Research Article

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Bioactive triterpenoids from Indonesian medicinal plant *Syzygium aqueum*

https://doi.org/10.1515/chem-2022-0138
received December 23, 2021; accepted February 15, 2022

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 anti-cancer 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.

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 chromosome 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-sulfo-phenyl)-2H-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 were 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 F254 (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 F254 (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 (MeOH, 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 CO2 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 \(\times\) 10^4 cell/well density and were incubated at 37°C in a CO2 incubator for 24 h. The cells were then treated with 100 \(\mu\)L of the prepared sample with different concentration series (1.5625–100 \(\mu\)g/mL) at 37°C in a CO2 incubator for 24 h. As much as 100 \(\mu\)L 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 CO2 incubator for 2–4 h until purple formazan crystals are formed. After the crystal formation, cells were observed with a microscope, and 100 \(\mu\)L 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 [19,20].

2.5.3 XTT assay

The anticancer test was carried out using XTT assay. Cells were seeded (10^5–10^5 cells/well) into 96 well plates and were incubated at 37°C in a CO2 incubator for 24 h. As much as 100 \(\mu\)L of the prepared sample with various concentrations (1.5625–100 \(\mu\)g/mL) was inserted to treat cancer cells. The plate was incubated at 37°C in a CO2 incubator for 24 h. And then, PMS solution (10 mM PMS solution and PBS (3 mg PMS + 1 mL PBS)) was prepared and 10 \(\mu\)L 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 \(\mu\)L of the XTT reagent (XTT/PMS solution) was filled in each well. After this, it was incubated for 2–4 h in a CO2 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 \(S.\ aquaeum\) (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, [\(\lambda_{max}\)] 245–29, 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 \(C_{30}H_{49}O_{4}–H⁻\), \(m/z\) 471.3474). The UV spectrum in MeOH was displayed at \(\lambda_{max}\) 182 nm. In the FTIR spectrum (KBr), hydroxyl group (3,423 cm⁻¹), symmetric and asymmetric sp³ C–H stretching (2,941–1,870 cm⁻¹), carbonyl acid group (1,699 cm⁻¹), CH₂ bending (1,456 cm⁻¹), and CH₃ bending (1,381 cm⁻¹) were recorded. The proton signals, both \(\delta_{HH}\) 3.60 ppm (H–β) and \(\delta_{HH}\) 2.89 ppm (H–α), 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–α. Nuclear Overhauser Effect Spectroscopy (NOESY) cross-peaks of H–α 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 (\(\delta_{H}\) 0.99 ppm) and C–24 (\(\delta_{C}\) 15.8 ppm), H–24 (\(\delta_{H}\) 0.78 ppm) and H–25 (\(\delta_{H}\) 0.92 ppm) and C–5 (\(\delta_{C}\) 55.2 ppm), H–26 (\(\delta_{H}\) 0.97 ppm) and C–7 (\(\delta_{C}\) 1.43 ppm), and H–27 (\(\delta_{H}\) 1.01 ppm)
and C-14 (δC: 42.3 ppm). Two vinylic protons (H-29) were observed at δH: 4.60 ppm and δH: 4.71 ppm. Their positions were confirmed by HMBC between these two H-29 protons with C-19 (δC: 47.1 ppm) and C-30 (δC: 18.2 ppm). Based on HSQC experiments, two carbinol methine carbons (R2-CH-OH) gave correlations H-2β-C-2 (δC: 68.4 ppm) and H-3α-C-3 (δC: 83.0 ppm). A vinlylic group was appeared at δC: 108.7 ppm (C-29) and δC: 150.6 ppm (C-20) in the 13C 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 (=CH2), which were connected by two protons [δH: 4.60 ppm (H-29) and δH: 4.71 ppm (H-29)] through the HSQC spectrum. Chemical shift of the carbonyl group appeared at δC: 178.7 ppm (C-28) in the 13C NMR spectrum. In the HMBC spectrum, C-28 showed correlations with H-18 (δH: 1.61 ppm) and H-22 (δH: 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 (δC: 39.1 ppm), H-27 to C-8 (δC: 40.6 ppm), H-1 to C-10 (δC: 38.1 ppm), H-15 to C-14 (δC: 42.3 ppm), and H-16 to C-17 (δC: 56.1 ppm). The remaining peaks at δC: 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 (1H-1H), NOESY (1H-1H), and HMBC (1H-13C) correlations of the structure are shown in Figure 1, as well as spectral data of both 1H NMR (600 MHz, methanol-d4) with their coupling constant and 13C NMR (150 MHz, methanol-d4) 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 λmax 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), CH2 bending (1,462 cm⁻¹) and CH3 bending (1,388–1,365 cm⁻¹), and C–O stretching (1,049 cm⁻¹) were observed. In the 1H NMR spectrum, four carbinol protons were shown at δH: 3.68 ppm (H-2β), δH: 3.35 ppm (H-3α), δH: 3.27 ppm (H-23), and δH: 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-β and H-2β. Thus, H-23 was correlated to C-3 (δC: 76.8 ppm) according to the HMBC spectrum. The olefinic protons were shown at δH: 5.25 ppm (H-12) and coupled to H-11 (δH: 1.95 ppm) in the DQF-COSY spectrum. Moreover, six methyl protons were appeared at δH: 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 (δC: 65.0 ppm), H-26 and C-8 (δC: 39.2 ppm) and C-14 (δC: 41.6 ppm), H-27 and C-8 (δC: 39.2 ppm), C-14 (δC: 41.6 ppm) and C-15 (δC: 27.4 ppm), and H-29 and H-30 and C-20 (δC: 30.2 ppm). On the 13C NMR spectrum, the three carbinol carbons were observed at δC: 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 (R2-CH-OH), and C-23 was suggested as methylene alcohol (R-CH2-OH) according to DEPT (DEPT 135 and DEPT 90) experiments. Chemical shifts at δC: 180.7 ppm in the 13C 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] |
|----------|------|------------|----------------|
|          |      | $\delta_C$ (ppm) | $\delta_H$ (ppm) (mult, $J$ (Hz)) | $\delta_C$ (ppm) | $\delta_H$ (ppm) (mult, $J$ (Hz)) |
| 1        | CH$_2$ | 46.9 | 0.82 (1H, m) | 46.5 | $-/-$ |
| 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$_3$ | 18.1 | 1.54 (1H, m) | 18.2 | $-/-$ |
| 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*. 
The presence of eight quaternary carbons, correlated with H-18 (δH: 2.86 ppm) on the HMBC spectrum. The double bond carbons at positions C-12 and C-13 were appeared at δC: 121.9 ppm (methine carbon) and δC: 144.1 ppm (quaternary carbon). The remaining quaternary carbon signals were shown at δC: 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 δC: 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 C30H48O5. D Q F

