Bioactive triterpenoids from Indonesian medicinal plant Syzygium aqueum

by Alfinda Novi Kristanti

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Research Article

Alfinda Novi Kristanti*, Ei Ei Aung, Nanik Siti Aminah, Yoshiaki Takaya, Hnin Thanda Aung, Rico Ramadhan

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Abstract: A large common species, *Syzygium aqueum*, belonging to the genus *Syzygium* possesses numerous bioactive phytochemical constituents. Moreover, the different parts of this species have been used as folk medicine since centuries ago. In this study, a phytochemical exploration was carried out on the plant's stem bark. Isolation of the compounds was carried out through the extraction step with some organic solvents, followed by separation and purification using chromatography techniques until the two triterpenoids were isolated from nonpolar and semipolar extracts. Structure elucidation was done using spectroscopic methods. These compounds were identified as alphitolic acid and arjunolic acid. Subsequently, these two compounds were used in anticancer tests against human cancer cells HeLa, T47D, and A549 using colorimetric assay. The result showed that both compounds showed more inhibition of the growth of HeLa and T47D than A549 cancer cells, with the highest activity shown by arjunolic acid against HeLa cell lines.

*** Corresponding author: Alfinda Novi Kristanti,** Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Kampus C UNAIR, Jl. Mulyorejo-60115, Surabaya, Indonesia; Biotechnology of Tropical Medicinal Plants Research Group, Universitas Airlangga, Surabaya, Indonesia, e-mail: alfinda-n-k@fst.unair.ac.id, tel: +62-31-5936501, fax: +62-31-5936502
Ei Ei Aung: Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Kampus C UNAIR, Jl. Mulyorejo-60115, Surabaya, Indonesia; Department of Chemistry, Yadanarbon University, Amarapura-05063, Mandalay, Myanmar
Nanik Siti Aminah: Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Kampus C UNAIR, Jl. Mulyorejo-60115, Surabaya, Indonesia; Biotechnology of Tropical Medicinal Plants Research Group, Universitas Airlangga, Surabaya, Indonesia
Yoshiaki Takaya: Faculty of Pharmacy, Meijo University, Tempaku-468-8502, Nagoya, Japan
Hnin Thanda Aung: Department of Chemistry, Mohnyin Degree College, Kachin state, Myitkyina, Myanmar
Rico Ramadhan: Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Kampus C UNAIR, Jl. Mulyorejo-60115, Surabaya, Indonesia

Keywords: *Syzygium aqueum*, alphitolic acid, arjunolic acid, MTT assay, XTT assay

1 Introduction

One of the probable medicament manufacturing countries, Indonesia, between the Pacific and Indian Oceans, is commonly known as a country with the second richest biodiversity of medicinal plants on the earth. Medicinal plants have therapeutic properties or provide beneficial pharmacological effects for the human body. Some examples are given here. The chemical composition of the essential oils extracted from *Elaeagnus umbellata* Thunb fruit demonstrated antioxidant, anticholinesterase, and antidiabetic activities that could be used as an alternative drug to treat oxidative stress-related diseases [1]. The essential oil of *Teucrium stocksianum* possesses a strong antinociceptive potential, which is further used as a topical analgesic [2]. The study of *Woodwardia unigemmata* (Makino) Nakai plant extracts, which were rich in polyphenolic compounds (total phenolic compounds and total flavonoid compounds) with efficient antioxidant activity, also showed remarkable antibacterial activity against plant and animal pathogenic bacteria [3]. The diverse Indonesian medicinal plants have been used as the sources of herbal medicines for the inhibition and treatment of human diseases for several thousand years [4].

The sixteenth largest genus of flowering plants in the Myrtaceae family, *Syzygium*, comprises about 1,200–1,800 species. Moreover, *Syzygium* species of tree and shrub types are distributed in tropical areas worldwide, especially Indonesia, Malaysia, Philippines, and Thailand. Some species of *Syzygium* are widely cultivated for economic importance, edible fruits, spices, flavoring agents, and pharmacological proprieties. *Syzygium* contains abundant diversity of bioactive phytochemical constituents such as terpenoids, steroids, chalcones, flavonoids, lignans, alkyl phloroglucinols, hydrolysable tannins, and chromone derivatives [5].

