Two Flavonoid Compounds as Antiproliferative Activity Against SP-C1 Cancer Tongue Cells from the Leaves of Rasamala (*Altingia excelsa* Nornha)

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Abstract

Two flavonoid compounds, kaempferol (**1**) and quercetin (**2**) have been isolated from the leaves of Rasamala (*Altingia excelsa* Nornha). The chemical structure of compounds **1** and **2** were identified by spectroscopic evidences including, UV, IR, 1D-NMR, 2D-NMR and MS as well as by comparing with previously reported spectral data.These compounds were isolated from this plant for the first time. Compounds **1** and **2** were evaluated for their antiproliferative activity against SP-C1 cancer tongue cells and showed IC_{50} values of 2.50 and 2.31 μM, respectively.

Keywords: *Altingia excelsa*, SP-C1 cancer tongue cells, kaempferol, quercetin.

1. INTRODUCTION

Oral squamous cell cancer (OSCC) has high morbidity and mortality rates across the world because it is frequently found in advanced stages before therapy (Chen *et al*., 2008; Chen *et al*., 2013). The major environmental risk factors responsible for the development of cancer cell include betel nut chewing, cigarette smoking, alcohol consumption, and exposure to high-risk human papillomavirus. This cancer cell is a difficult disease to treat because of multidisciplinary and diverse treatment strategies and the varied natural behavior of the cancer. Local invasion and frequent regional lymph node metastases together with relative resistance to chemotherapeutic. The conventional strategies of cancer cell management still depend on surgery, radiotherapy, chemotherapy and targeted therapy (Shah and Gil 2009). The poor outcome of chemotherapy to oral squamous cell cancer contributes to the poor prognosis for this diseases (Scully and Bagan 2009). Therefore, novel, effective therapy for oral squamous cell cancer treatment is still needed. Due to this high incidence, the identification of novel compounds that inhibit cancer development has become a crucial objective for scientists. Hundreds of chemicals that have been and are being evaluated for their anti-cancer activities, natural products derived from medicinal plants rank among the most promising (Tan *et al*., 2011). During the course of our continuing search for novel anticancer compounds from Indonesian plants, the ethanol extract of the leaves of *Altingia*

excelsa Nornha exhibited significant antiproliferative activity against SP-C1 cancer tongue cells. *A. excelsa* Nornha is known as Rasamala in Indonesia is higher plant. The plant is used in folklore and traditional medicine for the treatment of stomachache and coughs (Kanjijal *et al*., 2003; Pramono and Djam'an, 2002). Previous phytochemical study of this plant reported the presence of sesquiterpenoid from the leaves (Kanjijal *et al*., 2003), but antiproliferative compounds not yet reported. In this paper, we report the isolation and structure elucidation of two flavonoid compounds (**1** and **2**) as antiproliferative activity against SP-C1 cancer tongue cells.

2. MATERIAL AND METHODS
General Experimental Procedure

UV spectra were measured by using a TECAN Infinite M200 pro, with MeOH. The IR spectra were recorded on a SHIMADZU IR Prestige-21 in KBr. The mass spectra were recorded with a Waters Xevo QTOF MS. NMR data were recorded on JEOL JNM A-500 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. Column chromatography was conducted on silica gel 60. TLC plates were precoated with silica gel GF$_{254}$ (Merck, 0.25 mm) and detection was achieved by spraying with 10% H$_2$SO$_4$ in EtOH, followed by heating and under UV light at wavelenght at 254 and 367 nm.

Plant Material

The leaves of *A. excelsa* Nornha were collected in Wayang Windu mountain, Pangalengan, West Java Province, Indonesia in August 2015. The plant was identified by the staff of the Laboratory of Taxonomy, Department of Biology, Universitas Padjadjaran and a voucher specimen (No. 256/HB/06/2015) was deposited at the herbarium.

Plant Extraction

Dried ground leaves (2.5 kg) of *A. excelsa* Nornha were extracted with methanol exhaustively (15 L) at room temperature for 5 days. The combined methanol extracts were then concentrated in vacuo at 40 ^{o}C to yield 254.5 g of residue. The residue was suspended in water and then partitioned, in turn, with *n*-hexane, EtOAc, and *n*-BuOH. Evaporation

