H., Kenney S., Boyd M. R. (1990), New colorimetric cytotoxicity assay for anticancer-drug screening, *JNCI: Journal of the National Cancer Institute*, 82(13), 1107-1112. **14.** Hamdi O. A. A., Anouar E. H., Shilpi J. A., Trabolsy Z. B. K. A., Zain S. B. M., Zakaria N. S. S., Zulkefeli M., Weber J. F. F., Malek S. N. A., Rahman S. N. S. A., Awang K. (2015), A quantum chemical and statistical study of cytotoxic activity of compounds isolated from *Curcuma zedoaria*, *International Journal of Molecular Sciences*, 16(5), 9450-9468. **15.** Zhang F., Li Y., Zhu T., Zhang W., Qin B., You S. (2018), Absolute configuration of curdione and its three isomers by NMR, ECD and DFT calculations: an insight into the scope of unsaturated ketone helicity rule based on an ECD study, *Asian Journal of Traditional Medicines*, 13(1), 1-9. **16.** Inayama S., Gao J., Harimaya K., Iitaka Y., Guo Y., Kawamata T. (1985), Stereostructure of curdione, *Chemical and Pharmaceutical Bulletin*, 33(3), 1323-1326. **17.** Dar M. A., Khan A. H. (2023), Species of *Curcuma*: a source of anticancer agents, *International Journal of Current Research in Physiology and Pharmacology*, 7(4), 66-77. **18.** Zhang K. Y., Lyu L. L., Chen J. X., Xu J. Y., Li Q., Wu Y., Zheng L. (2021), Effect of curdione on MDA-MB-231 cell cycle and apoptosis, *Chinese Journal of Experimental Traditional Medical Formulae*, 24, 74-81. **19.** Sun X. R., Yang K., Lyu L. L., Chen J. X., Guo Y. B., Ruan M., Ying H. F., Shen X. H., Zheng L. (2019), Effect and mechanism of curdione on migration and invasion of breast cancer HCC1937 cells, *Chinese Journal of Experimental Traditional Medical Formulae*, 24, 66-73. **20.** Li J., Bian W. H., Wan J., Zhou J., Lin Y., Wang J. R., Wang Z. X., Shen Q., Wang K. M. (2014), Curdione inhibits proliferation of MCF-7 cells by inducing apoptosis, *Asian Pacific Journal of Cancer Prevention*, 15(22), 9997-10001. **21.** Islam M. T., Ali E. S., Uddin S. J., Shaw S., Islam M. A., Ahmed M. I., Shill M. C., Karmakar U. K., Yarla N. S., Khan I. N., Billah M. M. (2018), Phytol: A review of biomedical activities, *Food and Chemical Toxicology*, 121, 82-94.

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CHEMICAL CONSTITUENTS FROM THE LEAVES OF *ARDISIA GIGANTIFOLIA*

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Summary

Chemical Constituents from the Leaves of *Ardisia gigantifolia*

Phytochemical investigation of *Ardisia gigantifolia* Stapf leaves led to the isolation of five compounds, including nicotiflorin (**1**), rutin (**2**), 4'-*O*-methyl-myricetin 3-*O*-rutinoside (**3**), (*R*)-methyl mandelate (**4**), and (2*S*)-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-3-*O*-*β*-D-galactopyranosyl glycerol (**5**). Their chemical structures were elucidated by the analysis of NMR and MS data, compared with the previously reported data. This is the first time to report the existence of compounds **1 - 5** from the genus *Ardisia*.

Keywords: *Ardisia gigantifolia* Stapf *leaves, Flavonoids, Phenolic, Glycoglycerolipid.*

1. Introduction

The *Ardisia*, one of the largest genera in the family Myrsinaceae with approximately 732 species of evergreen shrubs and trees are distributed throughout the tropical and subtropical regions of the world, such as East and Southeast Asia, America, and Australia [1]. Many *Ardisia* species: *A. cornudetata*, *A. crenata*, *A. crispa*, *A. crassa*, *A. japonica*,... have been used for the treatment of liver disease, cough, diarrhea, anti-inflammatory/analgesic diseases, fracture coalescence, rheumatism, cancer, among others...[2]. The phytochemical investigations of *Ardisia* species indicate the discovery of flavonoids, triterpenoids [3],[4],[5],[6],[7],[8], quinones, coumarins, and phenolic compounds [8]. Pharmacological studies reported that the extracts and pure compounds display a wide range of bioactive effects: anti-inflammatory, antitumor, antioxidant, anticancer, antibacterial, and antidiabetic activities [8]...

