Journal of Medicinal Materials, 2024, Vol. 29, No. 6 (pp. 323 - 329)

ISOLATION, STRUCTURAL DETERMINATION OF FLAVONE GLYCOSIDES FROM THE LEAVES OF *SYZYGIUM CUMINI* AND THEIR CYTOTOXIC EVALUATION

Nguyen Thi Van,[1,2] Nguyen Thi Hue[1], Vu Van Nam[1], Trinh Thi Thanh Van[1], Nguyen Thuy Linh[1], Tran Thu Huong[2], Cao Duc Tuan[3], Pham Van Cuong[1,] *, Doan Thi Mai Huong[1,]*

[1]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam;*

[2]*Hanoi University of Science and Technology, Hanoi, Vietnam;*

[3]*Hai Phong University of Medicine and Pharmacy, Hai Phong, Vietnam*

*Corresponding author: phamvc@imbc.vast.vn; huongdm@imbc.vast.vn

(Received August 14th, 2024)

Summary

Isolation, Structural Determination of Flavone Glycosides from the Leaves of *Syzygium cumini* and their Cytotoxic Evaluation

Five flavone glycosides were isolated from the EtOAc and aqueous extracts of *Syzygium cumini* (L.) Skeels leaves. Their chemical structures were elucidated as quercetin 3-*O*-neohesperidoside (**1**), rutin (**2**), quercetin 3-*O*-*α*-L-rhamnopyranoside (**3**), kaempferol 3-*O*-neohesperidoside (**4**), and afzelin (**5**) on the basis of detailed analyses of the 1D and 2D NMR spectroscopic data and in comparison with the literature data. All isolated compounds (**1-5**) were estimated for their cytotoxicity against KB, MCF-7, HepG2, and A549 human cancer cell lines. Unfortunately, these compounds were inactive against all four tested human cancer cell lines at the concentration of 128 µg/mL. To the best of our knowledge, compounds **1** and **4** were first reported from the *Syzygium* genus, while compounds **3** and **5** were isolated from the leaves of *Syzygium cumini* for the first time.

Keywords: *Syzygium cumini*, *Myrtaceae*, *Flavone glycosides, Quercetin, Kaempferol, Cytotoxicity.*

1. Introduction

The genus *Syzygium* is one of the genera of the family Myrtaceae comprising 1200-1800 species, which spread out over the world [1, 2] and have been commonly found in Southeast Asia. Many species of the genus *Syzygium* are known for their traditional use in various diseases, such as burns and wounds (*S. aromaticum*), tooth infections and toothache [3]; abdominal pain, indigestion, and diarrhea (*S. cordatum* and *S. guineese*) [4]; dysentery, menorrhagia, asthma, and ulcers (*S. cumini*) [5]; hemorrhages, syphilis, leprosy, ulcers, and lung diseases (*S. jambos*) [6]; mouth ulcers and irregular menstruation (*S. malaccense*); coughs and colds (*S. suboriculare*) [7]; diabetes mellitus (*S. caryophyllatum*, *S. cumini*, *S. malaccense*, and *S. samarangens*) [8]; etc. A few *Syzygium* species have been extensively studied for their phytochemical as well as their biological activities. Of those, *S. cumini*, *S. samarangense*, and *S. jambos* were the most investigated species with the reported pharmacological activities, including antioxidant, antiviral, anti-diabetic, and hepatoprotective properties [9],[10]. Accordingly, flavonoids, terpenoids, alkaloids, and their glycosides were mainly found in the *Syzygium* genus [11],[12]. Recently, there have been few publications relating to the chemical constituents and biological activities of the *Syzygium* genus in Vietnam, for example: *S. myrsinifolium* [13], *S. attopeuense* [14], *S. cerasiforme* [15], etc.

In the course of a screening program of the flora in Vietnam, the ethyl acetate extract of *S. cumini* leaves showed significant cytotoxicity against the KB cell line (40.9% inhibition at the concentration of 1 µg/mL). Thus, herein we report the isolation and structural elucidation of five known compounds, including quercetin 3-*O*-neohesperidoside (**1**), rutin (**2**), quercetin 3-*O*-*α*-L-rhamnopyranoside (**3**), kaempferol 3-*O*-neohesperidoside (**4**), and afzelin (**5**) (Fig. 1). Also, they were evaluated for their cytotoxicity against four human cancer cell lines (KB, HepG2, A549, and MCF-7).

2. Materials and methods

2.1. Plant materials

The leaves of *Syzygium cumini* were collected in Vinh Linh, Quang Tri, Vietnam, in April 2022

and identified by Dr. Nguyen The Cuong of the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (VN-1236F) was deposited at the Institute of Marine Biochemistry, VAST.

