

GLYCOSIDE CONSTITUENTS FROM *ABROMA AUGUSTUM* AND THEIR *IN VITRO* ANTIPROLIFERATIVE ACTIVITY

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Received January 13th, 2026

Accepted March 4th, 2026

Summary

Using combined chromatographic experiments, five glycoside derivatives, 1-allyl-3-methoxy-4-[apiofuranosyl(1→2)-glucopyranosyloxybenzene] (1), phenylethyl rutinoside (2), kaempferol 3-*O*-β-D-glucopyranosyl-(2→1)-β-D-xylopyranoside (3), pinoreosinol 4-*O*-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside (4), and (7*S**,8*R**)-urologinside (5), were isolated from the methanolic extract of *Abroma augustum* (L.) L. f. (Malvaceae). Their chemical structures were elucidated on the basis of the 1D (¹H and ¹³C NMR) and 2D (HSQC and HMBC) NMR spectroscopic data in comparison with those published. This is the first report of compounds 1–5 from this species. In addition, compounds 2 and 4 exhibited moderate to weak antiproliferative activity against three human cancer cell lines (MCF-7, HepG2, and SK-LU-1) with IC₅₀ values ranging from 49.38 ± 3.64 to 84.55 ± 6.60 μM.

Keywords: *Abroma augustum*; Malvaceae; Antiproliferative activity.

1. Introduction

Abroma augustum (L.) L. f. is a medium-sized shrub or small tree, 1–3 m tall. Leaves are variable in shape, blade ovate, cordate, sometimes narrowly ovate, usually toothed, slightly velvety hairy, more densely so on the lower surface, 10–20 cm long, 5–24 cm wide. The plant grows scattered in open forests, shrubby hills, along roadsides, and near stream banks. Flowering occurs from May through June, with fruiting from July to November. The root bark is used as a folk medicine to treat paralysis, gonorrhea, and to regulate menstruation. In China, the roots and leaves are used medicinally to treat injuries from falls and broken bones, irregular menstruation, and red, swollen boils [1]. In 1993, maslinic acid and α-amyrin were isolated from this plant [2]. In addition, taraxerol isolated from this species was reported to attenuate diabetic nephropathy in type 2 diabetic rats [3] and acute inflammation via inhibition of NF-κB signaling [4]. This species also exhibits many noteworthy biological activities, including antidiabetic, anti-inflammatory, antibacterial, antioxidant, cytotoxic (against cancer cells), and wound healing effects... [5]. Within the frame of our research project on cytotoxic compounds from some plants growing in Phia Oac-Phia Den National Park, the MeOH extract of this plant was found to exhibit cytotoxic activity against the HepG2 human cancer cell line with IC₅₀ = 90.22 ± 7.54 μg/mL. In this paper, we report the isolation and structure elucidation of five

glycoside derivatives (Fig. 1) from this species and evaluate their *in vitro* antiproliferative activity against three human cancer cell lines such as MCF-7 (breast), HepG2 (liver), and SK-LU-1 (lung).

2. Materials and methods

2.1. Plant materials

Branches and leaves of *Abroma augustum* (L.) L. f. were collected at Phia Oac - Phia Den National Park, Thanh Cong commune, Nguyen Binh District, Cao Bang province, in July 2024. The species was identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources (now: Institute of Biology), Vietnam Academy of Science and Technology. The voucher specimen (No. KHCBBH.01/24-25-08) was deposited at the Institute of Chemistry.

2.2. General experimental procedures

The Nuclear Magnetic Resonance spectra (NMR) were recorded on Bruker AVANCE NEO 600 and AVANCE 500 FT-NMR spectrometers with TMS as an internal standard. Medium-pressure liquid chromatography (MPLC) was carried out on a Biotage Isolera One system. Column chromatography (CC) was carried out on *silica gel* (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck), reversed-phase *silica gel* (ODS-A, 12 nm S-150 μm, YMC Co., Ltd.), Sephadex LH-20 (Sigma), and Diaion HP-20 (Supelco). Pre-coated *silica gel* 60 F₂₅₄ (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F₂₅₄S plates (1.15685.0001, Merck) were used for TLC, and spots were visualized by

spraying with aqueous 10% H₂SO₄, followed by heating for 3–5 min at 120°C.