A medicinal species of *Syzygium* found in Indonesia, *Syzygium aqueum*, is a large common species in this genus found in Malaysia [6]. Fruits, leaves, and roots of this species have been used as folk medicine, especially for antibiotic properties, diabetic treatment, the treatment for a cracked tongue, relieving itches, stomach aches, dysentery, and reducing of swelling, respectively [7–11].

Some researchers were focused on bioactive phytochemical constituents based on the above medicinal values of this plant. Therefore, bioactive phytochemical constituents such as tannins, flavonoids, steroids, triterpenoids, and phenols from leaves and stem bark of this plant have already been reported in some research articles [12–17]. These articles also showed that some flavonoids from *S. aqueum* (leaves) strongly showed antidiabetic properties using α-glucosidase and α-amylase inhibition assays and showed their cytotoxicity against MCF-cell line using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Moreover, some triterpenoids and steroids from *S. aqueum* (stem bark) were active with high cytotoxicity on HeLa, T47D, and A549 cancer cell lines using MTT and 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) assays.

Based on the literature study, the stem bark of *S. aqueum* was designated for this research. Primarily, some extracts of stem bark of *S. aqueum* were separated to collect bioactive constituents with vacuum liquid chromatography and column chromatography. The structure of isolated compounds was identified with spectroscopic methods, including UV, Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR [1D and 2D]), and HR-DART-MS. After this, their anticancer activity was determined with colorimetric assay (MTT and XTT assays). The isolated bioactive constituents were triterpenoid compounds, first isolated from *S. aqueum*.

2 Materials and methods

2.1 Plant materials

The stem bark of *S. aqueum* was collected in December 2018 from Wage, Taman, Sidoarjo, East Java, Indonesia. The plant material was identified at the Department of Biology, Faculty of Science and Technology, Universitas Airlangga, and the voucher specimen (UA-MSa050918) was deposited at the Herbarium of Universitas Airlangga, Laboratory of Biosystematic, Department of Biology, Faculty of Science and Technology, Universitas Airlangga. The collected stem bark was cleaned, chopped, and crushed into small pieces of coarse powder.

2.2 Instrumentations

A polarimeter with φ 3.5 mm diameter (cell size) × 100 mm (cell length) was used to measure the specific rotation. For structure elucidation, some of the instruments used are a UV-vis 1800 spectrometer (Shimadzu), FTIR spectra on a Tracer-100 spectrophotometer (Shimadzu), NMR spectra on Bruker Avance III HD 600, and HR-DART-MS on an Exactive Plus Orbitrap DART Mass Spectrometer. Enzyme-linked immunosorbent assay (ELISA) reader was used for the anticancer test.

2.3 General procedure

For extraction, fractionation, isolation, and purification of a sample, organic solvents such as *n*-hexane (*n*-Hex), ethyl acetate (EtOAc), dichloromethane (DCM), and methanol (MeOH) were used. In analytical thin-layer chromatography (TLC), a precoated silica gel 60 F_{254} (Merck) was used and an anisaldehyde-sulfuric acid reagent was sprayed on a TLC plate for visualization. Silica gel 60 (700–200 mesh ASTM) was applied for column chromatography. Then, vacuum liquid chromatography (VLC) was performed using Kieselgel 60 (F_{254}, Merck). The melting point of pure compounds was measured on a Fisher-Johns melting point apparatus (Stuart SMP30). Spectroscopic methods, including UV-vis, IR, NMR, and MS, were used to determine the structure of isolated compounds. An anticancer test was carried out using MTT and XTT reagents (colorimetric assay), and the absorbance for an anticancer test was recorded on an ELISA reader.