In the ongoing research to discover new naturally occurring compounds for treating peptic ulcer diseases from medicinal plants in Vietnam, we are interested in *A. gigantifolia*, known as Khoi tia or Khoi nhung (in Vietnam). *A. gigantifolia* is used in traditional Vietnam medicine for colic and stomachache, however, as far as we know, most of the studies have focused on the chemical constituents of the rhizomes or roots and only a limited number of phytochemical studies about the leaves of this plant have been investigated or described in detail in the literature [9],[10],[11],[12],[13],[14]. Therefore, in this study, the chemical constituents from the leaves of *A. gigantifolia* were conducted and resulted in the isolation and structural elucidation of five compounds, including three flavonol glycosides (**1 - 3**), a phenolic compound (**4**), and a glycoglycerolipid (**5**).

2. Materials and methods

2.1. Plant material

A. gigantifolia leaves were collected in Yen Bai province and identified by Dr. Phuong Thien

Thuong, Biotechnology Department, Vietnam - Korea Institute of Science and Technology (VKIST). A specimen of this plant (KT-2023) is kept in the Herbarium of the Biotechnology Department, VKIST.

2.2. General experimental procedures

Optical rotations were measured by a Jasco P-1020 polarimeter. The ^{1}H- and ^{13}C-NMR were recorded using a Bruker Avance III 600 spectrometer (^{1}H at 600 MHz and ^{13}C at 150 MHz) with tetramethylsilane as the internal standard. ESI-MS analyses were conducted employing a Shimadzu LC-MS/MS system equipped with a DAD detector. *Silica gel* 60 (60-200 µm, Merck), and YMC RP-18 (120 Å, 150 µm) were used for column chromatography (CC). Preparative HPLC was performed on a Shimadzu Instrument apparatus equipped with a detector UV DAD and a Shim-pack GIS RP18 column (10 µm, 20 x 250 mm). Thin layer chromatography (TLC) was performed on Merck pre-coated 60 F_{254} *silica gel* plates and compounds were visualized by spraying the dried plates with 50% H_2SO_4, followed by heating.

2.3. Extraction and isolation

The dried leaves of *A. gigantifolia* (500.0 g) were extracted with EtOH 80° (5L) under reflux (60°C, 3h, 3 times). The ethanol extract (45.2 g) was suspended in water (452.0 mL) and partitioned with ethyl acetate (EtOAc, 2L/each time, 3 times). The EtOAc extract (17.0 g) was chromatographed on a *silica gel* CC and eluted with a gradient solvent system of methylene chloride (MC): methanol (MeOH) (99: 1 → 1:1, v/v) to give nine fractions (AG-1A - AG-1H). Fraction AG-1B (1.5 g) was applied to RP-18 gel CC and eluted with an isocratic solvent system of MeOH:H_2O (1:1, v/v) to obtain compound **4** (300.0 mg). Compound **5** (50.2 mg) was purified from fraction AG-1C (730.2 mg) by using RP-18 gel CC and eluting with MeOH:H_2O (2:1, v/v). Fraction AG-1E (4.7 g) was further separated by using RP-18 CC and eluting with a gradient solvent system of MeOH:H_2O ((1:1 - 3:1, v/v) to give five fractions (AG-2A - AG-2E). Fraction AG-2C (400.0 mg) was applied to *silica gel* CC and eluted with a gradient solvent system of EtOAc:MeOH:acetic acid (99:1:1 - 80:20:1, v/v/v) to afford three fractions (AG-3A - AG-3C). Fraction AG-3B (236.0 mg) was continuously separated by *silica gel* CC with an isocratic elution of EtOAc:MeOH:H_2O (8.5:1.5:0.5, v/v/v) to yield three fractions (AG-4A - AG-4C). Compounds **1** (44.2 mg) and **3** (15.2 mg) were isolated from fraction AG-4B (115.0 mg) by using a preparative HPLC system with a mobile phase of MeOH:H_2O (35:65, v/v). Fraction AG-2D (1.7g) was recrystallized in MeOH to obtain compound **2** (200.0 mg).