2.3. Extraction and isolation

Branches and leaves of *A. augustum* were cleaned, dried at 50°C, and ground into fine powder. This powder (10 kg) extracted three times with methanol under ultrasonic conditions. The resulting solutions were combined and concentrated using a rotary evaporator under vacuum to afford the methanol residue (M, 440 g). The residue M was suspended in water and extracted in turn with dichloromethane and ethyl acetate to give dichloromethane (D, 35 g), ethyl acetate (E, 22 g) extracts, water layer, and a portion of undissolved residue. The water layer was filtered and passed through a Diaion HP-20 CC using stepwise elution with 100% water, methanol-H₂O (30:70), methanol-H₂O (70:30) and methanol 100% to obtain four fractions W1-W4.

Fraction W4 (14 g) was divided into seven subfractions W4A-W4G using RP-18 MPLC with gradient eluent of MeOH-H₂O (1:3-1:0, v/v). Subfraction W4F (2.0 g) was then separated on *silica gel* CC with CH₂Cl₂-MeOH-H₂O (5:1:0.1, v/v/v) to give three smaller fractions W4F1-W4F3. Fraction W4F2 (100 mg) was purified on reversed phase *silica gel* CC with acetone - H₂O (1:2, v/v) to furnish compound **1** (4.0 mg). Subfraction W4E (2.5 g) was also separated on *silica gel* CC with CH₂Cl₂-MeOH-H₂O (5:1:0.1, v/v/v), giving six smaller fractions W4E1-W4E6. Fraction W4E4 (200 mg) was further separated on *silica gel* CC with CH₂Cl₂-acetone-H₂O (1:2:0.05, v/v), followed by Sephadex LH-20 CC with MeOH-H₂O (1:1, v/v) to get compound **4** (18 mg). Similarly, fraction W4E3 (250 mg) was also separated on *silica gel* CC with CH₂Cl₂-acetone-H₂O (1:2:0.05, v/v) to get two fractions W4E3A (100 mg) and W4E3B (130 mg). Fraction W4E3B was further separated into two fractions, W4E3B1 (80 mg) and W4E3B2 (40 mg), using reversed phase *silica gel* CC with acetone-H₂O (1:4, v/v). Purification of fraction W4E3B2 on *silica gel* CC with CH₂Cl₂-MeOH-H₂O (7:1:0.1, v/v) to obtain compound **5** (13 mg). Fraction W4E3B1 was purified on *silica gel* CC with CH₂Cl₂-MeOH-H₂O (5:1:0.1, v/v) to give compounds **2** (3 mg) and **3** (6.5 mg).

1-Allyl-3-methoxy-4-[apiofuranosyl(1→2)-glucopyranosyloxybenzene] (1): Pale yellow solid; ¹H-NMR (600 MHz, CD₃OD) and ¹³C-NMR (150 MHz, CD₃OD) see Table 1.

Phenylethyl rutinoside (2): Pale yellow solid; ¹H-NMR (600 MHz, CD₃OD) and ¹³C-NMR (150 MHz, CD₃OD) see Table 1.

Kaempferol 3-O-β-D-glucopyranosyl-(2→1)-β-D-xylopyranoside (3): Pale yellow powder; ¹H-NMR (600 MHz, CD₃OD) and ¹³C-NMR (150 MHz, CD₃OD) see Table 2.

Pinoresinol 4-O-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside (4): White powder; [α]_D²² +15 (c 0.05, MeOH); ¹H-NMR (600 MHz, CD₃OD) and ¹³C-NMR (150 MHz, CD₃OD) see Table 2.

(7S*,8R*)-Urolignoside (5): White powder; [α]_D²² -25 (c 0.05, MeOH); ¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (125 MHz, CD₃OD) see Table 2.

2.4. Antiproliferative assay

The *in vitro* antiproliferative activity of compounds **1–5** on three human cancer cell lines as MCF-7 (breast), HepG2 (liver), and SK-LU-1 (lung) was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) method [6],[7]. The cell lines are a kind gift from Prof. Chi-Ying F. Huang, National Yang Ming Chiao Tung University, Taipei, Taiwan. Detail:

- Compounds were dissolved in DMSO 100% to get the stock solution at 20 mM. Serial dilutions were performed on a 96-well plate using cell culture medium (Gibco, Thermo Fisher Scientific Inc.) without FBS to create 4 concentration series from high to low.