2.4 Extraction and isolation

At room temperature, 2 kg of coarse powder of stem bark was extracted with a polar solvent (MetOH, 40 L) for 3 × 24 h. This crude extract (450 g) was then mentioned to partition with a nonpolar solvent (*n*-hexane). It was separated into a nonpolar fraction and a methanol fraction. Then, 400 mL of aqueous was added to methanol fractions before partitioning with a semipolar solvent, EtOAc. After this, this mixture was separated into a semipolar fraction and an aqueous methanol residue. First, the

nonpolar fraction was chromatographed with gradient polarity solvents, *n*-Hex:EtOAc. Separation procedure was carried out for three times using column chromatography to obtain a pure compound. Compound 1 (30 mg) was acquired from a fraction eluted with *n*-Hex:EtOAc = 7:3. The semipolar fraction was separated by VLC using gradient solvent mixtures of *n*-Hex:DCM and DCM:EtOAc. Separation procedure using column chromatography was carried out twice to obtain a pure compound, Compound 2 (50 mg), that was eluted with DCM:EtOAc = 6:4.

2.5 Anticancer activity

2.5.1 Cell culture

Three cancer cell lines, HeLa (cervical cancer), T47D (breast cancer), and A459 (lung cancer), were obtained from American Type Culture Collection. Cells were cultured at 37°C in a CO_2 incubator for 24 h and 100% humidity in medium supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin. The cell culture process was carried out in the Cancer Chemoprevention Research Center, Faculty of Pharmacy at Universitas Gadjah Mada, Indonesia and in Physic Laboratory, Faculty of Science of University Putra Malaysia (UPM), Malaysia.

2.5.2 MTT assay

An anticancer test of pure compounds was performed using MTT assay. Cells were cultured in 96 well plates at 213 × 10^4 cell/well density and were incubated at 37°C in a CO_2 incubator for 24 h. The cells were then treated with 100 µL of the prepared sample with different concentration series (1.5625–100 µg/mL) at 37°C in a CO_2 incubator for 24 h. As much as 100 µL of the MTT reagent (50 mg in 10 mL of PBS) was filled in each well after incubation, and it was incubated again at 37°C in a CO_2 incubator for 2–4 h until purple formazan crystals are formed. After the crystal formation, cells were observed with a microscope, and 100 µL of 10% SDS stopper in 0.1 N HCl was added, and it was kept in a dark place overnight (room temperature). Next, the plate's absorbance value was read at a wavelength of 560 nm by using an ELISA reader [18,19].

2.5.3 XTT assay

The anticancer test was carried out using XTT assay. Cells were seeded (10^4–10^5 cells/well) into 96 well plates and were incubated at 37°C in a CO_2 incubator for 24 h. As much as 100 µL of the prepared sample with various concentrations (1.5625–100 µg/mL) was inserted to treat cancer cells. The plate was incubated at 37°C in a CO_2 incubator for 24 h. And then, PMS solution (10 mM PMS solution and PBS (3 mg PMS + 1 mL PBS)) was prepared and 10 µL of this solution was added in 4 mL of XTT (4 mg of XTT dissolved in 4 mL of 37°C cells culture medium). After incubation, 25 µL of the XTT reagent (XTT/PMS solution) was filled in each well. After this, it was incubated for 2–4 h in a CO_2 incubator at 37°C until the changing of the orange formazan. The next step was to read the absorbance value at a wavelength of 450 nm using an ELISA reader [19,20]. Each experiment was carried out in triplicate, and the number of viable cells was calculated using the following formula:

$$\%\ \text{Cell viability} = \frac{\text{Absorbance of treatment} - \text{Absorbance of media}}{\text{Absorbance of negative control} - \text{Absorbance of media}} \times 100\%.$$

3 Results and discussion

3.1 Isolation of compounds and structure elucidation

Nonpolar and semipolar fractions of *S. aqueum* (stem bark) gave one bioactive compound each, Compound 1 and Compound 2. Structures of these compounds were identified and elucidated using spectroscopic methods.

