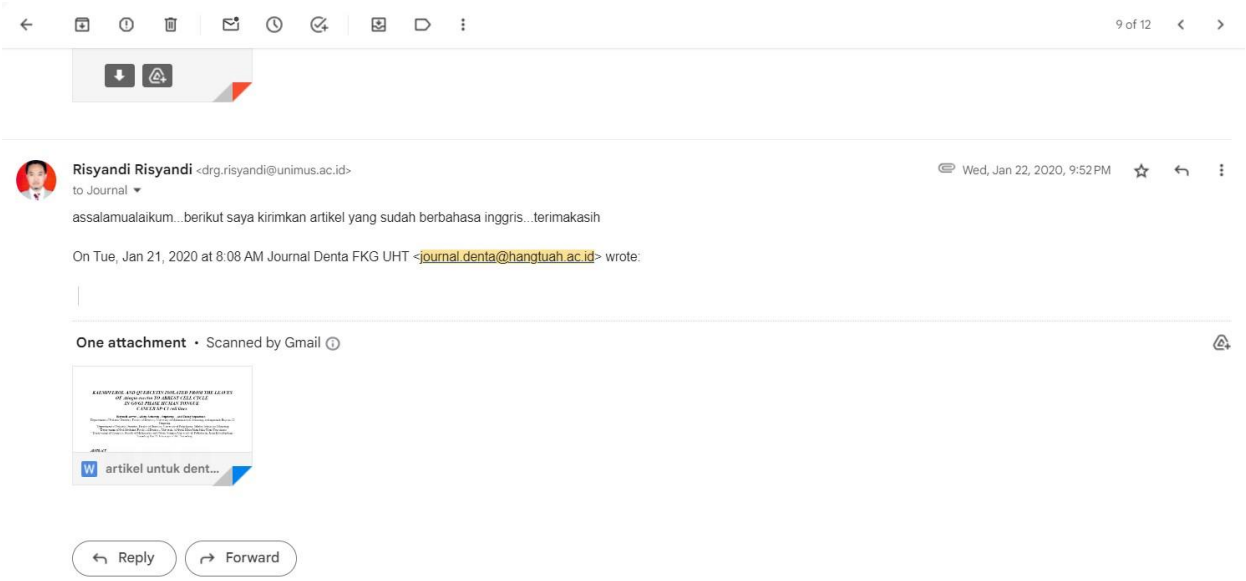
BUKTI KORESPONDENSI

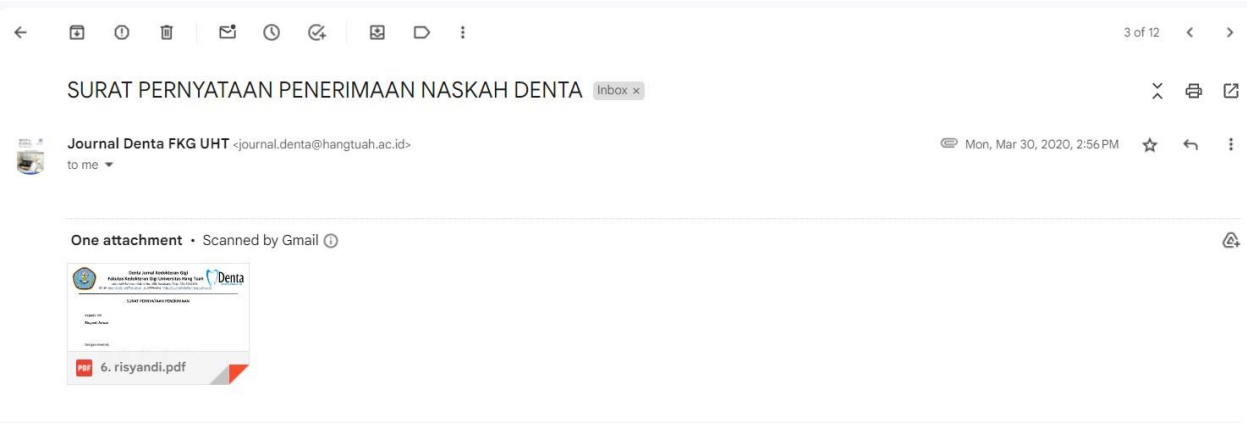
Kaempferol And Quercetin Isolated From The Leaves Of Atingia Excelsa To Arrest Cell Cycle In G0/G1 Phase Human Tongue Cancer Sp-C1 Cell Lines