Nicotiflorin (**1**): Yellow crystalline; ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H: 6.20 (1H, d, J = 1.8 Hz, H-6), 6.41 (1H, d, J = 1.8 Hz, H-8), 7.98 (2H, d, J = 9.0 Hz, H-2', H-6'), 6.88 (2H, d, J = 9.0 Hz, H-3', H-5'), 5.31 (1H, d, J = 7.2 Hz, H-1''), 5.06 (2H, m, H-6''), 4.38 (1H, d, J = 1.4 Hz, H-1'''), 3.00 – 4.50 (8H, H-2'' – H-5'', H-2''' – H-5'''), 0.98 (3H, d, J = 6.0 Hz, H-6'''); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C: 156.5 (C-2), 133.2 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.1 (C-7), 93.7 (C-8), 156.8 (C-9), 104.0 (C-10), 120.9 (C-1'), 130.8 (C-2', C-6'), 115.1 (C-3', C-5'), 159.9 (C-4'), 101.3 (C-1''), 74.1 (C-2''), 75.7 (C-3''), 69.9 (C-4''), 76.3 (C-5''), 66.9 (C-6''), 100.7 (C-1'''), 70.5 (C-2'''), 70.3 (C-3'''), 71.8 (C-4'''), 68.2 (C-5'''), 17.7 (C-6'''). ESI-MS: *m/z* 593.20 [M-H]$^-$.

Rutin (**2**): Yellow crystals; ^{1}H-NMR (600 MHz, CD_3OD) δ_H: 6.23 (1H, d, J = 2.4 Hz, H-6), 6.42 (1H, d, J = 2.4 Hz, H-8), 7.69 (1H, d, J = 1.2 Hz, H-2'), 6.79 (1H, d, J = 8.0 Hz, H-5'), 7.65 (1H, dd, J = 8.0, 1.2 Hz, H-6'), 5.13 (1H, d, J = 7.8 Hz, H-1''), 5.04 (1H, d, J = 1.8 Hz, H-1'''), 3.25 – 3.85 (10H, m, H-2'' – H-6'', H-2''' – H-5'''), 1.14 (3H, d, J = 6.0 Hz, H-6'''); ^{13}C-NMR (150 MHz, CD_3OD) δ_C: 158.3 (C-2), 135.7 (C-3), 179.4 (C-4), 163.0 (C-5), 100.0 (C-6), 166.1 (C-7), 94.9 (C-8), 159.5 (C-9), 105.7 (C-10), 123.2 (C-1'), 117.6 (C-2'), 145.8 (C-3'), 149.8 (C-4'), 123.6 (C-5'), 116.1 (C-6'), 104.7 (C-1''), 75.7 (C-2''), 77.2 (C-3''), 71.4 (C-4''), 78.2 (C-5''), 68.6 (C-6''), 102.4 (C-1'''), 72.1 (C-2'''), 72.3 (C-3'''), 74.0 (C-4'''), 69.7 (C-5'''), 17.9 (C-6'''). ESI-MS: *m/z* 627 [M+H_2O-H]$^-$.

4'-*O*-Methyl-myricetin 3-*O*-rutinoside (**3**): Yellow powder. ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H: 6.20 (1H, d, J = 1.8 Hz, H-6), 6.38 (1H, d, J = 1.8 Hz, H-8), 7.12 (2H, s, H-2' and H-6'), 5.42 (1H, d, J = 7.8 Hz, H-1''), 3.74 (2H, d, J = 10.4 Hz, H-6''), 4.41 (1H, d, J = 1.4 Hz, H-1'''), 3.05 - 3.33 (8H, H-2'' – H-5'', H-2''' – H-5'''), 1.00 (1H, d, J = 6.0 Hz, H-6'''), 4.41 (3H, s, 4′ -OCH_3); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C: 156.4 (C-2), 133.9 (C-3), 177.5 (C-4), 161.2 (C-5), 98.7 (C-6), 164.2 (C-7), 93.5 (C-8), 156.4 (C-9), 104.0 (C-10), 125.2 (C-1'), 108.6 (C-2', C-6'), 150.2 (C-3', C-5'), 137.9 (C-4'), 100.8 (C-1''), 73.9 (C-2''), 76.1 (C-3''), 70.0 (C-4''), 76.5 (C-5''), 67.0 (C-6''), 100.7 (C-1'''), 70.5 (C-2'''), 70.4 (C-3'''), 71.8 (C-

4'''), 68.2 (C-5'''), 17.7 (C-6'''), 59.8 (4′ -OCH_3). ESI-MS: *m/z* 639.20 [M-H]$^-$.