Compound 1 was acquired as white powder, $[\alpha]_D^{25}$-9, m.p. 279–280°C. According to the HR-DART-MS spectrum, the molecular formula was $C_{30}H_{48}O_4$, *m/z* 471.3462 $[M-H]^-$ (calc. mass for $C_{30}H_{48}O_4$–H^-, *m/z* 471.3474). The UV spectrum in MeOH was displayed at λ_{max} 182 nm. In the FTIR spectrum (KBr), hydroxyl group (3,423 cm^{-1}), symmetric and asymmetric sp^3 C–H stretching (2,941–1,870 cm^{-1}), carbonyl acid group (1,699 cm^{-1}), CH_2 bending (1,456 cm^{-1}), and CH_3 bending (1,381 cm^{-1}) were recorded. The proton signals, both δ_H: 3.60 ppm (H-2β) and δ_H: 2.89 ppm (H-3α), were suggested as carbinol methine protons, which coupled each other according to the Double Quantum Filtered-COSY (DFQ-COSY) experiment and relative configuration indicated *trans* configuration due to the 9.6 Hz coupling constant of H-3α. Nuclear Overhauser Effect Spectroscopy (NOESY) cross-peaks of H-3α and H-5α confirmed the presence of the *trans*-AB ring. Six methyl protons possessed some Heteronuclear Multiple Bond Correlation (HMBC) such as H-23 (δ_H: 0.99 ppm) and C-24 (δ_C: 15.8 ppm), H-24 (δ_H: 0.78 ppm) and H-25 (δ_H: 0.92 ppm) and C-5 (δ_C: 55.2 ppm), H-26 (δ_H: 0.97 ppm) and C-7 (δ_C: 1.43 ppm), and H-27 (δ_H: 1.01 ppm)

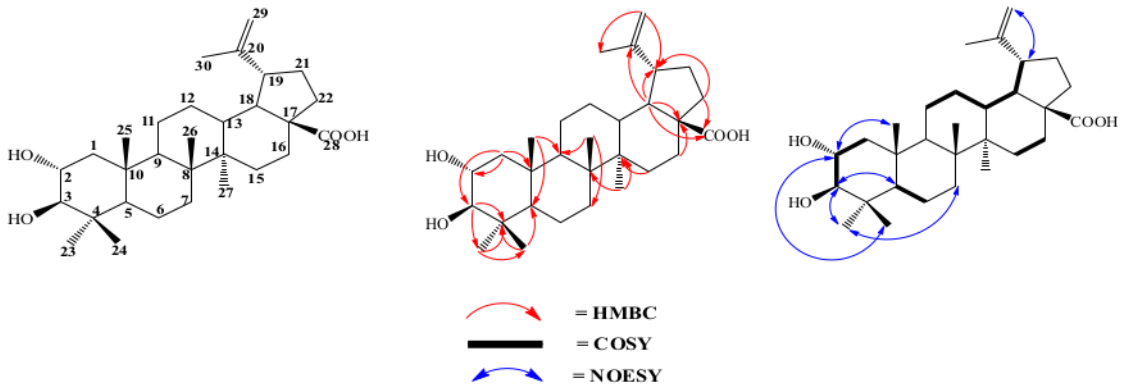

Figure 1: DQF-COSY, NOESY, and HMBC correlations of alphitolic acid from *S. aqueum*.

