″ | 70.1 | 3.75 (br d, 2.8) | 71.4 | 5.38 (br s) |
| 5″ | 75.5 | 3.63–3.66 (m) | 73.9 | 3.88–3.90 (m) |
| 6″ | 68.0 | 3.47–3.49 (m) | 67.1 | 3.21–3.25 (m) |
| | | 3.69–3.71 (m) | | 3.47–3.50 (m) |
| 6″-*O*Rha | | | | |
| 1‴ | 102.0 | 4.50 (br s) | 102.3 | 4.46 (br s) |
| 2‴ | 72.0 | 3.58 (br s) | 72.0 | 3.62 (br s) |
| 3‴ | 72.3 | 3.67 (dd, 2.0, 8.0) | 72.0 | 3.70 (dd, 2.0, 8.0) |
| 4‴ | 83.4 | 3.45–3.49 (m) | 82.8 | 3.42–3.46 (m) |
| 5‴ | 68.4 | 3.55–3.59 (m) | 68.2 | 3.41–3.45 (m) |
| 6‴ | 18.2 | 1.20 (d, 6.0) | 18.2 | 1.09 (d, 5.0) |
| 4‴-*O*Glc | | | | |
| 1⁗ | 105.7 | 4.48 (d, 7.6) | 105.2 | 4.54 (d, 7.6) |
| 2⁗ | 76.0 | 3.14–3.18 (m) | 75.9 | 3.19–3.23 (m) |
| 3⁗ | 78.3 | 3.30–3.32 (m) | 78.2 | 3.33–3.37 (m) |
| 4⁗ | 71.6 | 3.24–3.28 (m) | 71.7 | 3.22–3.26 (m) |
| 5⁗ | 78.0 | 3.19–3.23 (m) | 77.9 | 3.20–3.24 (m) |
| 6⁗ | 62.8 | 3.62 (dd, 4.8, 12.0) | 62.9 | 3.59 (dd, 4.0, 12.0) |
| | | 3.81 (br d, 12.0) | | 3.79 (br d, 12.0) |
| 4″-*O*Fer | | | | |
| 1‵‴ | | | 127.7 | – |
| 2‵‴ | | | 111.6 | 7.20 (s) |
| 3‵‴ | | | 149.4 | – |
| 4‵‴ | | | 150.8 | – |
| 5‵‴ | | | 116.5 | 6.83 (d, 8.0) |
| 6‵‴ | | | 124.5 | 7.10 (d, 8.0) |
| 7‵‴ | | | 147.8 | 7.64 (d, 16.0) |
| 8‵‴ | | | 115.1 | 6.38 (d, 16.0) |
| 9‵‴ | | | 168.8 | – |
| OMe | | | 56.3 | 3.93 (s) |

Assignments were done by HSQC, HMBC, and COSY experiments; Glc, glucopyranosyl; Rha, rhamnopyranosyl; Gal, galactopyranosyl; Fer, feruloyl.

ABX coupling system of B ring at $\delta_H$ 6.91 (1 H, d, $J$ = 8.4 Hz), 7.63 (1 H, d, $J$ = 8.4 Hz), and 8.05 (1 H, s); one methoxy group at $\delta_H$ 3.96 (3 H, s); three anomeric protons at $\delta_H$ 4.46 (1 H, br s), 4.54 (1 H, d, $J$ = 7.6 Hz), and 5.29 (1 H, d, $J$ = 6.0 Hz); the proton signals of (*E*)-feruloyl moiety at $\delta_H$ 7.20 (1 H, s), 6.83 (1 H, d, $J$ = 8.0 Hz),