2.2. General experiment procedures

NMR spectra were recorded on a Bruker 600 MHz spectrometer operating at 150 MHz for ^{13}C-NMR and at 600 MHz for ^{1}H-NMR. The ^{1}H chemical shift was referenced to CD_3OD at δ_H 3.31 ppm, respectively, while the ^{13}C chemical shift was referenced to the solvent peak at δ_C 49.0 (CD_3OD). TLC *silica gel* Merck 60 F_{254} was used as thin layer chromatography. Column chromatography (CC) was carried out using *silica gel* 40 - 63 µm, YMC RP-18 (30 - 50 µm), Sephadex LH-20, and Dianion HP-20. Medium pressure liquid chromatography (MPLC) was performed on a Biotage-Isolera One system (Sweden).

2.3. Extraction and isolation

Dried and ground leaves of *S. cumini* (1.0 kg) were extracted with MeOH (5 times × 4 L, 24 h) at room temperature. The MeOH solutions were combined and concentrated under reduced pressure to obtain 75 g of the MeOH residue. The MeOH residue was suspended in H_2O (1 L) and then partitioned successively with *n*-hexane (5 times × 1 L), CH_2Cl_2 (5 times × 1 L), and EtOAc (5 times × 1 L). The extracts were concentrated under reduced pressure to give *n*-hexane (H, 10 g), CH_2Cl_2 (D, 30 g), EtOAc (E, 15 g), and aqueous extracts (W, 15 g), respectively.

The ethyl acetate extract (E, 15 g) was subjected to MPLC over *silica gel* using mixtures of CH_2Cl_2 and MeOH (0% to 100% MeOH in CH_2Cl_2) to give 7 fractions E1-E7. Fraction E2 (2.2 g) was separated on a Sephadex LH-20 column (MeOH/CH_2Cl_2 8/2, *v/v*), giving 6 subfractions E2.1-E2.6. Subfraction E2.5 (0.31 g) was purified by C_{18}-reversed phase *silica gel* CC, eluting with H_2O/MeOH (from 0% to 100% MeOH in H_2O) and followed by CC (*n*-hexane/acetone 1/1, *v/v*) to provide **5** (2.0 mg). Fraction E4 (4.5 g) was subjected to CC on Sephadex LH-20 (100% MeOH), giving 8 subfractions E4.1-E4.8. Subfraction E4.7 (1.72 g) was separated by CC on *silica gel* using a mixture of CH_2Cl_2/MeOH (0% to 100% MeOH in CH_2Cl_2) and followed by CC on RP C_{18} eluting with H_2O/MeOH (from 0% to 100% MeOH in H_2O) to give **3** (4.2 mg). The aqueous extract (W, 15 g) was chromatographed on a Diaion HP-20 column, eluting with H_2O/MeOH (from 0% to 100% MeOH in H_2O) to give 4 fractions W1-W4. Fraction W4 (8.0 g) was separated on a Sephadex LH-20 column (MeOH), giving 6 subfractions W4.1-W4.6. Subfraction W4.2 (1.4 g) was subjected to RP C_{18} CC, eluting with H_2O/MeOH (from 0% to 100% MeOH in H_2O) to give 5 subfractions W4.2.1-W4.2.5. Subfraction W4.2.3 (0.18 g) was purified by CC on Sephadex LH-20 (MeOH), followed by CC on *silica gel* using a mixture of CH_2Cl_2/MeOH/HCOOH (9/1/0.1, *v/v/v*) to give **1** (2.0 mg). Subfraction W4.2.4 (0.19 g) was subjected to CC on Sephadex LH-20 column (MeOH) to give **2** (2.5 mg) and **4** (2.8 mg).

Quercetin 3-*O*-neohesperidoside (1): Yellow powder, α_D^{25} -213.63 (*c* 0.208, MeOH), ^{1}H-NMR (600 MHz, CD_3OD, (see Table 2), ^{13}C-NMR (150 MHz, CD_3OD) (see Table 1).

Rutin (2): Yellow powder, α_D^{25} +1.64 (*c* 0.60, MeOH) [16], ^{1}H-NMR (600 MHz, CD_3OD) (see Table 2), ^{13}C-NMR (150 MHz, CD_3OD) (see Table 1).

Quercetin 3-*O*-*α*-L-rhamnopyranoside (3): Yellow powder, α_D^{25} -145.13 (*c* 0.28, MeOH) [17], ^{1}H-NMR (600 MHz, CD_3OD) (see Table 2), ^{13}C-NMR (150 MHz, CD_3OD) (see Table 1).

Kaempferol 3-*O*-neohesperidoside (4): Yellow powder, α_D^{25} -128.95 (*c* 0.208, MeOH) [18], ^{1}H-NMR (600 MHz, CD_3OD) (see Table 2), ^{13}C-NMR (150 MHz, CD_3OD) (see Table 1).

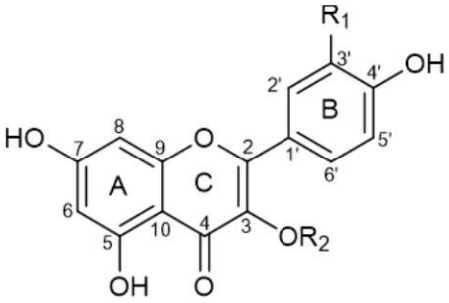
Afzelin (5): Yellow powder, α_D^{25} -183.10 (*c* 0.168, MeOH) [19], ^{1}H-NMR (600 MHz, CD_3OD) (see Table 2), ^{13}C-NMR (150 MHz, CD_3OD) (see Table 1).

