BUKTI KORESPONDENSI

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

1. Bukti mengirimkan dan balasan pengiriman artikel dari jurnal — 27 Februari 2018
2. Bukti penerimaan artikel oleh editor — 13 Maret 2018
3. Permintaan revisi artikel sesuai arahan reviewer — 27 Maret 2018
4. Pernyataan revisi diterima sesuai arahan dan artikel "accepted" dan akan di publish pada volume 4 no 2 bulan November — 2 April 2018
5. Pernyataan letter of Accepted — 1 Mei 2018

1. Pernyataan sudah menerima kiriman artikel oleh jurnal

41 of many

Fwd: [JKV] Submission Acknowledgement Inbox ×

Unang Supratman <unang.supratman@unpad.ac.id> — Tue, Feb 27, 2018, 5:15 PM

to me

---------- Forwarded message ----------
From: **Drs. Dede Sukandar, M.Si** <sukandarkimia@uinjkt.ac.id>
Date: Tue, Feb 27, 2018 at 3:41 PM
Subject: [JKV] Submission Acknowledgement
To: "Prof. Dr. Unang Supratman" <u_supratman@unpad.ac.id>

Prof. Dr. Unang Supratman:

Thank you for submitting the manuscript, "Flavonoid Compounds from the Leaves of Rasamala (Altingia excelsa Nornha) and their Antiproliferative Activity Against SP-C1 Cancer Tongue Cells" to Jurnal Kimia VALENSI. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL:
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Username: unpad_unang

2. Pernyataan artikel memenuhi ketentuan jurnal

42 of many

Fwd: [JKV] Editor Decision

Unang Supratman <unang.supratman@unpad.ac.id> Tue, Mar 13, 2018, 11:53 AM

to me

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From: **Adawiah, S.Si** <adawiah@uinjkt.ac.id>
Date: Tue, Mar 13, 2018 at 11:42 AM
Subject: [JKV] Editor Decision
To: "Prof. Dr. Unang Supratman" <u_supratman@unpad.ac.id>

Prof. Dr. Unang Supratman:

We have reached a decision regarding your submission to Jurnal Kimia VALENSI, "Flavonoid Compounds from the Leaves of Rasamala (Altingia excelsa Nornha) and their Antiproliferative Activity Against SP-C1 Cancer Tongue Cells".

Our decision is to:

Adawiah, S.Si
Program Studi Kimia
Fakultas sains dan teknologi
Universitas Islam Negeri Syarif Hidayatullah Jakarta

3. Permintaan revisi artikel sesuai arahan reviewer

40 of many

Unang Supratman <unang.supratman@unpad.ac.id> Tue, Mar 27, 2018, 1:37 PM

to me

tolong segera perbaiki.

salam
unang
---------- Forwarded message ----------
From: **Adawiah, S.Si** <adawiah@uinjkt.ac.id>
Date: Tue, Mar 27, 2018 at 10:27 AM
Subject: [JKV] Editor Decision
To: "Prof. Dr. Unang Supratman" <u_supratman@unpad.ac.id>

Prof. Dr. Unang Supratman:

We have reached a decision regarding your submission to Jurnal Kimia VALENSI, "Flavonoid Compounds from the Leaves of Rasamala (Altingia excelsa Nornha) and their Antiproliferative Activity Against SP-C1 Cancer Tongue Cells".

Our decision is to:

Adawiah, S.Si
Program Studi Kimia
Fakultas sains dan teknologi
Universitas Islam Negeri Syarif Hidayatullah Jakarta

4. Pernyataan revisi diterima sesuai arahan dan artikel “accepted” dan akan di publish pada volume 4 no 2 bulan november

39 of many

Fwd: [JKV] Two Flavonoid Compounds as Antiproliferative Activity Against SP-C1 Cancer Tongue Cells from the Leaves of Rasamala (Altingia excelsa Nornha) Inbox ×

Unang Supratman <unang.supratman@unpad.ac.id> Mon, Apr 2, 2018, 1:41 PM
to me

---------- Forwarded message ---------
From: Adawiah, S.Si <adawiah@uinjkt.ac.id>
Date: Fri, Mar 30, 2018, 10:18 AM
Subject: [JKV] Two Flavonoid Compounds as Antiproliferative Activity Against SP-C1 Cancer Tongue Cells from the Leaves of Rasamala (Altingia excelsa Nornha)
To: Unang Supratman <u_supratman@unpad.ac.id>