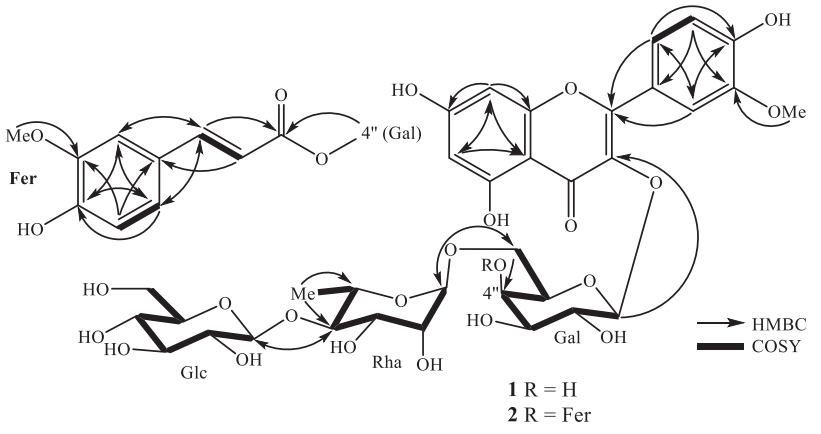



**Figure 2.** The key HMBC and COSY correlations of compounds **1** and **2**.

7.10 (1 H, d, $J = 8.0$ Hz), 6.38 (1 H, d, $J = 16.0$ Hz), and 7.64 (1 H, d, $J = 16.0$ Hz), and one methoxy group at $\delta_H$ 3.93 (s). The $^{13}$C-NMR and HSQC spectra of **2** showed the signals of 44 carbons, including 2 carbonyl, 12 non-protonated carbons, 25 methines, 2 methylenes, and 3 methyl carbons (Table 1). The $^{1}$H- and $^{13}$C-NMR data of **2** showed almost the same to those of fissmacoside A (1) except for the addition of feruloyl moiety at gal C-4ddition of feruloyl(arb**2** proved the presence of D-galactose, L-rhamnose, and D-glucose (identified as TMS derivatives by a gas chromatography method). The HMBC correlations between rha H-1‴ ($\delta_H$ 4.46) and gal C-6″ ($\delta_C$ 67.1); glc H-1⁗ ($\delta_H$ 4.54) and rha C-4‴ ($\delta_C$ 82.8); and between gal H-4″ ($\delta_H$ 5.38) and fer C-98) and $\delta_C$ 168.8) confirmed the sugar linkages as *O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranosyl and feruloyl group at gal C-4″. Finally, the HMBC correlation from gal H-1″ ($\delta_H$ 5.29) to C-3 ($\delta_C$ 135.4) indicated the trisaccharide position at C-3 of flavonol. Based on the above evidence, compound **2** was determined to be isorhamnetin 3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-[4-(*E*)-feruloyl]-*β*-D-galactopyranoside, a new compound and named as fissmacoside B.

The known compounds were elucidated as kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside (3) [8] and kaempferol 3-*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-[4-(*E*)-feruloyl]-*β*-D-galactopyranoside (4) [7] by comparing their observed and reported physical data.

All isolated compounds were evaluated for cytotoxic activity against three human cancer cell lines, A-549 (human lung cancer), MCF-7 (human breast cancer), and MKN-45 (human gastric cancer) at 10 μM. However, none of them showed cytotoxic activity (cell viability >50%).

## 3. Experimental

### 3.1. General experimental procedures

Optical rotations were determined on a JASCO P-2000 digital polarimeter (JASCO Ltd., Tokyo, Japan). UV spectra were obtained from Labtron LUS-B14 spectrophotometer (Labtron Equipment Ltd., Camberley, UK). The IR spectra were obtained from a Bruker TENSOR 37 FT-IR spectrometer (Bruker, Ettlingen, Germany). NMR spectra were

recorded on an Agilent 400 MHz (Agilent Technologies, Santa Clara, CA, USA). Data processing was carried out with the MestReNova ver. 9.0.1. HR-ESI-MS were recorded on an AGILENT 6550 iFunnel Q-TOF LC/MS system (Agilent Technologies). HPLC was carried out using an AGILENT 1200 HPLC system (Agilent Technologies). Column chromatography was performed on silica gel (Kieselgel 60, 230–400 mesh, Merck, Darmstadt, Germany) or RP-18 resins (30–50 $\mu$m, Fuji Silysia Chemical Ltd., Tokyo, Japan). For thin layer chromatography (TLC), pre-coated silica-gel 60 $F_{254}$ (0.25 mm, Merck) and RP-18 $F_{254}S$ (0.25 mm, Merck) plates were used.

### 3.2. Plant material

The leaves of *Fissistigma maclurei* Merr. were collected at Curut, Daknong, Vietnam in April 2018, and identified by Dr. Nguyen, The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (HVQY1804) was deposited at the Military Medical University.

### 3.3. Extraction and isolation

The dried powdered leaves of *F. maclurei* (2.0 kg) were sonicated with hot methanol two times (each 5 L, 2 h) and removed the solvent *in vacuo* to yield methanol (MeOH) extract (120.0 g). The MeOH extract was suspended in $H_2O$ (1.0 L) and successively partitioned with chloroform, and ethyl acetate (EtOAc) to yield chloroform (FM1, 18.0 g), EtOAc (FM2, 8.0 g) residues, and water layer (FM3).

The water-soluble fraction (FM3) was chromatographed on a Diaion HP-20P column eluting with water then increasing the concentration of MeOH in water (25, 50, 75, and 100%) to obtain four sub-fractions, FM3A (5.0 g), FM3B (8.0 g), FM3C (16.0 g), and FM3D (7.0 g). The FM3C fraction was chromatographed on a silica gel column eluting with gradient solvents of $CHCl_3$–MeOH (10: 1 M3C fraction was chromatographed on a silica, FM3C1–FM3C3. The FM3C2 fraction was chromatographed on a Sephadex LH-20 column eluting with MeOH to give two fractions, FM3C2A-FM3C2B. The fraction FM3C2A was chromatographed on HPLC using J'sphere ODS-H80 (150 mm × 20 mm ID) with solvent condition of 35% acetonitrile in water, the flow rate of 3 ml/min, and the wavelength of 254 nm to yield compound **2** (5.0 mg, $t_R$ 38.8 min). The FM3C3 fraction was chromatographed on a RP-18 column using MeOH–water (1: 1, v/v) as eluent solvent to give three fractions, FM3C3A-FM3C3C. The FM3C3B was chromatographed on HPLC using J'sphere ODS-H80 (150 mm × 20 mm ID) with solvent condition of 25% acetonitrile in water, the flow rate of 3 ml/min, and the wavelength of 254 nm to yield compounds **1** (5.0 mg, $t_R$ 44.2 min) and **3** (11.0 mg, $t_R$ 51.7 min). Compound **4** (5.0 mg, $t_R$ 32.3 min) was obtained from FM3C3C using J'sphere ODS-H80 column (150 mm × 20 mm ID) on Agilent HPLC 1290 with solvent condition of 30% acetonitrile in water, the flow rate of 3 ml/min, and the wavelength of 254 nm