(*R*)-Methyl maldelate (**4**): Yellow oil. $[\alpha]_D^{20}$ [– 43 (c 0.05, MeOH). ^{1}H-NMR (600 MHz, CD_3OD) δ_H: 7.53 (2H, m, H-2, H-6), 7.43 (2H, m, H-3, H-5), 7.38 (1H, m, H-4), 5.21 (1H, s, H-7), 3.70 (3H, s, 8-OCH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ_C: 140.3 (C-1), 129.3 (C-2, C-6), 129.5 (C-3, C-5), 127.8 (C-4), 74.3 (C-7), 174.8 (C-8), 52.7 (8-OCH_3). ESI-MS: *m/z* 165.15 [M-H]$^-$.

(2*S*)-1,2-Di-*O*-[9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-3-*O*-β-D-galactopyranosyl glycerol (**5**): Colorless oil; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 4.39 (1H, dd, *J* = 12.6, 3.0 Hz, H-1), 4.21 (1H, dd, *J* = 12.6, 6.6 Hz, H-1), 5.28 (1H, m, H-2), 3.72 (1H, dd, *J* = 10.8, 6.0 Hz, H-3), 3.99 (1H, dd, *J* = 10.8, 5.7 Hz, H-3), 4.27 (1H, d, *J* = 7.8 Hz, H-1'), 3.50 - 3.85 (6H, m, H-2' - H-6'), 2.32 (4H, m, H-2", H-2'"), 1.60 (4H, m, H-3", H-3'"), 1.32 (16H, m, H-4" - H-7" and H-4'" - H-7'"), 2.08 (8H, m, H-8", H-17", H-8'", H-17'"), 5.36 (12H, m, H-9", H-10", H-12", H-13", H-15", H-16"), 2.80 (8H, m, H-11", H-11'", H-14", H-14'"), 0.98 (6H, t, *J* = 7.5 Hz, H-18", H-18'"); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 68.3 (C-1), 70.2 (C-2), 62.8 (C-3), 104.0 (C-1'), 71.5 (C-2'), 73.5 (C-3'), 69.4 (C-4'), 74.6 (C-5'), 62.5 (C-6'), 173.8 (C-1"), 173.5 (C-1'"), 34.3 (C-2"), 34.2 (C-2'"), 24.9 (C-3'", C-3'"), 29.6 (C-4", C-4'"), 29.2 (C-5", C-6", C-5'", C-6'"), 29.1 (C-7", C-7'"), 27.2 (C-8", C-8'"), 132.0 (C-9", C-9'"), 130.2 (C-10", C-10'"), 25.6 (C-11", C-11'", C-14", C-14'"), 128.3 (C-12", C-13", C-12'", C-13'"), 127.8 (C-15" and C-15'"), 127.2 (C-16" and C-16'"), 20.6 (C-17", C-17'"), 14.3 (C-18", C-18'"). ESI-MS: *m/z* 773.52 [M-H]$^-$

*2.4. Acid hydrolysis of compounds **1** - **3** and **5**.*

Following the process published in reference [15, 16]. The monosaccharide units were purified by preparative TLC with a solvent system of methylene chloride:MeOH: H_2O = 8: 5: 1. The optical rotations of sugars were measured and determined to be β-D-glucose (R_f 0.40, $[\alpha]_D^{20}$ + 43 (H_2O, c 0.05), α-L-rhamnose (R_f 0.60, $[\alpha]_D^{20}$ – 12 (H_2O, c 0.05), β-D-galactose (R_f 0.33, $[\alpha]_D^{20}$ + 50 (H_2O, c 0.05).

3. Results and discussion

The ethanol extract of the leaves of *A. gigantifolia* was suspended in water and exhaustively partitioned with EtOAc. The EtOAc extract was separated by *silica gel*, RP-18 gel, and preparative HPLC to obtain five compounds, including nicotiflorin (**1**), rutin (**2**), 4'-*O*-methyl-myricetin 3-*O*-rutinoside (**3**), (*R*)-methyl maldelate (**4**), (2*S*)-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-3-*O*-α-D-galactopyranosyl glycerol (**5**). The structures of compounds **1** - **5** were identified by the analysis of their spectroscopic data and comparison with the previous publications.