Yth. Prof. Dr. Unang Supratman
Chemisty Department, Padjajaran University

based on the confirmation with reviewers, we decided that your article could be published in the jurnal Kimia VALENSI for the next issue (Volume 4 No. 2 November 2018). we will send the accepted letter along with proofreading.
Thank you

salam
adawiah
Jurnal Kimia Valensi

5. Pernyataan letter of Accepted

38 of many

Accepted letter Inbox ×

tarso rudiana <tarso.rudiana@gmail.com> Tue, May 1, 2018, 3:50 PM
to me

Dear Risyandi Anwar

Here we attached the accepted letter of your manuscript to Jurnal Kimia VALENSI.

We also attached your edited manuscript, so you can check before it is published.

Thank you for choosing us as your partner to share your knowledge.

Sincerely,

Editor Jurnal Kimia Valensi

website: http://journal.uinjkt.ac.id/index.php/valensi/
e-mail: kimia@uinjkt.ac.id
Alternative email: tarso.rudiana@uinjkt.ac.id

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Flavonoid Compounds from Rasamala (*Altingia excelsa* Nornha) Leaves and Their Antiproliferative Activity Against SP-C1 Cancer Tongue Cells

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

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 tested for their antiproliferative activity against SP-C1 cancer tongue cells and showed IC_{50} values of 2.50 and 2.31 □□, 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 OSCC include betel nut chewing, cigarette smoking, alcohol consumption, and exposure to high-risk human papillomavirus. OSCC 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 OSCC management still depend on surgery, radiotherapy, chemotherapy and targeted therapy (Shah and Gil 2009). The poor outcome of chemotherapy to OSCC contributes to the poor prognosis for OSCC (Scully and Bagan 2009). Therefore, novel, effective therapy for OSCC 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 dan Djam'an, 2002). Previous phytochemical study of this plant reported the presence of sesquiterpenoid from the leaves (Kanjijal *et al*., 2003). In this

paper, we report the isolation and structure elucidation of two flavonoid compounds (**1** and **2**) along with their antiproliferative activity against SP-C1 cancer tongue cells.
Note: OSCC sangat banyak dalam paragraf ini, agar dicarikan kata ganti yang sesuai, menghidari sesuatau yang berulangulang

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 ECZ-600 spectrometer at 600 MHz for ^1H and 125 MHz for ^{13}C, chemical shifts are given on a □ (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_2SO_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 was deposited at the herbarium. Cantumkan specimen number

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 ^oC 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

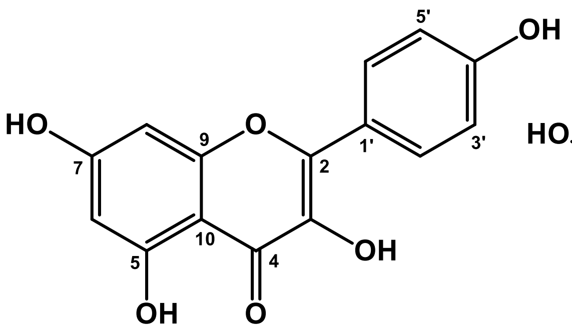
The SP-C1 human tongue cancer cell line used in this study were cultured in RPMI-1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). For cell treatments, various concentrations of the sample were added to the 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

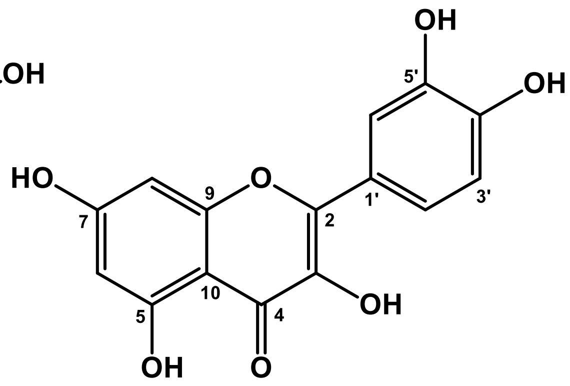
The methanol extract from the dried leaves of *A. excelsa* Nornha were concentrated and extracted successively with *n*-hexane, ethyl acetate and *n*-butanol. The ethyl acetate extracts showed strongest antiproliferative activity against SP-C1 cancer tongue cells.. By using antiproliferative activity assay to guide separations, the ethyl acetate fraction was separated by combination of column chromatography on silica gelG60 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, 1260, 720 *apa cukup signifikan sebagai karaktersitik suatu senyawa* ??;^{1}H-NMR (CD_3OD, 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_3OD, 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, 1250; ^{1}H-NMR (CD_3OD, 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_3OD, 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).