#### 3.3.1. Fissmacoside A (1)

Yellow amorphous powder. $[\alpha]_D^{25}$ −67.0 (*c* 0.1 MeOH). UV (MeOH) $\lambda_{max}$: 264, 370 nm. IR (KBr) $\nu_{max}$: 3390, 1658 $cm^{-1}$. $^1H$- and $^{13}C$-NMR spectral data (methanol-

$d_4$): see Table 1. HR-ESI-MS *m/z*: 809.2110 $[M+Na]^+$ (calcd for $C_{34}H_{42}NaO_{21}$, 809.2111).

#### 3.3.2. Fissmacoside B (2)

Yellow amorphous powder. $[\alpha]_D^{25}$ −86.0 (*c* 0.1 MeOH). UV (MeOH) $\lambda_{max}$: 265, 370 nm. IR (KBr) $\nu_{max}$: 3410, 1675, 1658 $cm^{-1}$. $^1$H- and $^{13}$C-NMR spectral data (methanol-$d_4$): see Table 1. HR-ESI-MS *m/z*: 985.2606 $[M+Na]^+$ (calcd for $C_{44}H_{50}NaO_{24}$, 985.2584).

### 3.4. Acid hydrolysis

Each compound (**1** and **2**, 2.0 mg) was separately dissolved in 1.0 N HCl (dioxane–$H_2O$, 1:1, v/v, 1.0 ml) and heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate; the solvent was thoroughly removed under a nitrogen stream overnight. After extraction with $CHCl_3$, the water layer was concentrated to dryness using $N_2$. The residue was dissolved in pyridine (0.1 ml) and then added L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 ml). The reaction mixture was heated at 60 °C for 2 h and then added trimethylsilylimidazole solution (0.1 ml), heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and $H_2O$ (0.1 ml each), and the organic layer was analyzed by gas chromatography: column DB-5 (0.32 mm ID × 30 m length), detector FID, column temperature 210 °C, injector temperature 270 °C, detector temperature 300 °C, carrier gas He (2 ml/min). Under these conditions, the standard sugars gave peaks at $t_R$ (min) 14.11 and 14.26 for D- and L-glucose, 18.71 and 22.92 for D- and L-galactose, and 4.50 for L-rhamnose, respectively. Peaks at $t_R$ (min) 14.11, 18.71, and 4.50 of D-glucose, D-galactose, and L-rhamnose for all compounds were observed.

### 3.5. Cytotoxic assays

See Reference [9].

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This article's data dues to the state level research "Research on the intervention increasing the ability to protect the public health in the national frontier in Tay Nguyen and to create goods from the local herbs". Code: TN16/T03.

## References

[1] Y.H. Lan, Y.T. Peng, T.D. Thang, T.L. Hwang, D.N. Dai, Y.L. Leu, W.C. Lai, and Y.C. Wu, *Chem. Pharm. Bull.* **60**, 280 (2012).
[2] S.T. Lu, Y.C. Wu, and S.P. Leou, *Phytochemistry* **24**, 1829 (1985).

[3] A. Porzel, T.P. Lien, J. Schmidt, S. Drosihn, C. Wagner, K. Merzweiler, T.V. Sung, and G. Adam, *Tetrahedron* **56**, 865 (2000).
[4] T.D. Thang, H.V. Luu, V.C. Dung, N.N. Tuan, N.H. Hung, D.N. Dai, and I.A. Ogunwande, *Nat. Prod. Res.* **28**, 174 (2014).
[5] N.V. Hung, D.N. Dai, T.H. Thai, T.D. Thang, and I.A. Ogunwande, Chem. Sci. Int. J. **17**, 1 (2016).
[6] M.A. Beck and H. Häberlein, *Phytochemistry* **50**, 329 (1999).
[7] X. Liu, W. Ye, B. Yu, S. Zhao, H. Wu, and C. Che, *Carbohydr. Res.* **339**, 891 (2004).
[8] A. Hasan, I. Ahmed, M. Jay, and B. Voirin, *Phytochemistry* **39**, 1211 (1995).
[9] V.K. Thu, N.V. Thang, N.X. Nhiem, B.H. Tai, N.H. Nam, P.V. Kiem, C.V. Minh, H.L.T. Anh, N. Kim, S. Park, and S.H. Kim, *Phytochemistry* **116**, 213 (2015).