and C-14 (δ_C: 42.3 ppm). Two vinylic protons (H-29) were observed at δ_H: 4.60 ppm and δ_H: 4.71 ppm. Their positions were confirmed by HMBC between these two H-29 protons with C-19 (δ_C: 47.1 ppm) and C-30 (δ_C: 18.2 ppm). Based on HSQC experiments, two carbinol methine carbons (R_2-CH-OH) gave correlations H-2β-C-2 (δ_C: 68.4 ppm) and H-3α–C-3 (δ_C: 83.0 ppm). A vinylic group was appeared at δ_C: 108.7 ppm (C-29) and δ_C: 150.6 ppm (C-20) in the ^{13}C NMR spectrum. The C-20 was supposed to be a quaternary carbon of the olefinic system. And then, the C-29 was suggested as methylene carbon of the same olefinic system (=CH_2), which were connected by two protons [δ_H: 4.60 ppm (H-29) and δ_H: 4.71 ppm (H-29)] through the HSQC spectrum. Chemical shift of the carbonyl group appeared at δ_C: 178.7 ppm (C-28) in the ^{13}C NMR spectrum. In the HMBC spectrum, C-28 showed correlations with H-18 (δ_H: 1.61 ppm) and H-22 (δ_H: 1.43 ppm). Furthermore, the positions of quaternary carbons were verified based on the HMBC spectrum by observing the correlations of H-23 and H-24 to C-4 (δ_C: 39.1 ppm), H-27 to C-8 (δ_C: 40.6 ppm), H-1 to C-10 (δ_C: 38.1 ppm), H-15 to C-14 (δ_C: 42.3 ppm), and H-16 to C-17 (δ_C: 56.1 ppm). The remaining peaks at δ_C: 27.7 ppm (C-23), 15.8 ppm (C-24), 16.5 ppm (C-25), 15.3 ppm (C-26), 13.7 ppm (C-27), and 18.2 ppm (C-30) were suggested as primary carbons through Distortionless Enhancement by Polarization Transfer (DEPT) 135 information. Moreover, six methyl carbons, ten methylene carbons, seven methine carbons, and seven quaternary carbons were confirmed with the DEPT experiment (DEPT 90 and DEPT 135). Therefore, Compound 1 was verified as alphitolic acid according to the above data. DQF-COSY (^{1}H-^{1}H), NOESY (^{1}H-^{1}H), and HMBC (^{1}H-^{13}C) correlations of the structure are shown in Figure 1, as well as spectral data of both ^{1}H NMR (600 MHz, methanol-d_4) with their coupling constant and ^{13}C NMR (150 MHz, methanol-d_4) were compared with the literature data in Table 1.

Compound 2 was acquired as white powder, m.p. 338–340°C. The UV spectrum in MeOH appeared at λ_{max} 220 nm. In FTIR spectra (KBr), hydroxyl group (3,371 cm^{-1}), asymmetric and symmetric sp^3 C–H stretching vibration (2,941 cm^{-1}), carbonyl (COOH) group (1,693 cm^{-1}), CH_2 bending (1,462 cm^{-1}) and CH_3 bending (1,388–1,365 cm^{-1}), and C–O stretching (1,049 cm^{-1}) were observed. In the ^{1}H NMR spectrum, four carbinol protons were shown at δ_H: 3.68 ppm (H-2β), δ_H: 3.35 ppm (H-3α), δ_H: 3.27 ppm (H-23), and δ_H: 3.50 ppm (H-23). The H-3α and H-2β were coupled through the DQF-COSY spectrum. The *trans* configuration was confirmed due to the 10.2 Hz coupling constant of H-3α and NOESY correlation of H-2β and H-24β. Thus, H-23 was correlated to C-3 (δ_C: 76.8 ppm) according to the HMBC spectrum. The olefinic protons were shown at δ_H: 5.25 ppm (H-12) and coupled to H-11 (δ_H: 1.95 ppm) in the DQF-COSY spectrum. Moreover, six methyl protons were appeared at δ_H: 0.70 ppm (H-24), 1.03 ppm (H-25), 0.82 ppm (H-26), 1.18 ppm (H-27), 0.91 ppm (H-29), and 0.94 ppm (H-30). These methyl protons have correlations observed on the HMBC spectrum, especially between H-24 and C-23 (δ_C: 65.0 ppm), H-26 and C-8 (δ_C: 39.2 ppm) and C-14 (δ_C: 41.6 ppm), H-27 and C-8 (δ_C: 39.2 ppm), C-14 (δ_C: 41.6 ppm) and C-15 (δ_C: 27.4 ppm), and H-29 and H-30 and C-20 (δ_C: 30.2 ppm). On the ^{13}C NMR spectrum, the three carbinol carbons were observed at δ_C: 68.8 ppm (C-2), 76.8 ppm (C-3), and 65.0 ppm (C-23). The carbons of C-2 and C-3 were identified as methine alcohol (R_2-CH-OH), and C-23 was suggested as methylene alcohol (R-CH_2-OH) according to DEPT (DEPT 135 and DEPT 90) experiments. Chemical shifts at δ_C: 180.7 ppm in the ^{13}C NMR spectrum indicated the presence of the carboxylic acid functional group (C-28), which was