1.	Bukti pengiriman artikel ke jurnal	22 januari 2020
2.	Balasan dari jurnal bahwa artikel sudah diterima oleh jurnal	30 Maret 2020
3.	Permintaan revisi sesuai arahan reviewer	24 April 2020
4.	Permintaan perbaikan hasil cek plagiasi	22 juli 2020
5.	Pengiriman naskah hasil perbaikan	5 Agustus 2020

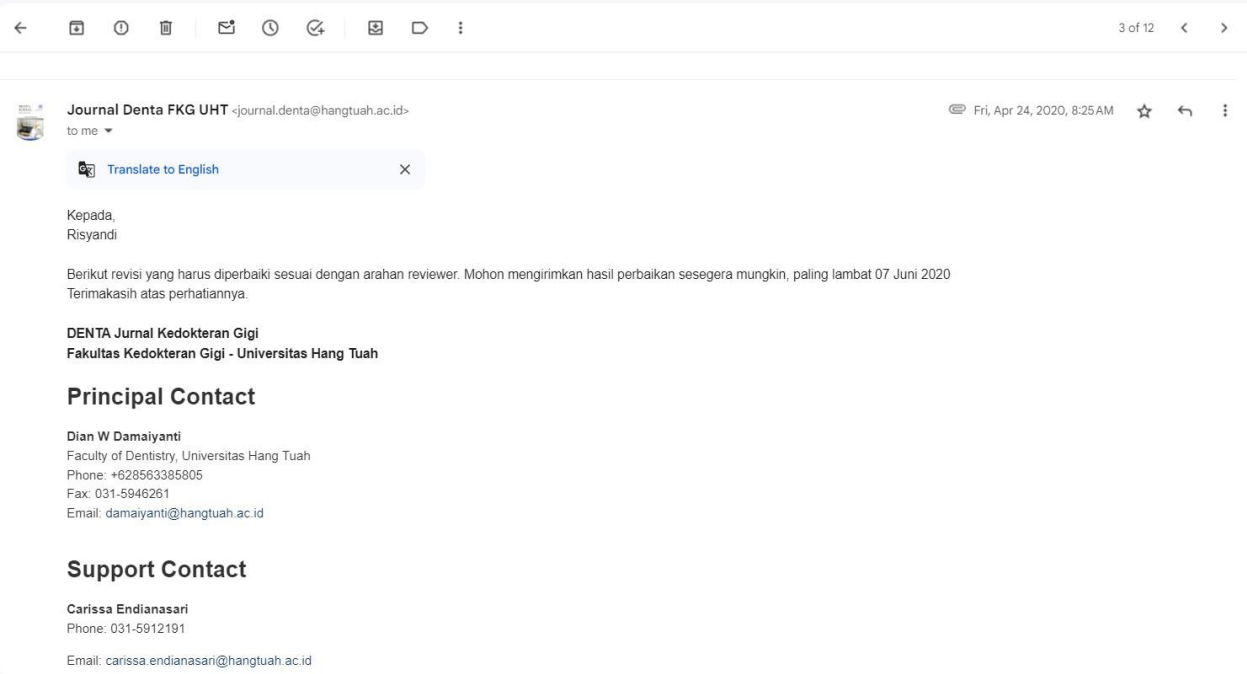
1. Bukti pengiriman nahkah atau artikel ke jurnal