- Trypsinize the experimental cells to detach them, then count the cells using a hemocytometer to adjust the cell density to the appropriate level for the experiment. Add 5700 cells/well were added in 190 μL of complete culture medium (supplemented with 5% FBS) into each well of a 96-well plate and incubate in a CO₂ incubator to allow stable growth for 18–20 hours.

- After this stabilization period, the cells were treated with test samples as follows: 10 μL of the pre-diluted test sample (at various concentrations prepared earlier) is added to the wells containing cells. Negative control wells contain cancer cells (190 μL) + 1% DMSO (10 μL) without any test compound. Blank wells contain only culture medium (no cells, no test sample). All treatments are performed in triplicate to ensure reliability. Ellipticine at concentrations of 10, 2, 0.4, and 0.08 μg/mL is used as the positive control.

- After 48 hours of incubation with the test compounds in a CO₂ incubator (37°C, 5% CO₂), 10 μL of MTT (Bio Basic Canada Inc.) solution (final concentration in the well = 0.5 mg/mL) is added to each well.

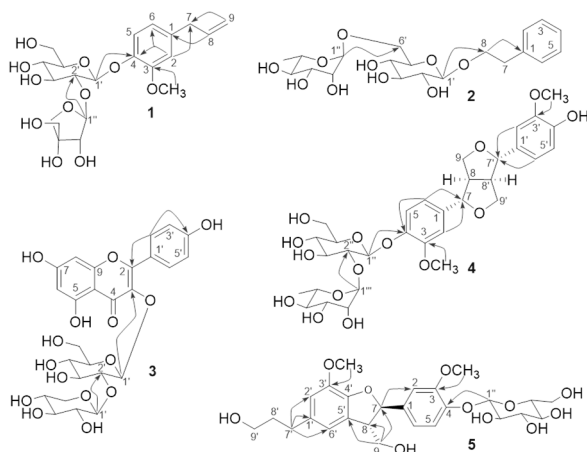


Fig. 1. Structures and key HMBC (H→C) correlations of compounds 1–5

- After 4 hours, the medium is carefully removed, and the formazan crystals are dissolved in 50 μ L of 100% DMSO. The optical density (OD) is measured at 540 nm using a BioTek ELx800 microplate reader. The IC₅₀ value (concentration that inhibits 50% of cell growth) is calculated using TableCurve 2D v4 software.

- The percentage of cell growth inhibition in the presence of the test compound is calculated using the following formula:

$$\% \text{ inhibition (\%)} = 100 - \frac{OD(\text{sample}) - OD(\text{blank})}{OD(\text{DMSO}) - OD(\text{blank})} \times 100$$

Table 1. The ¹H- (CD₃OD, 600 MHz) and ¹³C-NMR (CD₃OD, 150 MHz) spectral data of 1 and 2

Pos.	δ_c^a	δ_c^b	1		δ_c^c	2	
			δ_c	δ_H mult. (J in Hz)		δ_c	δ_H mult. (J in Hz)
1	136.4	133.8	136.06	-	140.1	140.03	-
2	114.1	113.0	114.07	6.82 d (1.8)	129.5	129.38	7.28 m
3	150.7	149.2	150.78	-	130.1	130.01	7.27 m
4	146.3	145.1	146.32	-	127.3	127.21	7.19 m
5	118.2	115.6	117.50	7.04 d (8.4)	130.1	130.01	7.27 m
6	122.1	120.6	121.78	6.71 dd (1.8, 8.4)	129.5	129.38	7.28 m
7	40.8	39.5	40.75	3.33 m	37.4	37.30	2.96 m
8	139.0	138.4	139.06	5.96 m	71.9	71.84	3.78 m/4.05 m
9	115.9	116.0	115.77	5.04 dd (1.8, 17.4) 5.08 dd (1.8, 10.2)			
3-OCH ₃	56.7	55.9	56.44	3.83 s			
<i>Glc</i>							
1'	103.0	98.8	101.16	4.97 d (7.8)	104.7	104.50	4.31 d (7.8)
2'	74.9	77.7	77.59	3.70 m	75.3	75.08	3.19 dd (7.8, 9.0)
3'	78.1	76.5	78.79	3.60 m	78.3	78.07	3.35 m
4'	71.3	70.5	71.49	3.42 t (8.4)	71.8	71.66	3.30 t (9.0)
5'	77.8	75.4	78.00	3.40 m	77.1	76.84	3.42 m
6'	62.5	61.1	62.52	3.69 dd (3.4, 12.0) 3.87 dd (1.8, 12.0)	68.4	68.12	3.63 dd (6.0, 12.0) 4.00 dd (1.8, 12.0)
<i>Api</i>							
1''		108.	110.26	5.56 d (1.2)	102.4	102.25	4.76 d (1.8)
2''		77.3	77.85	4.00 d (1.2)	72.3	72.20	3.85 dd (1.8, 3.6)
3''		79.8	80.82	-	72.5	72.37	3.68 m
4''		74.4	75.49	3.76 d (9.6) 4.19 d (9.6)	74.2	74.02	3.38 m
5''		64.9	66.21	3.55 d (11.4) 3.60 d (11.4)	69.9	69.81	3.69 m
6''					18.5	18.04	1.27 d (6.0)
<i>Rha</i>							