Table 1: NMR spectra of alphitolic acid by comparing to literature

Position	DEPT	Experiment			Literature [21]		
		δ_C (ppm)	δ_H (ppm) {mult, J (Hz)}		δ_C (ppm)	δ_H (ppm) {mult, J (Hz)}	
1	CH_2	46.9	0.82	(1H, m)	46.5	—	—
			2.00	(1H, m)			
2	CH	68.4	3.60	(1H, m)	68.6	3.46	(1H, dd, J = 4.0, 9.5 Hz)
3	CH	83.0	2.89	(1H, d, J = 9.6 Hz)	83.2	2.74	(1H, d, J = 9.5 Hz)
4	C	39.1	—		40.6	—	
5	CH	55.2	0.82	(1H, m)	55.2	—	
6	CH_2	18.1	1.54	(1H, m)	18.2	—	
			1.43	(1H, m)			
7	CH_2	34.1	1.43	(2H, m)	34.1	—	
8	C	40.6	—		39.2	—	
9	CH	50.6	1.39	(1H, m)	50.3	—	
10	C	38.1	—		38.2	—	
11	CH_2	20.8	1.43	(2H, m)	20.9	—	
12	CH_2	25.4	1.73	(1H, m)	25.3	1.52	(1H, m)
			1.08	(1H, m)		1.64	(1H, m)
13	CH	38.2	2.32	(1H, m)	38.1	—	
14	C	42.3	—		40.6	—	
15	CH_2	29.4	1.30	(1H, m)	30.4	—	
			1.15	(1H, m)			
16	CH_2	32.0	0.90	(2H, m)	32.1	—	
17	C	56.1	—		56.1	—	
18	CH	49.1	1.61	(1H, m)	48.1	—	
19	CH	47.1	3.03	(1H, m)	48.9	2.86	(1H, m)
20	C	150.6	—		150.7	—	
21	CH_2	30.3	1.39	(2H, m)	29.5	—	
22	CH_2	36.7	1.91	(1H, m)	37.0	—	
			1.43	(1H, m)			
23	CH_3	27.7	0.99	(3H, s)	28.2	0.81	(3H, s)
24	CH_3	15.8	0.78	(3H, s)	16.4	0.60	(3H, s)
25	CH_3	16.5	0.92	(3H, s)	17.1	0.71	(3H, s)
26	CH_3	15.3	0.97	(3H, s)	15.7	0.80	(3H, s)
27	CH_3	13.7	1.01	(3H, s)	14.5	0.75	(3H, s)
28	COOH	178.7	—		179.3	—	
29	CH_2	108.7	4.60	(1H, s)	109.4	4.42	(1H, s)
			4.71	(1H, s)		4.54	(1H, s)
30	CH_3	18.2	1.70	(3H, s)	19.1	1.51	(3H, s)

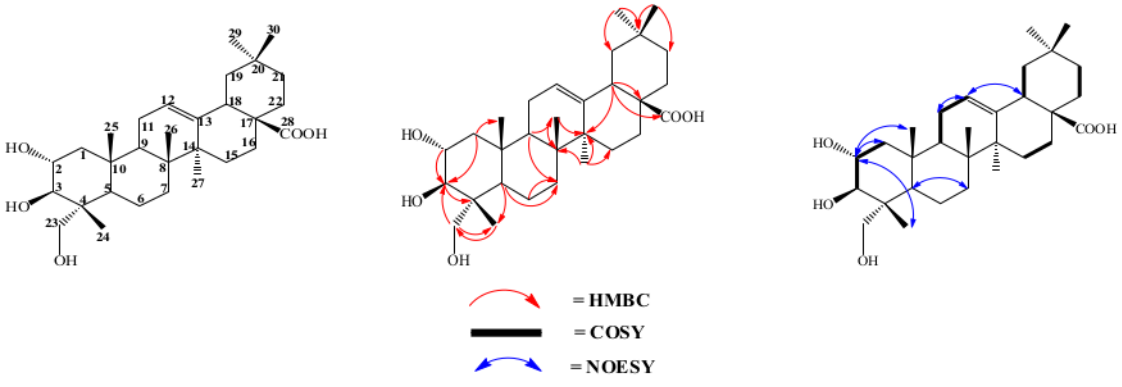

Figure 2: DQF-COSY, NOESY, and HMBC correlations of arjunolic acid from *S. aqueum*.