2.4. Cytotoxic assay

The cytotoxic activities of compounds **1-5** were evaluated against KB, HepG2, A549, and MCF-7 human cancer cell lines. The cells were cultured at 37°C in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin in a 5% CO_2 incubator. Cells between 5 and 20 passages were used for the assays. The cytotoxic activity was measured by using a modified MTT assay [20]. Viable cells were seeded in 96-well plates at a density of 3×10^4 cells/mL. Cells were treated with four concentrations of test compounds (2, 8, 32, and 128 µg/mL) and then incubated at 37°C

for 72 h in fresh DMEM medium. Cells were subsequently incubated at 37°C with MTT (0.5 mg/mL) for 4 h. After removal of the supernatant, formazan crystals were dissolved in DMSO, and the optical density was measured at 540 nm. Ellipticine was used as a positive control. All cytotoxicity assays were performed in triplicate in three independent experiments.

3. Results and discussion



Compound	Aglycone	R_1	R_2
1	Quercetin	OH	Rha-(1→2)-Glc
2	Quercetin	OH	Rha-(1→6)-Glc
3	Quercetin	OH	Rha
4	Kaempferol	H	Rha-(1→2)-Glc
5	Kaempferol	H	Rha

Fig. 1. Structures of the isolated compounds **1-5**

Compound **1** was isolated as a yellow solid. The ^{1}H NMR spectrum of **1** revealed the proton signals of an ABX aromatic ring system at δ_H 7.63 (1H, dd, J = 2.4, 8.4 Hz, H-6′), 6.89 (1H, d, J = 8.4 Hz, H-5′), 7.64 (1H, d, J = 2.4 Hz, H-2′) and two *meta*-coupled aromatic protons at δ_H 6.19 (1H, d, J = 2.4 Hz, H-6), 6.38 (1H, d, J = 2.4 Hz, H-8). Additionally, the ^{1}H-NMR spectrum of compound **1** exhibited the signals of two sugar moieties, including two anomeric protons at δ_H 5.76 (1H, d, J = 7.2 Hz, H-1″) and 5.25 (1H, br s, H-1‴), eight oxymethines at δ_H 3.25-4.07, one oxymethylene at δ_H 3.75 (1H, dd, J = 2.4, 12.0 Hz, Ha-6″), 3.56 (1H, dd, J = 4.2, 12.0 Hz, Hb-6″), one doublet methyl at δ_H 1.00 (3H, d, J = 6.0 Hz, CH_3-6‴), which were characteristic of a *β*-D-glucopyranosyl and an *α*-L-rhamnopyranosyl moiety. The ^{13}C-NMR spectrum of compound **1** showed the presence of twenty-seven carbon signals, including twelve carbons at δ_C 62.3 (C-6″), 71.7 (C-4″), 78.3 (C-5″), 78.9 (C-3″), 80.1 (C-2″), and 100.4 (C-1″) and 17.6 (C-6‴), 70.0 (C-5‴), 72.3 (C-3‴), 72.4 (C-2‴), 73.2 (C-4‴), and 102.6 (C-1‴) of the glucopyranosyl and rhamnopyranosyl moieties, respectively; fourteen aromatic carbons, and one ketone carbon at δ_C 179.3 (C-4) which were assigned for a flavonoid aglycone. This assignment was supported by the COSY spectrum of **1** which showed three spin-spin interaction systems of protons: H-5′/H-6′, H-1″/H-2″/H-3″/H-4″/H-5″/H-6″, and H-1‴/H-2‴/H-3‴/H-4‴/H-5‴/H-6‴. In the HMBC spectrum of **1**, the correlation of H-2″ (δ_H 3.68) with C-1‴ (δ_C 102.6), H-1‴ (δ_H 5.25) with C-2″ (δ_C 80.1) revealed that the rhamnopyranosyl moiety was connected to the glucopyranosyl moiety at C-2″. Next, the HMBC correlation of the proton H-1″ (δ_H 5.76) with C-3 (δ_C 134.6) determined that the glucopyranosyl moiety was connected to the flavonoid unit at C-3. Thus, the detailed analysis of the 1D and 2D-NMR spectra of **1** and comparison with the literature led to the assignment of **1** as quercetin 3-*O*-neohesperidoside [21].