Compound **1** was isolated as a yellow powder and its molecular formula was determined as $C_{27}H_{30}O_{15}$ by the ESI-MS pseudomolecular-ion peak at *m/z* 593.20 [M - H]$^-$. The ^{1}H- and ^{13}C-NMR data of **1** showed the characteristics of a flavonol glycoside. The ^{1}H-NMR spectrum of **1** revealed signals of two *meta*-coupled doublet protons at δ_H 6.20 (1H, d, *J* = 1.8 Hz, H-6) and 6.41 (1H, d, *J* = 1.8 Hz, H-8), an AA'BB' system at δ_H 7.98 (2H, d, *J* = 9.0 Hz, H-2', H-6') and 6.88 (2H, d, *J* = 9.0 Hz, H-3', H-5'), and two anomeric protons at δ_H 5.31 (1H, d, *J* = 7.2 Hz, H-1") and 4.38 (1H, d, *J* = 1.4 Hz, H-1'") of a glucosyl and a rhamnosyl units, respectively. The large coupling constant of H-1" (*J* = 7.2 Hz) suggested the β-configuration of the D-glucosyl moiety whereas the α-form of the L-rhamnosyl moiety was deduced by the small *J* value of H-1'" (*J* = 1.4 Hz). After the hydrolysis reaction, the absolute configurations of β-glucose and α-rhamnose units were determined as D and respectively by comparing their R_f and $[\alpha]_D$ values with those of the authentic samples: β-D-glucose (R_f 0.40, $[\alpha]_D^{20}$ + 43 (H_2O, c 0.05) and α-L-rhamnose (R_f 0.60, $[\alpha]_D^{20}$ – 12 (H_2O, c 0.05) [15]. The ^{13}C-NMR spectrum of **1** indicated signals of 27 carbons, including a carbonyl carbon at δ_C 177.4 (C-4), six aromatic methines at δ_C 98.7 (C-6), 93.7 (C-8), 130.8 (C-2', C-6'), 115.1 (C-3', C-5'), two quaternary carbons at δ_C 104.0 (C-10), 120.9 (C-1'), and six oxygenated carbons at δ_C 156.5 (C-2), 133.2 (C-3), 161.2 (C-5), 164.1 (C-7), 156.8 (C-9), and 159.9 (C-4') for the aglycone part; six carbon signals of the glucosyl moiety at δ_C 101.3 (C-1"), 74.1 (C-2"), 75.7 (C-3"), 69.9 (C-4"), 76.3 (C-5"), and 66.9 (C-6"), and six remaining resonances of the rhamnosyl moiety at δ_C 100.7 (C-1'"), 70.5 (C-2'"), 70.3 (C-3'"), 71.8 (C-4'"), 68.2 (C-5'"), and 17.7 (C-6'"). The 1D-NMR data of **1** was consistent with those of kaemferol-3-*O*-rutinoside (nicotiflorin) [17]. Thus, compound **1** was determined as nicotiflorin.

Compound **2** was obtained as a yellow powder. The ESI-MS spectrum of **2** exhibited an

ion peak at *m/z* 627.20 $[M+H_2O-H]^-$, suggesting the molecular formula of **2** as $C_{27}H_{29}O_{16}$. The ^{1}H-NMR data of **2** also showed the presence of a 5,7-disubstituted A ring of a flavonol at δ_H 6.23 (1H, d, J = 2.4 Hz, H-6) and 6.42 (1H, d, J = 2.4 Hz, H-8), an ABX system of B ring at δ_H 7.69 (1H, d, J = 1.2 Hz, H-2'), 6.79 (1H, d, J = 8.0 Hz, H-5'), 7.65 (1H, dd, J = 8.5, 2.4 Hz, H-6'), and two anomeric protons at δ_H5.42 (1H, d, J = 7.8 Hz, H-1''), 4.41 (1H, d, J = 1.4 Hz, H-1'''). The ^{13}C-NMR of **2** displayed 31 carbons, including 15 carbons for a flavonol and 12 carbons for two sugar moieties, suggesting that **2** was a flavonol glycoside. The 1D-NMR of **2** was almost identical to those of **1**, except for the replacement of an AA'BB' system in the B ring of **1** by an ABX system of **2**. Particularly, the ^{1}H-NMR spectrum of **2** displayed the presence of three aromatic protons at δ_H7.69 (1H, d, J = 1.2 Hz, H-2'), 6.79 (1H, d, J = 8.0 Hz, H-5'), and 7.65 (1H, dd, J = 8.0, 1.2 Hz, H-6') instead of four signals at δ_H7.98 (2H, d, J = 9.0 Hz, H-2', H-6'), 6.88 (2H, d, J = 9.0 Hz, H-3', H-5') in **1**. By careful analysis of NMR data and comparing it to the published data, compound **2** was determined as rutin [18].