correlated with H-18 (δ_H: 2.86 ppm) on the HMBC spectrum. The double bond carbons at positions C-12 and C-13 were appeared at δ_C: 121.9 ppm (methine carbon) and δ_C: 144.1 ppm (quaternary carbon). The remaining quaternary carbon signals were shown at δ_C: 42.7 ppm (C-4), 39.2 ppm (C-8), 41.6 ppm (C-14), 46.3 ppm (C-17), and 30.2 ppm (C-20). Carbon signals at δ_C: 12.4 ppm (C-24), 16.2 ppm (C-25), 16.4 ppm (C-26), 25.1 ppm (C-27), 32.1 ppm (C-29), and 22.6 ppm (C-30) were suggested as primary carbons. The presence of eight quaternary carbons, six methine carbons, ten methylene carbons, and six methyl carbons was also confirmed by the DEPT experiment. According to the above data, the structure of Compound 2 was verified as arjunolic acid, and its molecular formula was $C_{30}H_{48}O_5$. DQF-COSY (^1H–^1H), NOESY (^1H–^1H), and HMBC (^1H–^{13}C) correlations of the structure are demonstrated in Figure 2. And then, the spectral data of both ^1H NMR (600 MHz, methanol-d_4) with their coupling constants and ^{13}C NMR (150 MHz, methanol-d_4) are shown in Table 2, completed with the reported literature data.

Table 2: NMR spectra of arjunolic acid by comparing to literature

Position	DEPT	Experiment			Literature [22]		
		δ_C (ppm)	δ_H (ppm) (mult, *J* in Hz)		δ_C (ppm)	δ_H (ppm) (mult, *J* in Hz)	
1	CH_2	46.5	0.89	(1H, m)	47.8	—	
			1.95	(1H, m)			
2	CH	68.8	3.68	(1H, m)	69.6	3.66	(1H, m)
3	CH	76.8	3.35	(1H, d, *J* = 10.2 Hz)	78.1	3.36	(1H, m)
4	C	42.7	—		43.0	—	
5	CH	46.8	1.28	(1H, m)	48.3	—	
6	CH_2	17.7	1.39	(1H, m)	19.1	—	
			1.48	(1H, m)			
7	CH_2	32.0	1.28	(1H, m)	33.8	—	
			1.64	(1H, m)			
8	C	39.2	—		40.7	—	
9	CH	47.6	1.69	(1H, m)	48.1	—	
10	C	37.7	—		38.5	—	
11	CH_2	23.2	1.95	(1H, m)	24.0	—	
			1.24	(1H, m)			
12	CH	121.9	5.25	(1H, m)	123.4	5.23	(1H, m)
13	C	144.1	—		144.3	—	
14	C	41.6	—		42.7	—	
15	CH_2	27.4	1.07	(1H, m)	29.1	—	
			1.78	(1H, m)			
16	CH_2	22.6	1.59	(1H, m)	24.4	—	
			2.24	(1H, m)			
17	C	46.3	—		46.6	—	
18	CH	41.4	2.86	(1H, dd, *J* = 3.9, 13.7 Hz)	43.3	—	
19	CH_2	45.9	1.13	2H (m)	44.1	—	
20	C	30.2	—		31.6	—	
21	CH_2	33.5	1.39	(1H, m)	34.8	—	
			1.21	(1H, m)			
22	CH_2	32.5	1.75	(1H, m)	33.5	—	
			1.54	(1H, m)			
23	CH_2	65.0	3.50	(1H, d, *J* = 11.0 Hz)	66.2	3.50	(1H, s)
			3.27	(1H, d, *J* = 11.0 Hz)		3.31	(1H, s)
24	CH_3	12.4	0.70	(3H, s)	13.9	0.69	(3H, s)
25	CH_3	16.2	1.03	(3H, s)	17.7	0.81	(3H, s)
26	CH_3	16.4	0.82	(3H, s)	17.5	0.91	(3H, s)
27	CH_3	25.1	1.18	(3H, s)	26.8	1.17	(3H, s)
28	COOH	180.7	—		181.8	—	
29	CH_3	32.1	0.91	(3H, s)	25.3	1.03	(3H, s)
30	CH_3	22.6	0.94	(3H, s)	24.6	0.94	(3H, s)