Table 1. ^{13}C-NMR data of compounds **1-5**

Carbon	δ_C[a]				
	1	**2**	**3**	**4**	**5**
2	158.4	159.4	158.2	161.3	158.6
3	134.6	135.6	136.2	134.4	136.2
4	179.3	179.5	179.4	179.4	179.7
5	163.2	163.5	166.0	161.3	161.6
6	99.9	100.0	99.9	100.3	99.9
7	166.0	166.1	166.1	166.0	165.9
8	94.6	95.0	94.8	94.7	94.8
9	158.4	158.6	159.3	158.5	159.3
10	105.9	105.7	106.0	105.9	106.0

Carbon	δ_C[a]				
	1	2	3	4	5
1′	123.2	123.2	123.0	123.1	122.7
2′	117.2	117.7	117.0	132.1	131.9
3′	146.0	145.9	146.4	116.1	116.5
4′	149.6	149.8	149.8	158.5	163.2
5′	116.0	116.1	116.4	116.1	116.5
6′	123.5	123.6	122.8	132.1	131.9
1″	100.4	104.7	103.6	100.3	103.5
2″	80.1	75.7	72.0	80.1	72.0
3″	78.9	78.2	72.2	78.9	72.2
4″	71.7	71.4	73.2	71.9	73.2
5″	78.3	77.3	71.9	78.4	71.9
6″	62.3	68.6	17.7	62.3	17.7
1‴	102.6	102.4		102.6	
2‴	72.4	72.3		72.4	
3‴	72.3	72.1		72.3	
4‴	74.6	74.0		74.1	
5‴	70.0	69.7		69.9	
6‴	17.6	17.9		17.5	

[a]: recorded in methanol-d_4 at 150 MHz.

Compound **2** was isolated as a yellow powder. The ^{1}H-NMR spectrum of **2** was similar to those of **1**, including the characteristic signals of a quercetin aglycone at δ_H 6.21 (1H, d, J = 2.4 Hz, H-6), 6.40 (1H, d, J = 2.4 Hz, H-8), 7.62 (1H, dd, J = 2.4, 8.4 Hz, H-6′), 6.87 (1H, d, J = 8.4 Hz, H-5′), and 7.76 (1H, d, J = 2.4 Hz, H-2′). Besides, the ^{1}H-NMR spectrum of **2** also showed the proton signals of a β-D-glucopyranosyl moiety at δ_H 3.47 (1H, m, H-2″), 3.41 (1H, m, H-3″), 3.26 (1H, m, H-4″), 3.33 (1H, m, H-5″), 3.80 (1H, dd, J = 1.8, 11.4 Hz, H-6″a), 3.38 (1H, m, H-6″ b), 5.10 (1H, d, J = 7.8 Hz, H-1″) and an α-L-rhamnopyranosyl moiety at δ_H 3.62 (1H, dd, J = 1.8, 3.6 Hz, H-2‴), 3.52 (1H, dd, J = 3.6, 9.6 Hz, H-3‴), 3.28 (1H, m, H-4‴), 3.45 (1H, m, H-5‴), 1.11 (1H, d, J = 6.6 Hz, CH_3-6‴), and 4.52 (1H, d, J = 1.8 Hz, H-1‴). Analysis of the ^{13}C-NMR spectrum with the aid of the HSQC experiment revealed the resonances of twenty-seven carbons, including one carbonyl carbon at δ_C 179.5 (C-4), fourteen aromatic carbon signals, and twelve carbon signals of a rhamnopyranosyl and a glucopyranosyl moieties. In the HMBC spectrum of **2**, the correlation of H-1″ (δ_H 5.10) with C-3 (δ_C 135.6) determined that the glucopyranosyl moiety was connected to the flavonoid unit at C-3. Next, the correlation of H-1‴ (δ_H 4.52) with C-6″ (δ_C 68.6) revealed that the rhamnopyranosyl moiety was connected to the glucopyranosyl moiety at C-6″ instead of the C-2″ position in compound **1**. Complete analysis of the 1D, 2D-NMR of **2**, and comparison with the reported values indicated that compound **2** was quercetin-3-*O*-rutinoside or rutin [12],[16],[22].

Table 2. ^{1}H-NMR data of compounds **1-5**

Proton	δ_H[b] mult. (J in Hz)				
	1	2	3	4	5
6	6.19 d (2.4)	6.21 d (2.4)	6.22 d (1.8)	6.20 d (2.4)	6.22 d (1.8)
8	6.38 d (2.4)	6.40 d (2.4)	6.39 d (1.8)	6.40 d (2.4)	6.40 d (1.8)
2′	7.64 d (2.4)	7.76 d (2.4)	7.36 d (2.4)	8.06 d (9.0)	7.78 dd (2.4, 7.2)
3′	-	-	-	6.91 d (9.0)	6.96 dd (2.4, 7.2)
5′	6.89 d (8.4)	6.87 d (8.4)	6.93 d (8.4)	6.91 d (9.0)	6.96 dd (2.4, 7.2)
6′	7.63 dd (2.4, 8.4)	7.62 dd (2.4, 8.4)	7.32 dd (2.4, 8.4)	8.06 d (9.0)	7.78 dd (2.4, 7.2)
1″	5.76 d (7.2)	5.10 d (7.8)	5.37 d (1.8)	5.74 d (7.8)	5.40 d (1.8)
2″	3.68 dd (7.2, 9.0)	3.47 m	4.23 dd (3.0, 1.8)	3.63 t (7.8)	4.24 dd (1.8, 3.0)
3″	3.58 d (9.0)	3.41 m	3.76 dd (3.0, 9.6)	3.57 t (7.8)	3.72 dd (1.8, 3.0)
4″	3.45 dd (2.4, 9.0)	3.26 m	3.36 m	3.32 m	3.35 m
5″	3.25 m	3.33 m	3.44 dd (6.0, 9.6)	3.25 m	3.33 m