^a δ_c of eugenyl-O- β -glucopyranoside in CD₃OD [8]; ^b δ_c of 1-allyl-3-methoxy-4-[apiofuranosyl(1→2)-glucopyranosyloxy]benzene in DMSO-d₆ [9]; ^c δ_c of phenylethyl-rutinoside in CD₃OD [10].

where OD (DMSO): absorbance of well with untreated cells; OD (sample): absorbance of well with treated cells; and OD (blank): absorbance of well was medium only.

3. Results and discussion

Compound 1 was isolated as a pale yellow solid. The ¹H- and ¹³C-NMR spectra revealed typical signals of an ABX coupled aromatic ring [δ_H 6.82 (d, J = 1.8 Hz, H-2), 7.04 (d, J = 8.4 Hz, H-5), and 6.71 (dd, J = 1.8, 8.4 Hz, H-6)]/ δ_C 136.06 (C-1), 114.07 (C-2), 150.78 (C-3), 146.32 (C-4), 117.50 (C-5), and 121.78 (C-6)], a methylene group attached to an aromatic ring [δ_H 3.33 (m, H-7)]/ δ_C 40.75 (C-7)], a monosubstituted double bond [δ_H 5.96 (m, H-8), 5.04 (dd, J = 1.8, 10.2 Hz, H-9), and 5.08 (dd, J = 1.8, 17.4 Hz, H-9)]/ δ_C 139.06 (C-8) and 115.77 (C-9)] and a methoxy group [δ_H 3.83 (s, 3-OMe)]/ δ_C 56.44 (3-OMe)]. The obtained data are indicative of the eugenyl aglycon [8]. In addition, the presence of two anomeric protons at δ_H 4.97 (d, J = 7.8 Hz, H-1') and 5.56 (d, J = 1.2 Hz, H-1''), showed HSQC correlations with two relevant anomeric carbons at δ_C 101.16 (C-1') and 110.26 (C-1'') confirmed two sugar units. Detailed analysis of the HSQC and HMBC correlations allowed assignment of the ¹H- and ¹³C-NMR spectral data of the two sugars, as shown in Table 1.