Compound **3** was isolated as a yellow powder and its molecular formula was determined to be $C_{28}H_{32}O_{17}$ by the ESI-MS pseudomolecular-ion peak at *m/z* 639.20 $[M-H]^-$. A comparison of NMR data of **2** and **3** indicated that their structures were similar. The main differences between **2** and **3** were the addition of a hydroxy group at C-3' and a methoxy group at C-4' in the B ring of **3**. This was demonstrated by the appearance of only two singlet aromatic protons at δ_H 7.12 (2H, s, H-2', H-6') and an additional methoxy group at δ_H3.77 (3H, s, 4'-OCH_3) in the ^{1}H-NMR spectrum of **3**. The NMR data of **3** were compared with those of 4'-*O*-methyl-myricetin 3-*O*-rutinoside and found to be well-matched. Thus, compound **3** was elucidated as 4'-*O*-methyl-myricetin 3-*O*-rutinoside [19].

Compound **4** was afforded as a colorless oil and had a molecular formula of $C_9H_{10}O_3$ as identified by the ESI-MS peak at *m/z* 165.15 $[M-H]^-$. The ^{1}H-NMR data of **4** showed signals of five aromatic protons at δ_H7.53 (2H, m, H-2, H-6), 7.43 (2H, m, H-3, H-5), 7.38 (1H, m, H-5), an oxygenated methine at δ_H5.21 (1H, s, H-7), and a methoxy group at δ_H 3.70 (3H, s, OCH_3). The ^{13}C-NMR spectrum of **4** exhibited the presence of a benzene ring at δ_C 140.3 (C-1), 129.3 (C-2, C-6), 129.5 (C-3, C-5), 127.8 (C-4), a methine group at δ_C 74.3 (C-7), a carboxyl group at δ_C 174.8 (C-8), and a methoxy group at δ_C 52.7 (OCH_3). The stereochemistry of C-7 was identified as 7*R* by comparing the $[\alpha]_D^{20}$ value of **4** [– 43 (c 0.05, MeOH)] with that in the literature [– 130 (c 0.8, MeOH)] [20]. Based on the above evidence and reported data, the structure of **4** was elucidated as (*R*)-methyl maldelate.

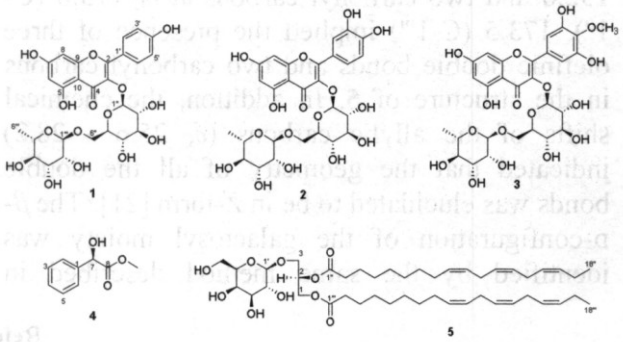

Fig. 1. Chemical structures of compounds **1** - **5**

Compound **5** was obtained as a colorless oil and possessed the molecular formula $C_{45}H_{74}O_{10}$, as determined by ESI-MS pseudomolecular-ion peak at *m/z* 773.52 $[M-H]^-$. Its NMR data showed the characteristics of a glycoglycerolipid with three spin systems, including a glycerol moiety, two long-chain fatty acids, and a galactosyl unit. Particularly, the ^{1}H-NMR spectrum of **5** revealed the presence of two methyl groups at δ_H 0.98 (6H, t, J = 7.5 Hz, H-18'' and H-18'''), a number of methylene groups at δ_H 1.32 (m), and deshielded methylene groups at δ_H 2.32 (4H, t, J = 7.0 Hz, H-2'' and H-2'''), 1.60 (4H, m, H-3'' and H-3'''), 2.08 (4H, m, H-8'' and H-8'''), 2.80 (8H, m, H-11'', H-11''', H-14'', and H-14'''), and olefinic double bonds at δ_H5.66 (12H, m, H-9'', H-9''', H-10'', H-10''', H-12'', H-12''', H-13'', H-13''', H-15'', H-15''', H-16'', and H-16''') for two fatty acyl residues. The presence of oxygenated methine and methylene groups of glycerol and the galactosyl unit in the structure of **5** was deduced by the downfield signals at δ_H 4.39 (1H, dd, J = 12.6, 3.0 Hz, H-1), 4.21 (1H, dd, J = 12.6, 6.6 Hz, H-1), 5.28 (1H, m, H-2), 3.72 (1H, dd, J = 10.8, 6.0 Hz, H-3), 3.99 (1H, dd, J = 10.8, 5.7 Hz, H-3), 3.66 (1H, d, J = 9.6, 7.8 Hz, H-2'), 3.54 (1H, t, J = 6.0 Hz, H-3'), 3.92 (1H, m, H-4'), 3.59 (1H, dd, J = 9.6, 3.0 Hz, H-5'), 3.90 (2H, m, H-6'). The ^{13}C-NMR data were also consistent with the ^{1}H-NMR data indicating two methyl carbons at δ_C 14.3 (H-18'' and H-18''') and several