Proton	δ_H[b] mult. (*J* in Hz)				
	1	**2**	**3**	**4**	**5**
6″	3.75 dd (2.4, 12.0) 3.56 dd (4.2, 12.0)	3.80 dd (1.8, 11.4) 3.38 m	0.96 d (6.0)	3.52 dd (5.4, 12.0) 3.74 dd (2.4, 12.0)	0.94 d (6.0)
1‴	5.25 br s	4.52 d (1.8)	-	5.25 br s	-
2‴	4.02 dd (1.8, 3.6)	3.62 dd (1.8, 3.6)	-	4.02 dd (1.8, 3.0)	-
3‴	3.80 dd (3.0, 9.6)	3.52 dd (3.6, 9.6)	-	3.80 dd (3.0, 9.6)	-
4‴	3.38 m	3.28 m	-	3.35 m	-
5‴	4.07 dd (6.0, 9.6)	3.45 m	-	4.05 dd (6.6, 9.6)	-
6‴	1.00 d (6.0)	1.11 d (6.6)	-	0.98 d (6.6)	-

[b] recorded in methanol-d_4 at 600 MHz

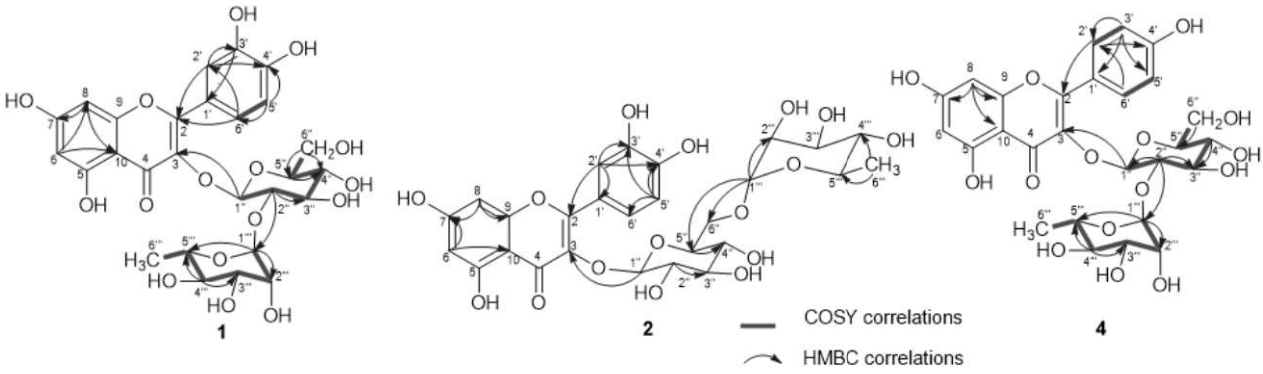


Fig. 2. HMBC and COSY interactions of compounds **1**, **2**, and **4**

Compound **3** was isolated as a yellow powder. The ^{1}H-NMR spectrum of **3** exhibited the presence of an ABX aromatic ring system at δ_H 7.32 (1H, dd, J = 2.4, 8.4 Hz, H-6′), 6.93 (1H, d, J = 8.4 Hz, H-5′), 7.36 (1H, d, J = 2.4 Hz, H-2′); two *meta*-coupled aromatic protons at δ_H 6.19 (1H, d, J = 1.8 Hz, H-6), 6.39 (1H, d, J = 2.4 Hz, H-8) and one α-L-rhamnopyranosyl moiety at δ_H 5.37 (1H, d, J = 1.8 Hz, H-1″), 4.23 1H, dd, J = 1.8, 3.0 Hz, H-2″), 3.76 (1H, dd, J = 3.0, 9.6 Hz, H-3″), 3.36 (1H, m, H-4″), 3.44 (1H, dd, J = 6.0, 9.6 Hz, H-5‴), 0.96 (1H, d, J = 6.0 Hz, CH_3-6″). Analysis of the ^{1}H-NMR and ^{13}C-NMR spectra of compound **3** showed similarities with those of compound **1**, except for the disappearance of one glucopyranosyl moiety in compound **3**. On the basis of these data and in comparison with the literature, compound **3** was determined as quercetin-3-*O*-α-L-rhamnopyranoside [23].