resulted in the crude extracts of *n*-hexane (20.90 g), EtOAc (35.18 g), and *n*-butanol (128.50 g), respectively. The EtOAc soluble fraction (30.0 g) was fractionated by column chromatography on silica gel using a gradient *n*-hexane, EtOAc and MeOH to give eleven fractions (A–K). Fraction D (1.85 g) was subjected to column chromatography over silica gel using a gradient mixture of *n*-hexane-acetone (10:0-1:1) as eluting solvents to afford eight subfractions (D1-D8). Subfraction D5 (380 mg) was separated on a column of silica gel, eluted with CHCl$_3$:MeOH (9:1), to give six subfractions (D5.1–D.5.6). Subfraction D5.3 (124.5 mg) was separated on preparative TLC on silica gel GF$_{254}$ eluted with CHCl$_3$:MeOH (9.5:0.5) to give **1** (28.5 mg). Subfraction D.5.4 (92.6 mg) was chromatographed on a column chromatography of silica gel, eluted with CHCl$_3$:MeOH (9.75:0.25), to give **2** (32.4 mg).

Cell culture and treatment

The SP-C1 human tongue cancer cell line used in this study were cultured in RPMI-1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin). For cell treatments, various concentrations of the sample were added to the cell culture medium. After 24 h, the cells were released from treatment, the medium was replaced, and cells were subsequently collected at the indicated times (Prayitno *et al*., 2013).

3. RESULT AND DISCUSSION

The methanol extract from the dried leaves of *A. excelsa* Nornha were concentrated and extracted successively with *n*-hexane, ethyl acetate and *n*-butanol. The ethyl acetate extracts showed strongest antiproliferative activity against SP-C1 cancer tongue cells. By using antiproliferative activity assay to guide separations, the ethyl acetate fraction was separated by combination of column chromatography on silica gel G60 and preparative TLC on silica gel GF$_{254}$ to afford two flavonoid compounds **1** and **2**.

Kaempferol (1) – Yellow amorphous powder; UV (MeOH): λ_{max} (log ε) 272 (4.0), 364 (3.7) nm; IR (KBr) ν_{max} cm^{-1}: 3420, 1690, 1605;^{1}H-NMR (CD$_3$OD, 500 MHz): δ_H7.15 (2H, d, *J*=6.80 Hz, H-2′ and H-6′), 7.01 (2H, d,
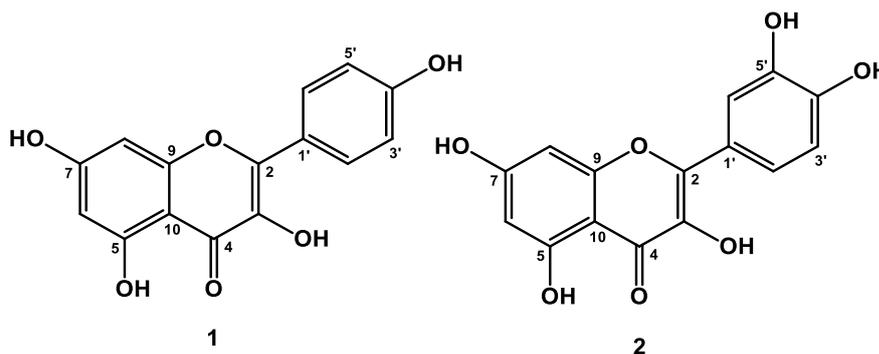
J=6.80 Hz, H-3′ and H-5′), 6.52 (1H, d, J=1.95 Hz, H-8), 6.28 (1H, d, J=1.95 Hz, H-6); ^{13}C-NMR (CD$_3$OD, 125 MHz): δ_C 176.6 (C-4), 164.9 (C-7), 162.3 (C-5), 160.1 (C-4′), 157.7 (C-2), 146.9 (C-9), 136.6 (C-3), 130.4 (C-2′), 123.3 (C-1′), 116.3 (C-3′), 104.1 (C-10), 99.2 (C-6), 99.1 (C-6′), 94.5 (C-5′), 94.4 (C-8); LC-MS (*m/z* 286).

Quercetin (2) – Yellow amorphous powder; UV (MeOH): λ_{max} (log ε) 274 (3.8), 360 (3.6) nm; IR (KBr) ν_{max} cm^{-1}: 3430, 1680, 1610; ^{1}H-NMR (CD$_3$OD, 500 MHz): δ_H7.73 (1H, d, J=2.5 Hz, H-2′), 7.62 (1H, dd, J=8.5, 2.5 Hz, H-6′), 6.87 (1H, d, J=8.5 Hz, H-5′), 6.38 (1H, d, J=2.5 Hz, H-8), 6.17 (1H, d, J=2.5 Hz, H-6); ^{13}C-NMR (CD$_3$OD, 125 MHz): δ_C176.6 (C-4), 165.0 (C-7), 162.4 (C-5), 157.8 (C-2), 148.4 (C-9), 147.0 (C-3′), 145.8 (C-4′), 136.8 (C-3), 124.5 (C-6′), 121.5 (C-1′), 116.2 (C-2′), 116.0 (C-5′), 104.2 (C-10), 99.2 (C-6), 94.5 (C-8); LC-MS spectral data (*m/z* 302).