methylene carbons at δ_C 20.6 - 34.3. In addition, the ^{13}C-NMR spectrum of **5** displayed nine downfield signals at δ_C 62.8 (C-1), 70.2 (C-2), 68.3 (C-3), 104.1 (C-1'), 71.5 (C-2'), 73.5 (C-3'), 69.4 (C-4'), 74.6 (C-5'), 62.5 (C-6'), suggesting the presence of three oxygenated methylene and six methine carbons. The downfield and overlapped methine carbons from δ_C 127.2 to 132.0 and two carbonyl carbons at δ_C 173.8 (C-1"), 173.5 (C-1'") implied the presence of three olefinic double bonds and two carbonyl carbons in the structure of **5**. In addition, the chemical shifts of the allylic carbons (δ_C 25.6 - 28.2) indicated that the geometry of all the double bonds was elucidated to be in *Z*-form [21]. The *β*-D-configuration of the galactosyl moiety was identified by the same method described in compound **1**. The *S*-configuration of C-2 was suggested by comparing the ^{13}C-NMR chemical shifts **5** with those reported in the literature [21]. Based on the above spectral data and compared with those of references [21], the structure of compound **5** was identified as (2*S*)-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-3-*O*-*α*-D-galactopyranosyl glycerol.

4. Conclusion

Five compounds, including three flavonol glycosides (**1** - **3**), a phenolic compound (**4**), and a glycoglycerolipid (**5**) were isolated from the leaves of *A. gigantifolia*. All of the compounds were reported for the first time from *Ardisia*.