Compound **4** was isolated as a yellow powder. The ^{1}H-NMR spectrum of **4** was similar to those of **1** including two anomeric protons at δ_H 5.74 (1H, d, J = 7.8 Hz, H-1″) and 5.25 (1H, br s, H-1‴), eight oxymethines at δ_H 3.25-4.05, one methylene at δ_H 3.52 (1H, dd, J = 5.4, 12.0 Hz, Ha-6″), 3.74 (1H, dd, J = 2.4, 12.0 Hz, Hb-6″), one doublet methyl at δ_H 0.98 (3H, d, J = 6.6 Hz, CH_3-6‴), which were characteristic of a β-D-glucopyranosyl and an α-L-rhamnopyranosyl moieties, respectively. Additionally, the ^{1}H-NMR spectrum with the support of the HSQC spectrum of **4** showed the signals of a kaempferol aglycone at δ_H 6.20 (1H, d, J = 2.4 Hz, H-6), 6.40 (1H, d, J = 2.4 Hz, H-8), 6.91 (2H, d, J = 9.0 Hz, H-3′, H-5′) and 8.10 (2H, d, J = 9.0 Hz, H-2′, H-6′). The ^{1}H-^{1}H COSY spectrum of **4** revealed four spin-spin interaction systems for the following protons: H-2′/H-3′, H-5′/H-6′, and H-1″/H-2″/H-3″/H-4″/H-5″/H-6″, and H-1‴/H-2‴/H-3‴/H-4‴/H-5‴/H-6‴, as shown in Fig. 2. In the HMBC spectrum of **4**, the correlation of H-2″ (δ_H 3.63) with C-1‴ (δ_C 102.6), H-1‴ (δ_H 5.25) with C-2″ (δ_C 80.1) revealed that the rhamnopyranosyl moiety was connected to the glucopyranosyl moiety at C-2″. Next, the correlation of H-1″ (δ_H 5.74) with C-3 (δ_C 134.4) determined that the glucopyranosyl moiety was connected to the kaempferol unit at C-3. Complete analysis of the 1D, 2D-NMR of **4**, and comparison with the reported values indicated that compound **4** was kaempferol 3-*O*-neohesperidoside [21].

Compound **5** was isolated as a yellow powder.

The ^{1}H-NMR and ^{13}C-NMR spectra of compound **5** showed similarities with those of compound **4**, except for the disappearance of one glucopyranosyl moiety in compound **5**. Thus, on the basis of analysis of the NMR data of **5**, and comparison with the literature, compound **5** was structurally elucidated as kaempferol 3-*O*-*α*-L-rhamnoside or afzelin [19].

The cytotoxic activities of compounds **1**-**5** were evaluated *in vitro* against KB, HepG2, A549, and MCF-7 cell lines. Unfortunately, all the compounds were inactive against all tested cell lines at a 128 µg/mL concentration. These results were consistent with those of compounds **3** and **5**, which were previously reported [24],[25].

4. Conclusion

Investigation of the chemical constituents of the EtOAc and water extracts from the leaves of *Syzygium cumini* resulted in the isolation and structural elucidation of five flavone glycosides: quercetin 3-*O*-neohesperidoside (**1**), rutin (**2**), quercetin 3-*O*-*α*-L-rhamnopyranoside (**3**), kaempferol 3-*O*-neohesperidoside (**4**), and afzelin (**5**) on the basis of detailed analyses of their 1D and 2D NMR spectroscopic data and in comparison with the literature values. All isolated compounds (**1**-**5**) were estimated for their cytotoxicity against KB, MCF-7, HepG2, and A549 human cancer cell lines. However, they were inactive against four tested human cancer cell lines at 128 µg/mL concentration. To the best of our knowledge, compounds **1** and **4** were first reported from the *Syzygium* genus, while compounds **3** and **5** were isolated from the leaves of *S. cumini* for the first time.

Acknowledgments: *This research was financially supported by the Vietnam Academy of Science and Technology (VAST) (Grant No. KHCBSS.03/22-24).*