Table 3: Anticancer activity against HeLa, T47D, and A549 cell lines

Compound name	MTT assay (IC_{50} ± SD µg/mL)		XTT assay (IC_{50} ± SD µg/mL)
	HeLa	T47D	A549
Alphitolic acid	16.12 ± 0.681	7.37 ± 0.388	84.41 ± 0.048
Arjunolic acid	6.74 ± 0.015	27.51 ± 0.036	168.22 ± 0.090
Doxorubicin	2.67 ± 0.25	0.035 ± 0.012	NT

The meaning of NT is not test, SD = standard deviation.

3.2 Anticancer activity

Isolated compounds in this study were evaluated their anticancer activity against the human cancer cells as HeLa (cervical cancer), T47D (breast cancer), and A549 (lung cancer) cell lines using MTT and XTT assays, which are the colorimetric measurement of cell viability. In the present anticancer activity test, doxorubicin was used as the standard. The pure compound is deemed highly toxic if the half-maximal inhibitory concentration (IC_{50}) is ≤4 µg/mL and toxic if the IC_{50} is ≤20 µg/mL. The compound is moderately toxic if the IC_{50} is 20–100 g/mL and if IC_{50} above 100 g/mL is recommended as nontoxic [23,24].

Based on anticancer activity data obtained, alphitolic acid was suggested as a toxic compound against HeLa and T47D cell lines, with IC_{50} being 16.12 µg/mL and 7.37 µg/mL, respectively. However, its activity against the A549 cell line was presented as moderate inhibition (IC_{50} = 84.41 µg/mL). Moreover, arjunolic acid with an IC_{50} of 6.74 µg/mL was considered a toxic compound against HeLa cell lines. Arjunolic acid was a moderate compound against the T47D cancer cell line with an IC_{50} of 27.15 µg/mL, although it was a nontoxic compound against the A549 cancer cell line. According to the data mentioned earlier, the two types of triterpenoids inhibited higher toxicity against HeLa and T47D cell lines than the A549 cell line, and their activities may improve to obtain the natural origin drug from this species. The IC_{50} values of the two compounds are listed in Table 3.

Many terpene compounds have interesting bioactivities, so it is necessary to carry out other activity tests such as agar-well diffusion methods to evaluate the minimum inhibitory concentrations, minimum bactericidal concentration, IC_{50}, and zone of inhibitions that could determine the potential antimicrobial and antifungal efficacy. The significant antioxidant potential could be evaluated through 1,1-diphenyl-2-picryl-hydrazyl and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The inhibiting of the α-glucosidase represents an antidiabetic activity that also could be visualized through molecular docking simulations. The effective anti-inflammatory agent could be determined via the carrageenan-induced assay, while an appreciable analgesic activity could be observed through the acetic acid-induced writhing bioassay [25].

4 Conclusion

This study reports the success of isolating two bioactive triterpenoid compounds from stem bark of *S. aqueum*, an Indonesian medicinal plant, namely alphitolic acid and arjunolic acid. These two isolated triterpenoids were significantly toxic against cervical cancer and breast cancer, shown by small IC_{50} values. Based on this result, we recommended continuing exploring the potential of this plant so that it can be used as a source of secondary metabolite compounds with interesting bioactivity.

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