Table 2. The ^1H - and ^{13}C -NMR spectral data of **3–5** in CD_3OD

Pos.	$\delta_{\text{C}}^{\text{a}}$		3		4		5	
	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{c}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{d}}$	$\delta_{\text{H}}^{\text{e}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{H}}^{\text{e}}$ mult. (J in Hz)	
1				137.23	-	138.32	-	
2	158.5	158.40	-	111.26	7.01 d (1.8)	111.20	7.05 br s	
3	135.0	134.98	-	151.44	-	150.92	-	
4	179.7	179.58	-	147.01	-	147.58	-	
5	163.1	163.10	-	117.84	7.10 d (8.4)	118.01	7.15 d (8.5)	
6	99.8	99.80	6.20 br s	119.23	6.89 dd (1.8, 8.4)	119.38	6.95 br d (8.5)	
7	165.8	165.80	-	87.13	4.76 d (4.2)	88.46	5.57 d (6.0)	
8	94.6	94.66	6.40 br s	55.51	3.14 m	55.61	3.46 m	
9	158.4	158.37	-	72.67	3.89 m/4.26 m	65.04	3.77 dd (7.5, 11.0) 3.87 m	
10	105.9	105.80	-					
1'	122.9	122.86	-	133.78	-	137.05	-	
2'	132.3	132.29	8.08 d (8.4)	111.02	6.96 d (1.8)	114.17	6.75 br s	
3'	116.2	116.17	6.91 d (8.4)	149.12	-	145.22	-	
4'	161.5	161.42	-	147.31	-	147.47	-	
5'	116.2	116.17	6.91 d (8.4)	116.12	6.79 d (7.8)	129.59	-	
6'	132.3	132.29	8.08 d (8.4)	120.05	6.82 dd (1.8, 7.8)	117.93	6.73 br s	
7'				87.49	4.72 d (4.8)	32.87	2.64 t (8.0)	
8'				55.32	3.14 m	35.77	1.83 m	
9'				72.69	3.89 m/4.26 m	62.22	3.58 t (7.0)	
3-OMe				56.41	3.86 s	56.70	3.85 s	
3'-OMe				56.46	3.87 s	56.78	3.89 s	
1''	100.8	100.77	5.50 d (7.8)	100.53	5.07 d (7.8)	102.75	4.90 d (7.0)	
2''	82.4	82.33	3.69 dd (7.8, 9.0)	78.41	3.75 dd (7.8, 9.0)	74.88	3.50 m	
3''	78.3	78.18	3.62 m	79.43	3.61 t (9.0)	78.15	3.41 m	
4''	71.1	71.12	3.38 m	71.48	3.42 t (9.0)	71.31	3.41 m	
5''	77.1	77.07	3.40 m	77.96	3.40 m	77.81	3.48 m	
6''	62.5	62.43	3.55 dd (5.4, 12.0) 3.72 dd (1.8, 12.0)	62.44	3.68 m/3.84 m	62.47	3.69 dd (3.0, 12.0) 3.87 m	
1'''	105.5	105.37	4.78 d (7.2)	101.95	5.38 d (1.2)			
2'''	75.0	74.91	3.39 m	72.32	3.96 dd (1.8, 3.0)			
3'''	78.4	78.30	3.24 m	72.21	3.68 m			
4'''	71.2	71.02	3.54 m	70.01	4.12 m			
5'''	66.7	66.66	3.26 dd (11.4, 9.6) 3.96 dd (11.4, 5.4)	73.98	3.40 m			
6'''				18.11	1.23 d (6.0)			

^a δ_{C} of kaempferol 3-O- β -D-glucopyranosyl-(2 \rightarrow 1)- β -D-xylopyranoside [11], ^b150 MHz, ^c600 MHz, ^d125 MHz, ^e500 MHz.

Comparison of these data with reported values led to the identification of the sugar chain as apiofuranosyl(1 \rightarrow 2)-glucopyranoside [9]. The HMBC cross-peak of H-1'' (δ_{H} 5.56) with C-2' (δ_{C} 77.59) confirmed the attachment of the apiofuranose unit at C-2' of the glucopyranose moiety. In addition, the HMBC cross-peak of the anomeric proton H-1' (δ_{H} 4.97) with carbon C-4 (δ_{C} 146.32) indicated the attached position of the sugar chain at this carbon (Fig. 1). From the above evidence, compound **1** was identified as 1-allyl-3-methoxy-4-[apiofuranosyl(1 \rightarrow 2)-glucopyranosyl-oxybenzene] [9].

The ^1H - and ^{13}C -NMR spectra of **2** also revealed two anomeric protons/carbons at δ_{H} 4.31 (d, $J = 7.8$ Hz, H-1') and 4.76 (d, $J = 1.8$ Hz, H-1'')/ δ_{C} 104.50 (C-1') and 102.25 (C-1''), confirming the presence of two sugar moieties.

Detailed analysis of the HSQC and HMBC correlations in comparison of the ^1H - and ^{13}C -NMR spectral data for the sugar part with the reported values led to the assignment of the two sugars as rhamnopyranose and glucopyranose [10]. The ^{13}C -NMR signal at C-6' of **2** was strongly shifted downfield at δ_{C} 68.12 (relative to that of **1** at δ_{C} 62.52), confirming the attached position of the rhamnose unit at C-6' of the glucose moiety. This was further confirmed by the HMBC cross-peak between H-1'' (δ_{H} 4.76) and C-6' (δ_{C} 68.12). In addition, the aglycon part contained signals of a monosubstituted benzene ring, a methylene group attached to an aromatic ring, and an oxymethylene group (Table 1). The anomeric proton H-1' (δ_{H} 4.31) exhibited an HMBC cross-peak with the oxymethylene carbon C-8 (δ_{C} 71.84) indicating the attachment of the

sugar chain at this carbon. Thus, compound **2** was identified as phenylethyl-rutinoside [10].