References

1. Ahmad B., Baider C., Bernardini B., Biffin E., Brambach F., Burslem D., Byng J. W., Christenhusz M., Florens F., Lucas E. (2016), *Syzygium* (Myrtaceae): Monographing a taxonomic giant via 22 coordinated regional revisions. *PeerJ Preprints*. 4: e1930v1. **2**. Reza A. A., Haque M. A., Sarker J., Nasrin M. S., Rahman M. M., Tareq A. M., Khan Z., Rashid M., Sadik M. G., Tsukahara T. (2021), Antiproliferative and antioxidant potentials of bioactive edible vegetable fraction of *Achyranthes ferruginea* Roxb. in cancer cell line. *Food Science Nutrition*. 9(7), 3777-3805. **3**. Batiha G. E.-S., Alkazmi L. M., Wasef L. G., Beshbishy A. M., Nadwa E. H., Rashwan E. K. (2020), *Syzygium aromaticum* L.(Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*. 10(2), 352. **4**. Dharani N. (2016), A review of traditional uses and phytochemical constituents of indigenous *Syzygium* species in East Africa. *Pharmaceutical Journal of Kenya*. 22(4), 123-127. **5**. Jadhav V., Kamble S., Kadam V. (2009), Herbal medicine: *Syzygium cumini*: A review. *Journal of Pharmacy Research*. 2(8), 1212-1219. **6**. Reis A. S., De Sousa Silva L., Martins C. F., De Paula J. R. (2021), Analysis of the volatile oils from three species of the gender *Syzygium*. *Research, Society Development*. 10(7), e13510716375. **7**. Cock I. E., Cheesman M. (2018), Plants of the genus *Syzygium* (Myrtaceae): A review on ethnobotany, medicinal properties and phytochemistry. *Bioactive Compounds of Medicinal Plants: Properties Potential for Human Health*, 35-84. **8**. Ediriweera E., Ratnasooriya W. (2009), A review on herbs used in treatment of diabetes mellitus by Sri Lankan ayurvedic and traditional physicians. *Ayuveda*. 30(4), 373-391. **9**. Sobeh M., Mahmoud M. F., Petruk G., Rezq S., Ashour M. L., Youssef F. S., El-Shazly A. M., Monti D. M., Abdel-Naim A. B., Wink M. (2018), *Syzygium aqueum*: A polyphenol-rich leaf extract exhibits antioxidant, hepatoprotective, pain-killing and anti-inflammatory activities in animal models. *Frontiers in Pharmacology*. 9, 566. **10**. Sobeh M., Youssef F. S., Esmat A., Petruk G., El-Khatib A. H., Monti D. M., Ashour M. L., Wink M. (2018), High resolution UPLC-MS/MS profiling of polyphenolics in the methanol extract of *Syzygium samarangense* leaves and its hepatoprotective activity in rats with CCl_4-induced hepatic damage. *Food Chemical Toxicology*. 113, 145-153. **11**. Famuyide I. M., Fasina F. O., Eloff J. N., Mcgaw L. J. (2020), The ultrastructural damage caused by *Eugenia zeyheri* and *Syzygium legatii* acetone leaf extracts on pathogenic *Escherichia coli*. *BMC Veterinary Research*. 16(1), 326. **12**. Rahim E. N. A., Ismail A., Omar M. N., Rahmat U. N., Ahmad W. A. N. W. (2018), GC-MS analysis of phytochemical compounds in *Syzygium polyanthum* leaves extracted using ultrasound-assisted method. *Pharmacognosy Journal*. 10(1), 110-119. **13**. Trang D. T., Tai B. H., Hoang N. H., Cuc N. T., Bang N. A., Dung D. T., Yen D. T. H., Huong P. T. T., Dung N. V., Hang D. T. T., (2024), Undescribed triterpenes from the leaves of *Syzygium myrsinifolium* with their *α*-glucosidase and *α*-amylase inhibition activity. *Chemistry & Biodiversity*. 21(3), e202400124. **14**. Van K. P., Hai N. B., Huu T. B., Xuan N. N, Hai Y. P, Huy H. N., Thi T. D., Thi D. D., Van T. N., Tuan A. L., (2023), Undescribed phenolic glycosides from *Syzygium attopeuense* and their inhibition of nitric oxide production. *Chemistry & Biodiversity*. 20(9), e202301037. **15**. Hai N. B., Thi D. D., Huu T. B., Hai Y. P., Xuan N. N., Thi T. H. T., Thi T. D., Van T. N., Tuan A. L., Thi H. N., (2023), New isopropyl chromone and flavanone glucoside compounds from the leaves of *Syzygium cerasiforme* (Blume) Merr. & LM Perry and their inhibition of nitric oxide production. *Chemistry & Biodiversity*. 20(3), e202201048. **16**. Ganbaatar C., Gruner M., Mishig D., Duger R., Schmidt A. W., Knölker H. J. (2015), Flavonoid glycosides from the aerial parts of *Polygonatum odoratum* (Mill.) Druce growing in Mongolia. *The Open Natural Products Journal*. 8(1), 1-7. **17**. Kiem P. V., Minh C. V., Dat N. T., Cai X. F., Lee J. J., Kim Y. H. (2003). Two new phenylpropanoid glycosides from the stem bark of *Acanthopanax trifoliatus*. *Archives of Pharmacal Research*, 26(12), 1014-1017. **18**. Gamez E. J., Luyengi L., Lee S. K., Zhu L. F., Zhou B. N., Fong H. H., Pezzuto J. M., Kinghorn A. D. (1998). Antioxidant flavonoid glycosides from *Daphniphyllum calycinum*. *Journal of Natural Products*. 61(5), 706-708. **19**. Joo S. J.,