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References

1. *http://www.powo.science.kew.org.* **2.** Kobayashi H., de Mejía E. (2005), The genus *Ardisia*: a novel source of health-promoting compounds and phytopharmaceuticals, *Journal of Ethnopharmacology*, 96(3), 347-354. **3.** Yin X., Hu R., Zhou Y., Zhu W., & Zhou Y. (2022), Cytotoxic 13,28-epoxy bridged oleanane-type triterpenoid saponins from the roots of *Ardisia crispa*, *Molecules*, 27(3), 1061. **4.** Anjum B., Kumar R., Kumar R., Rrakash O. M. (2020), Chemical composition, insect antifeeding, insecticidal, herbicidal, antioxidant and anti-inflammatory potential of *Ardisia solonaceae* Roxb. root extract, *Trends in Phytochemical Research*, 4(4), 177-192. **5.** Podolak I., Żuromska-Witek B., Grabowska K., Żebrowska S., Galanty A., & Hubicka U. (2021), Comparative quantitative study of ardisiacrispin A in extracts from *Ardisia crenata* Sims varieties and their cytotoxic activities, *Chemistry & Biodiversity*, 18(7), e2100335. **6.** Lin Z., Yang X., Li L., Ma C., Kong L., Li H., Xiong S., Zhong H., Pu S., Liang F. & Hu J. (2023), A rapid and effective method for obtaining ardicrenin from *Ardisia crenata* Sims var. *bicolor* plants, *Brazilian Journal of Botany*, 46(3), 505-512. **7.** Song N. N., Yang L. M., Zhang M. J., An R. F., Liu W., Huang X. F. (2021), Triterpenoid saponins and phenylpropanoid glycoside from the roots of *Ardisia crenata* and their cytotoxic activities, *Chinese Journal of Natural Medicines*, 19(1), 63-69. **8.** Liu B., Liu R., Liu Q., Ashby C. R. Jr., Zhang H., & Chen Z. S. (2022), The ethnomedicinal and functional uses, phytochemical and pharmacology of compounds from *Ardisia* species: An updated review, *Medicinal Research Reviews*, 42(5), 1888-1929. **9.** Tian L., Xi Gu Ri G., Yu J., Qu S., Xie Q., Shama R., Bao M., Su H., Liu B., & Borjigidai A. (2023), *Ardisia gigantifolia* Stapf (Primulaceae): A review of ethnobotany, phytochemistry, pharmacology, clinical application, and toxicity, *Journal of Ethnopharmacology*, 305, 116079. **10.** Chen Z. Y., Ling Y., Zhang Q., Wan H. (2017), Characterization of the major chemical constituents *in Ardisia gigantifolia* by high performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry, *Analytical Methods*, 9 (39), 5816-5825. **11.** Guan Y. F., Song X., Qiu M. H., Luo S. H., Wang B. J., Van Hung N., Cuong N. M., Soejarto D. D., Fong H. H., Franzblau S. G., Li S. H., He Z. D., & Zhang H. J. (2016), Bioassay-guided isolation and structural modification of the anti-TB resorcinols from *Ardisia gigantifolia*, *Chemical Biology & Drug Design*, 17(1), 40-46. **12.** Liu H.W., Zhao F., Yang R., Wang M., Zheng M., Zhao Y., Zhang X., Qiu F., & Wang H. (2009), Dimeric 1,4-benzoquinone derivatives and a resorcinol derivative from *Ardisia gigantifolia*, *Phytochemistry* 70 (6), 773-778. **13.** Lou F. M., Li Q. F., Zhang Q. R., Qiu W. W., (2010), Research on volatile oils from rhizoma *Ardisiae gigantifoliae* by microwave-assisted hydrodistillation, *Journal of Chinese Medicinal Materials*, 33 (5), 815-819. **14.** Zhao B., Xiong C., Wu L., Xiang L., Shi YH., Sun W., Chen S. L. (2021), DNA barcoding coupled with high resolution melting for rapid identification of *Ardisia gigantifolia* and its toxic adulterants, *Biotechnology & Biotechnological Equipment*, 35 (1), 641-649. **15.** Voutquenne-Nazabadioko L., Gevrenova R., Borie N., Harakat D., Sayagh C., Weng A., Thakur M., Zaharieva M., Henry M. (2013), Triterpenoid saponins from the roots of *Gypsophila trichotoma* Wender, *Phytochemistry*, 90, 114-127. **16.** Vu T. O., Tran P. T., Seo W., Lee J. H., Min B. S., Kim J. A. (2021), Flavonoids from the peels of *Citrus unshiu* Markov. and their inhibitory effects on RANKL-induced osteoclastogenesis through the downregulation of c-Fos signaling *in vitro*, *Bioorganic Chemistry*, 107, 104613. **17.** Osw P. S., Hussain F. H. S. (2020), Isolation of kaempferol 3-*O*-rutinoside from Kurdish plant *Anchusa italica* Retz. and bioactivity of some extracts, *Eurasian Journal of Science and Engineering*, 6(2), 141-156. **18.** Selvaraj K. S. V., Chowdhury R., Bhattacharjee C. (2013), Isolation and structural elucidation of flavonoids from aquatic fern *Azolla microphylla* and evaluation of free radical scavenging activity, *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3), 743-749. **19.** Bedir E., Tatli I. I., Khan, R. A., Zhao J., Takamatsu S., Walker L. A., Goldman P., Khan I. A. (2002), Biologically active secondary metabolites from *Ginkgo biloba, Journal of Agricultural and Food Chemistry*, 50(11), 3150-3155. **20.** Chen Y., Xu J., Xu X., Lin H., Xia S., Wang L. (2007), Enantiocomplementary preparation of (*S*)- and (*R*)-mandelic acid derivatives via α-hydroxylation of 2-arylacetic acid derivatives and reduction of α-ketoester using microbial whole cells, *Tetrahedron: Asymmetry*, 18(21), 2537-2540. **21.** Larsen E., Kharazmi A., Christensen L. P., Christensen S. B. (2003), An anti-inflammatory galactolipid from rose hip (*Rosa canina*) that inhibits chemotaxis of human peripheral blood neutrophils *in vitro*, *Journal of Natural Products*, 66(7), 994-995.