1



2

~~Figure 1. Chemical Structures of Compounds 1 and 2~~ *"tidak perlu structures sebagai Gambar, cukup nomor senyawa saja"*

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.

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.

ACKNOWLEDGEMENTS

This investigation was financially supported by Lembaga Pengelola Dana Pendidikan (LPDP) and Directorate General of Higher Education, Ministry of Science, Technology and Higher Education, Indonesia (2015 ~~by Risyandi Anwar~~). *Tidak ada salahnya jika ucapan terima ksih disampaikan juga ke institusi dan operator/petugas yang mengukur data NMR, bioaktivitas*

REFERENCE

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NOTE *Jika peneliti atau kelompok peneliti dari penulis, telah menghasilkan publikasi di Journal/prosidin seminar perlu juga jadi rujukan agar dpt meningkatkan jumlah citasinya*.

FORMULIR PEMERIKSAAN NASKAH

JURNAL KIMIA VALENSI

Terakreditasi Kemenristek Dikti No. 48/E/KPT/2017

Prodi Kimia FST UIN Syarif Hidayatullah Jakarta

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

SUBSTANSI NASKAH
JUDUL : 1. Apakah Judul telah secara jelas mencerminkan isi naskah? (~~Ya~~/tidak) Komentar : Penempatan " **from the Leaves of Rasamala (*Altingia excelsa* Nornha)**, lebih baik di bagian akhir dari judul
ABSTRAK : 2. Apakah abstrak telah secara jelas dan padat meringkaskan isi naskah? (Ya/~~tidak~~) Komentar : cukup ………………………………………………………………………………………………………..
ISI NASKAH : 3. Apakah *state of the art* telah dinyatakan secara jelas? (Ya/~~Tidak~~) Komentar Cukup perlu perbaikan kecil seperti terdapat dalam naskah : ……….. 4. Apakah maksud dan ruang lingkup dari naskah diuraikan dengan jelas? (Ya/~~Tidak~~) Komentar Sudh dielaborasi sebatas data yang tersedia. : 5. Apakah argumentasi dari penulis cukup jelas dan tersusun dengan baik dan benar? (Ya/~~tidak)~~ Komentar ; baik : ……………………….. 6. Apakah maksud dan tujuan penulis dalam penulisan naskah ini benar-benar tercapai? (Ya/t~~idak~~)

Komentar : Sudah…………………….. 7. Apakah isi uraian/pernyataan dalam naskah cukup jelas dan ada keterkaitan dengan sumber referensi yang diacu? (Ya/~~tidak~~) Komentar : sudah ……………………….. 8. Apakah keterangan gambar/table telah dinyatakan dengan jelas dan telah diacu dengan baik dalam badan tulisan ? (Ya/~~tidak~~) Komentar : Figure 1 dihilangkan saja, kaena cukup diberi no senyawa saja. Struktur senyawa bukan gambar ……………………..
PERSPEKTIF KARYA ILMIAH : 9. Apakah *Novelty* penelitian ini telah dinyatakan/dideklarasikan secara jelas? (Ya/tidak) Komentar : Novelty belum terlihat …………………….. 10. Apakah gambar/grafik yang disajikan cukup representative dan efektif sesuai dengan isi dan argumentasi penelitian? (Ya/tidak) Komentar : cukup ……………………………..
KESIMPULAN : 11. Apakah kesimpulan telah dinyatakan dengan jelas dan sistematis? (ya/~~tidak~~) Komentar : cukup jelas …………………….. 12. Apakah ada korelasi yang signifikan antara latar belakang, metode kerja dan kesimpulan yang dihasilkan? (Ya/~~tidak~~) Komentar : sudah ada ……………………………..
DAFTAR PUSTAKA :

~~13.~~ Apakah semua pustaka yang diacu di dalam naskah sudah dianggap cukup layak dan *up to date*? (Ya/~~tidak)~~

Komentar :Kelihatannya penulis atau kelompok penelitian penulis belumpernah melakukan publikasi di Journal atau di prosiding

………………………..

) Mohon naskah hasil review dilampirkan dengan Track Changes (softcopy/MsWord) atau hard copy naskah.