Park H. J., Park J. H., Cho J. G., Kang J. H., Jeong T. S., Kang H. C., Lee D. Y., Kim H. S., Byun S. Y. (2014), Flavonoids from *Machilus japonica* stems and their inhibitory effects on LDL oxidation. *International Journal of Molecular Sciences*. 15(9), 16418-16429. **20**. Mosmann T. (1983), Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*. 65(1-2), 55-63. **21**. Kazuma K., Noda N., Suzuki M. (2003), Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. *Phytochemistry*. 62(2), 229-237. **22**. Halim M. A., Kanan K. A., Nahar T., Rahman M. J., Ahmed K. S., Hossain H., Mozumder N. R., Ahmed M., (2022), Metabolic profiling of phenolics of the extracts from the various parts of blackberry plant (*Syzygium cumini* L.) and their antioxidant activities. *Lwt-Food Science and Technology*, 167(113813), 1-10. **23**. Wang K. J., Yang C. R., Zhang Y. J. (2007), Phenolic antioxidants from Chinese toon (fresh young leaves and shoots of *Toona sinensis*). *Food Chemistry*. 101(1), 365-371. **24**. Sun Y. J., Chen H. J., Xue G. M., Chen H., Zhang Y. L., Li M., Du K., Wang J. M., Feng W. S., (2021), Two new flavonoid glucosides from the fruits of *Sinopodophyllum hexandrum*. *Natural Product Research*. 35(13**),** 2164-2169. **25**. Ngoc N. T., Quang T. H., Hanh T. T. H., Cuong N. X., Cuong N. T., Ha C. H., Nam N. H., Van M. C., (2022), Phenolic glycosides from the leaves of *Iodes cirrhosa* Turcz. with cytotoxic and antimicrobial effects. *Chemistry & Biodiversity*. 19(9), e202200182.

Journal of Medicinal Materials, 2024, Vol. 29, No. 6 (pp. 329 - 334)

CHEMICAL CONSTITUENTS OF THE DICHLOROMETHANE EXTRACT OF *GANODERMA COCHLEAR*

Vu Thi Hanh Yen[1], Nguyen Van Vinh Ha[2], Tran Viet Hung[2], Bui Hong Cuong[3], Pham Thi Huong[3], Nguyen Thi Duyen[4,*]

[1]The Representative Office of Pharmascience Inc. Ho Chi Minh City, Vietnam;
[2]Institute of Drug Quality Control Ho Chi Minh City, Vietnam; [3]Hanoi University of Pharmacy, Vietnam;
[4]National Institute of Medicinal Materials (NIMM), Hanoi, Vietnam
*Corresponding author: nguyenduyen6784@gmail.com
(Received September 07th, 2024)

Summary

Chemical Constituents of the Dichloromethane Extract of *Ganoderma cochlear*

From the dichloromethane extract of *Ganoderma cochlear*, five compounds (**1** - **5**) were isolated by column chromatography. Their chemical structures were identified as fornicatin F (**1**), 1-tetracosanoylglycerol (**2**), (22*E*)-ergosta-7,9(11),22-trien-3*β*-ol (**3**), polycarpol (**4**), and lucidenic acid N (**5**) based on spectral data and literature references. Compounds **2** - **5** were isolated from *G. cochlear* for the first time.

Keywords: *Ganoderma cochlear*, *1-tetracosanoylglycerol, (22E)-ergosta-7,9(11),22-trien-3β-ol, Lucidenic acid N.*

1. Introduction

Ganoderma cochlear (Nees) Merr., belongs to the Ganodermataceae family. The species is popular in Tay Nguyen and is often used as a substitute for *G. lucidum* to enhance immunity and hepatoprotective effects [1]. Up-to-date phytochemical studies have shown over 90 compounds, including mainly triterpenoids, sterols, phenols, and especially alkaloids [2],[3],[4],[5],[6],[7],[8],[9],[10],[11],[12]. Scientific studies *in vitro* have shown the species has valuable effects such as anti-inflammatory [4], hepatoprotective [2], renoprotective [3],[6],[8], neuroprotective [7], anti-oxidant [11], and cytotoxic activity [5],[13],[14]. These activities are mainly attributed to terpenoids and phenolic compounds. However, in Vietnam, there is only one study on the chemical constituents of this mushroom [15]. In this study, we isolated and identified five compounds (**1** - **5**) from the dichloromethane extract of *Ganoderma cochlear*: fornicatin F (**1**), 1-tetracosanoylglycerol (**2**), (22*E*)-ergosta-7,9(11),22-trien-3*β*-ol (**3**), polycarpol (**4**), and lucidenic acid N (Fig.1). Among them, compounds **2** - **5** are reported for the first time from *G. cochlear*.

2. Materials and methods

2.1. Plant materials

The samples were collected in Kon Ka Kinh Park, Gialai, in December 2023. The mushroom was identified as *Ganoderma cochlear* (Nees) Merr., which belongs to the Ganodermataceae family, by Assoc. Prof. Nguyen Phuong Dai Nguyen. A voucher specimen (GC122023) was deposited at the National Institute of Medicinal Materials.

2.2. General experiment procedures

The NMR measurements were conducted using CD_3OD and $CDCl_3$ as solvents on a Bruker NMR spectrometer with a frequency of 500 or 600 MHz. Tetramethylsilane was used as an internal