\[ \text{COSY (} \^\text{H}-^\text{H}\text{), NOESY (} \^\text{H}-^\text{H}\text{), and HMBC (} \^\text{H}-^\text{C}\text{) correlations of the structure are demonstrated in Figure 2.} \]

And then, the spectral data of both 1H NMR (600 MHz, methanol-\( d_4 \)) with their coupling constants and 13C 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] |
|----------|------|------------|-----------------|
|          | δC (ppm) | δH (ppm) (mult, J in Hz) | δC (ppm) | δH (ppm) (mult, J in Hz) |
| 1        | CH₂   | 46.5 | 0.89 (1H, m) | 47.8 | — |
| 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        | CH₂   | 17.7 | 1.39 (1H, m) | 19.1 | — |
| 7        | CH₂   | 32.0 | 1.28 (1H, m) | 33.8 | — |
| 8        | C     | 39.2 | — | 40.7 | — |
| 9        | CH    | 47.6 | 1.69 (1H, m) | 48.1 | — |
| 10       | C     | 37.7 | — | 38.5 | — |
| 11       | CH₂   | 23.2 | 1.95 (1H, m) | 24.0 | — |
| 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       | CH₂   | 27.4 | 1.07 (1H, m) | 29.1 | — |
| 16       | CH₂   | 22.6 | 1.59 (1H, m) | 24.4 | — |
| 17       | C     | 46.3 | — | 46.6 | — |
| 18       | CH    | 41.4 | 2.86 (1H, dd, J = 3.9, 13.7 Hz) | 43.3 | — |
| 19       | CH₂   | 45.9 | 1.13 (2H, m) | 44.1 | — |
| 20       | C     | 30.2 | — | 31.6 | — |
| 21       | CH₂   | 33.5 | 1.39 (1H, m) | 34.8 | — |
| 22       | CH₂   | 32.5 | 1.75 (1H, m) | 33.5 | — |
| 23       | CH₂   | 65.0 | 3.50 (1H, d, J = 11.0 Hz) | 66.2 | 3.50 (1H, s) |
| 24       | CH₂   | 12.4 | 0.70 (3H, s) | 13.9 | 0.69 (3H, s) |
| 25       | CH₂   | 16.2 | 1.03 (3H, s) | 17.7 | 0.81 (3H, s) |
| 26       | CH₂   | 16.4 | 0.82 (3H, s) | 17.5 | 0.91 (3H, s) |
| 27       | CH₂   | 25.1 | 1.18 (3H, s) | 26.8 | 1.17 (3H, s) |
| 28       | COOH  | 180.7 | — | 181.8 | — |
| 29       | CH₂   | 32.1 | 0.91 (3H, s) | 25.3 | 1.03 (3H, s) |
| 30       | CH₂   | 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 (IC50 ± 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 (IC50) is ≤4 µg/mL and toxic if the IC50 is ≤20 µg/mL. The compound is moderately toxic if the IC50 is 20–100 g/mL and if IC50 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 IC50 being 16.12 µg/mL and 7.37 µg/mL, respectively. However, its activity against the A549 cell line was presented as moderate inhibition (IC50 = 84.41 µg/mL). Moreover, arjunolic acid with an IC50 of 6.74 µg/mL was considered a toxic compound against HeLa cell line. Arjunolic acid was a moderate compound against the T47D cancer cell line with an IC50 of 27.51 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 IC50 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, IC90, 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 IC50 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.

Acknowledgments: All authors express big thanks to the Indonesian Ministry of Education and Culture for the Research Grant (Penelitian Dasar, 2021), to the Faculty of Pharmacy, Meijo University, Tempaku, Nagoya, Japan, for NMR and MS data, to the Faculty of Pharmacy, Gajah Mada University, Indonesia, and the Faculty of Science, UPM, Malaysia for an anticancer test.

Funding information: This work was supported by the Indonesian Ministry of Education and Culture for the Research Grant (Penelitian Dasar, 2021).

Author contributions: Alfinda Novi Kristanti: conceptualization, formal analysis, funding acquisition, investigation, resources, supervision, and writing – review and editing; Ei Ei Aung: data curation, formal analysis, investigation, project administration, validation, visualization, and writing – original draft; Nanik Siti Aminah: conceptualization, formal analysis, funding acquisition, methodology, resources, and supervision; Yoshiaki Takaya: data curation, resources, software, validation, and visualization; Hnin Thanda Aung: data curation, formal analysis, methodology, and supervision; and Rico Ramadhan: data curation, formal analysis, software, and validation.

Conflict of interest: Authors state no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.
Data availability statement: All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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