Compound **1** was obtained as yellow amorphous powder. The molecular formula was established to be $C_{15}H_{10}O_6$ from its LC-MS spectral data (*m/z* 286) and NMR spectra, thus requiring eleven degrees of unsaturations. The UV spectrum of **1** showed λ_{max} at 272 and 364 nm and exhibited a bathochromic shift by added NaOH and AlCl$_3$ suggested the presence of flavonoid structure with 4′ and 5-hydroxyl groups. The IR spectrum of **1** showed the absorption band correspond to hydroxyl (3420 cm^{-1}), carbonyl (1690 cm^{-1}) and double bond (1605 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** showed the presence of *meta*-coupled of aromatic protons at δ_H 6.28 (1H, d, J=1.95 Hz) and 6.52 (1H, d, J=1.95 Hz) corresponds to H-6 and H-8, respectively. The ^{1}H-NMR spectrum of **1** also showed the presence of two doublet signals at δ_H 7.15 (2H, d, J=6.80 Hz, H-2′ and H-6′) and 7.01 (2H, d, J=6.80 Hz, H-3′ and H-5′) corresponds to four aromatic

protons in ring B, characteristics for the 1′,4′-disubstituted flavone. A total fifteen carbon signals were observed in the ^{13}C-NMR spectrum. These were assigned by DEPT experiments to fourteen sp^2 carbons and a carbonyl signal at δ_C 176.6. The degree of unsaturation was accounted for eight out of the total eleven double bond equivalents. The remaining three degree of unsaturation were consistent to flavonol structure (Kim *et al.*, 2016; Aisyah *et al.*, 2017). A comparison of the NMR data of **1** with those of kaempferol (Castenada *et al.*, 2016; Aisyah *et al.*, 2017), revealed that the structures of the two compounds are very similar, therefore, compound **1** was identified as kaempferol, which shown in this plant for the first time.

Compound **2** was obtained as a yellow amorphous powder. The LC-MS of **1** gave a ion peak at *m/z* 300, compatible with the molecular formula $C_{15}H_{10}O_7$. Its UV absorptions in MeOH were consistent with the presence of a 3, 5, 7, 3′, 4′-pentahydroxyflavone structure (Kim *et al.*, 2016; Aisyah *et al.*, 2017). The ^{1}H- and ^{13}C-NMR spectra of **1** exhibited resonances due to aromatic systems. The ^{13}C-NMR signals of **1** were assigned with the help of a DEPT experiment. In the ^{1}H-NMR spectrum of **1**, the aromatic region exhibited an ABX system at δ_H 7.73 (1H, d, J=2.0 Hz, H-2′), 7.62 (1H, dd, J=2.0, 7.5 Hz, H-6′), and 6.87 (1H, d, J=8.0 Hz, H-5′) due to a 3′, 4′ disubstitution of ring B and a typical *meta*-coupled pattern for H-6 and H-8 protons (δ_C 6.17 and 6.37, d, J=2.5 Hz). The ^{13}C-NMR spectrum of **1** showed the presence of 15 aromatic carbon signals. Based on the NMR data and comparison of the data given in the literature previously, the structure of compound **2** was identified as quercetin (Huang *et al.*, 2013), which shown in this plant for the first time.

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The effect of kaempferol and quercetine on the viability of SP-C1 cells was evaluated according to the methodology described in previous papers (Prayitno *et al*., 2013). The treatment of cancer SP-C1 cell lines with kaempferol and quercetine resulted in a dose-dependent inhibition of cell growth, as demonstrated by the MTT assay. Twenty-four hours of treatment with kaempferol and quercetine inhibited the proliferation of SP-C1cells with an IC_{50} value of 2.50 and 2.31 µM, indicating that both compounds are potential for further application in cancer treatment.

4. CONCLUSIONS

Two known flavonoid componds kaempferol (**1**) and quercetin (**2**) have been isolated from the leaves of *Altingia excelsa* Nornha. Quercetin showed stronger antiproliferative activity against SP-C1 cancer tongue cells, suggested the presence of additional hydroxyl group in flavonoid structure can increase antiproliferative activity.

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