The spot of compound **3** on the TLC plate turned orange when sprayed with 10% H₂SO₄ reagent and heated, suggesting that it is a flavonoid. The presence of two pairs of symmetric *ortho* coupled aromatic protons [δ_{H} 8.08 (d, $J = 8.4$ Hz, H-2' and H-6') and 6.91 (d, $J = 8.4$ Hz, H-3' and H-5')] and two *meta* coupled aromatic protons [δ_{H} 6.20 (br s, H-6) and 6.40 (br s, H-8)] indicated the presence of the kaempferol skeleton. In addition, signals of one glucose and one xylose moieties (see Table 2) [11] were also recorded. The ¹³C-NMR chemical shifts for C-3 of the aglycon at δ_{C} 134.98 and C-2' of the glucose moiety at δ_{C} 82.33 suggested attachments of the xylose unit at C-2' and the sugar chain at C-3 [11]. This was further supported by the HMBC cross-peaks of H-1'' (δ_{H} 4.78) with C-2' (δ_{C} 82.33) and H-1' (δ_{H} 5.50) with C-3 (δ_{C} 134.98). Consequently, compound **3** was identified as kaempferol 3-*O*- β -D-glucopyranosyl-(2 \rightarrow 1)- β -D-xylopyranoside [11].

Compound **4** was isolated as a white powder. The ¹H-NMR spectrum revealed signals of two ABX coupled aromatic rings [δ_{H} 7.01 (d, $J = 1.8$ Hz, H-2), 7.01 (d, $J = 8.4$ Hz, H-5), 6.89 (dd, $J = 1.8, 8.4$ Hz, H-6), 9.96 (d, $J = 1.8$ Hz, H-2'), 6.79 (d, $J = 7.8$ Hz, H-5'), and 6.82 (dd, $J = 1.8, 7.8$ Hz, H-6')], two oxymethines [δ_{H} 5.57 (d, $J = 6.0$ Hz, H-7) and 4.72 (d, $J = 4.8$ Hz, H-7')], two oxymethylenes [δ_{H} 3.89 and 4.26, m, H-9 and H-9'], and two methoxy groups [δ_{H} 3.85 (s, 3-OMe) and 3.89 (s, 3'-OMe)]. In addition, signals of one glucose and one rhamnose units (see Table 2) [12] were also observed. The ¹H- and ¹³C-NMR data of **4** were found to be similar to those of pinoresinol 4-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)- β -D-glucopyranoside [12]. Protons H-1'' (δ_{H} 5.38), 3-OMe (δ_{H} 3.85), and 3'-OMe (δ_{H} 3.89) revealed HMBC cross-peaks with the corresponding carbons C-2'' (δ_{C} 78.41), C-3 (δ_{C} 151.44), and C-3' (δ_{C} 149.12), respectively, confirming attachments of the rhamnose at C-2'' and two methoxy groups at C-3 and C-3'. Moreover, the HMBC cross-peaks of H-6 (δ_{H} 6.89) and H-1' (δ_{H} 5.07) with C-4 (δ_{C} 147.01) confirmed the location of the sugar chain at this carbon. Thus, **4** was identified as pinoresinol 4-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)- β -D-glucopyranoside [12].

The ¹H-NMR spectrum of **5** exhibited signals of three ABX [δ_{H} 7.05 (br s, H-2), 7.25 (d, $J = 8.5$ Hz, H-5), 6.95 (br d, $J = 8.5$ Hz, H-6)] and two *meta* coupled [δ_{H} 6.75 (br s, H-2) and 6.73

(br s H-6)] aromatic protons, one oxymethine [δ_{H} 5.57 (d, $J = 6.0$ Hz, H-7)], two oxymethylenes [δ_{H} 3.77 (dd, $J = 7.5, 11.0$ Hz, H_a-9), 3.87 (m, H_b-9), and 3.58 (t, $J = 7.0$ Hz, H-9')], and two methoxy groups [δ_{H} 3.85 (s, 3-OMe) and 3.89 (s, 3'-OMe)]. In addition, signals of one glucose moiety were also recorded. The ¹H- and ¹³C-NMR data of **5** closely resembled those of (7*S**,8*R**)-urologninside [13],[14]. The structure of **5** was further confirmed by the HMBC experiment, as shown in Fig. 1. Without CD data, only the relative configuration of **5** was assigned by comparison of its NMR and $[\alpha]_{\text{D}}$ data with the reported values. Consequently, compound **5** was identified to be (7*S**,8*R**)-urologninside [13],[14].