Reviewer (Mitra bestari),

REKAPITULASI HASIL PEMERIKSAAN NASKAH

<table>
<tr><td colspan="4">JURNAL KIMIA VALENSI</td></tr>
<tr><td>JUDUL NASKAH</td><td colspan="3">Two Flavonoid Compounds as Antiproliferative Activity Against SP-C1 Cancer Tongue Cells from the Leaves of Rasamala (Altingia excelsa Nornha) ???????</td></tr>
<tr><td>PENULIS</td><td colspan="3"></td></tr>
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<tr><td colspan="4">HASIL PEMERIKSAAN NASKAH OLEH REVIEWER</td></tr>
<tr><td></td><td>Ya</td><td>Tidak</td><td>Lihat Komentar</td></tr>
<tr><td>Apakah Naskah ini dapat diterbitkan tanpa perbaikan?</td><td>v</td><td></td><td>v</td></tr>
<tr><td>Apabila penulis bersedia mengubah naskah sesuai dengan anjuran yang diberikan, apakah naskah ini dapat diterima?</td><td>v</td><td></td><td></td></tr>
<tr><td>Apakah anda merekomendasikan bahwa beberapa aspek dari makalah ini memerlukan review tambahan? Bila ya, berikan keterangan yang lebih jelas.</td><td></td><td>v</td><td></td></tr>
<tr><td>NAMA PEMERIKSA (REVIEWER)</td><td colspan="3"></td></tr>
<tr><td>TANGGAL PEMERIKSAAAN</td><td colspan="3">25 Maret 2018</td></tr>
<tr><td>TANDA TANGAN RIVIEWER</td><td colspan="3">KOMENTAR DARI REVIEWER :

Perbaikan setelah dilakukan cukup diputuskan oleh tim editor untuk diterbitkan</td></tr>
</table>

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

Risyandi Anwar[1], Arlette Setiawan[2], Supriatno[3], Unang Supratman[4,5]

[1]Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Muhammadiyah, Semarang 50273, Central Java, Indonesia.
[2]Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Padjadjaran, Jatinangor 45363, West Java, Indonesia.
[3]Department of Oral Medicine, Faculty of Dentistry, Universitas Gadjah Mada, Jogjakarta 50125, Indonesia.
[4]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, West Java, Indonesia.
[5]Central Laboratory, Universitas Padjadjaran, Jatinangor 45363, West Java, Indonesia.

E-mail: unang.supratman@unpad.ac.id

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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.

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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*

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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_2SO_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

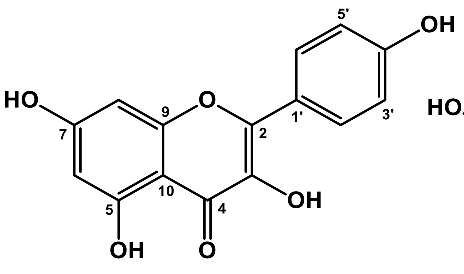
The methanol extract from the dried leaves of *A. excelsa* Nornha were concentrated and extracted successively with *n*-hexane, ethyl acetate and *n*-butanol. The ethyl acetate extracts showed strongest antiproliferative activity against SP-C1 cancer tongue cells. By using antiproliferative activity 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_3OD, 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_3OD, 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).

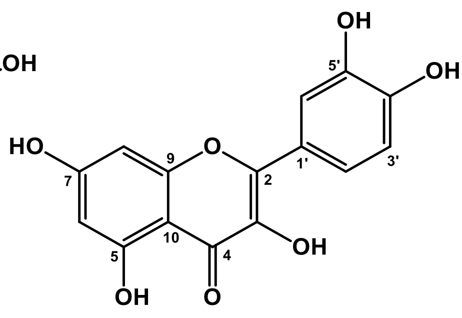
Quercetin (2) – Yellow amorphous powder; UV (MeOH): λ_{max} (log ε) 274 (3.8), 360 (3.6) nm; IR (KBr) ν_{max} cm^{-1}: 3430, 1680, 1610; ^{1}H-NMR (CD_3OD, 500 MHz): δ_H7.73 (1H, d, *J*=2.5 Hz, H-2′), 7.62 (1H, dd, *J*=8.5, 2.5 Hz, H-6′), 6.87 (1H, d, *J*=8.5 Hz, H-5′), 6.38 (1H, d, *J*=2.5 Hz, H-8), 6.17 (1H, d, *J*=2.5 Hz, H-6); ^{13}C-NMR (CD_3OD, 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.



1



2

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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