Table 3. The antiproliferative activity of compounds **1–5**

Compounds	IC ₅₀ values (μM)		
	MCF-7	HepG2	SK-LU-1
1	(-)	(-)	(-)
2	67.17 \pm 5.97	49.38 \pm 3.64	71.03 \pm 5.08
3	(-)	(-)	(-)
4	82.35 \pm 5.39	63.01 \pm 5.14	84.55 \pm 6.60
5	(-)	(-)	(-)
Ellip. ^a	1.46 \pm 0.12	1.34 \pm 0.12	1.54 \pm 0.12

^a Ellipticine was used as the positive control, (-): IC₅₀ > 100 μM .

Compounds **1–5** were evaluated for their antiproliferative activity against three human cancer cell lines, MCF-7 (breast), HepG2 (liver), and SK-LU-1 (lung) using the MTT assay [6],[7]. As the results (Table 3), phenylethyl-rutinoside (**2**) exhibited moderate activity on the HepG2 cell line (IC₅₀ = 49.38 \pm 3.64 μM) and weak effect on the two MCF-7 (IC₅₀ = 67.17 \pm 5.97 μM) and SK-LU-1 (IC₅₀ = 71.03 \pm 5.08 μM) cell lines, relative to that of the positive control ellipticine (with IC₅₀ values of 1.34 \pm 0.12, 1.46 \pm 0.12 and 1.54 \pm 0.12 μM). Compound **2** was reported to have anti-inflammatory activity via inhibition of IL-12 p40 production in LPS-stimulated BMDCs (IC₅₀ = 29.23 \pm 0.75 μM) [15], inhibitory effect of enzymes COX-1 (IC₅₀ = 41.2 \pm 1.5 μM), COX-2 (IC₅₀ = 35.4 \pm 1.6 μM) [16], and less cytotoxic activity against the human lung cancer cell line A549 (IC₅₀ > 100 μM) [17]. Pinoresinol 4-*O*- α -L-rhamnopyranosyl (1 \rightarrow 2)- β -D-glucopyranoside (**4**) revealed weak effect against all the three MCF-7, HepG2 and SK-LU-1 cell lines with the IC₅₀ values of 82.35 \pm 5.39, 63.01 \pm 5.14 and 84.55 \pm 6.60 μM , respectively. This compound was reported to inhibit lipid peroxidation in rat liver microsomes (IC₅₀ = 27.0 μM) [18]. The other compounds were inactive

with $IC_{50} > 100 \mu M$. Compounds **2** and **4** exhibited only moderate to weak antiproliferative activity; nevertheless, this finding provides a scientific basis for directing further investigations into the pharmaceutical effects of the isolated compounds. In general, glycoside compounds could participate in detoxification and elimination processes in animals and humans, where toxic substances are often conjugated to sugars for safer transport and excretion, while some exhibit broader effects such as cytotoxic, antioxidant, anti-inflammatory, or signaling functions in cellular processes.

4. Conclusion

From the branches and leaves of *Abroma augustum*, five glycoside derivatives 1-allyl-3-methoxy-4-[apiofuranosyl(1→2)-glucopyranosyloxybenzene] (**1**), phenylethyl

rutinoside (**2**), kaempferol 3-*O*- β -D-glucopyranosyl-(2→1)- β -D-xylopyranoside (**3**), pinoresinol 4-*O*- α -L-rhamnopyranosyl-(1→2)- β -D-glucopyranoside (**4**), and (7*S**,8*R**)-urologinside (**5**) were isolated using combined chromatographic experiments. Their chemical structures were elucidated by one (1H - and ^{13}C -NMR) and two-dimensional (HSQC and HMBC) NMR spectra. This is the first report of compounds **1–5** from *A. augustum*. Compounds **2** and **4** exhibited moderate to weak antiproliferative activity against three human cancer cell lines (MCF-7, HepG2, and SK-LU-1) with IC_{50} values ranging from 49.38 ± 3.64 to $84.55 \pm 6.60 \mu M$.

Acknowledgments: This study is funded by the Vietnam Academy of Science and Technology (grant number: KHCBHH.01/24-25).

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