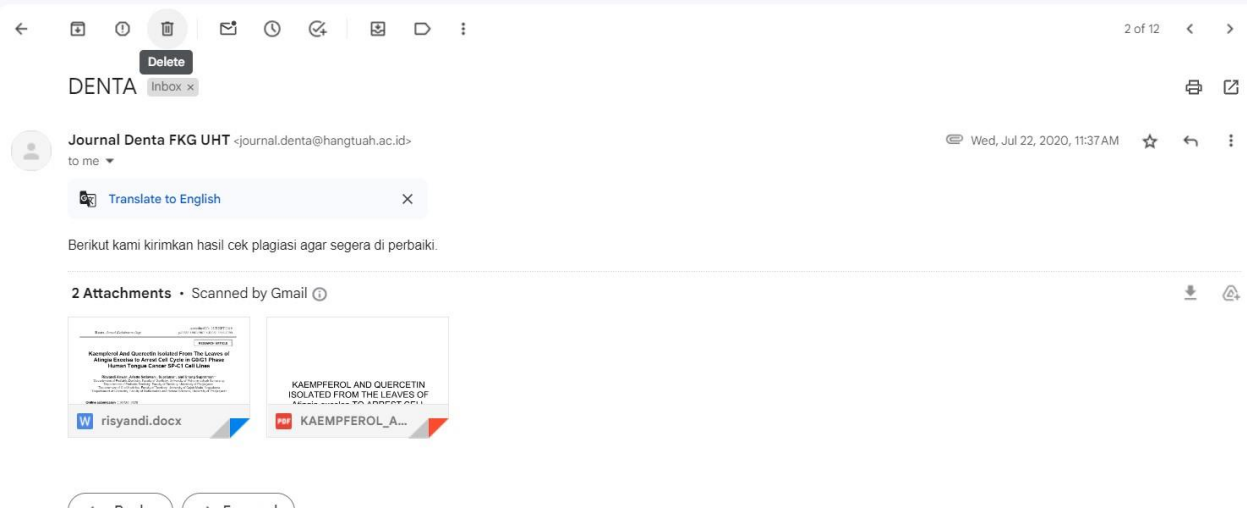
2. Surat pernyataan penerimaan naskah oleh jurnal



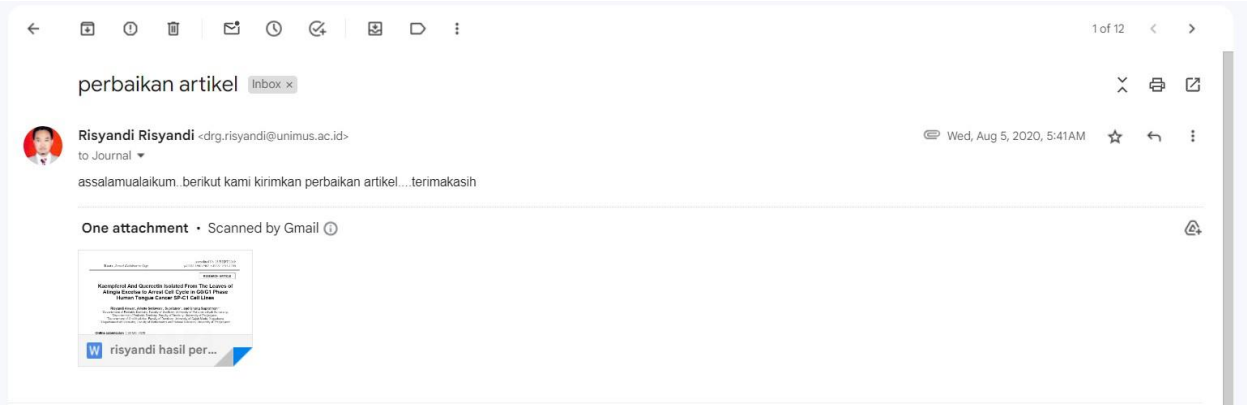
3. Permintaan naskah harus direvisi sesuai permintaan reviewer



4. Permintaan perbaikan naskah hasil cek plagiasi



5. Pengiriman naskah hasil perbaikan



KAEMPFEROL AND QUERCETIN ISOLATED FROM THE LEAVES OF Atingia excelsa TO ARREST CELL CYCLE IN G0/G1 PHASE HUMAN TONGUE CANCER SP-C1 cell lines

ABSTRACT

The leaves of Altingia excelsa were found to strongly inhibit SP-C1 human tongue cancer cell lines. This study was focused on identifying the antiproliferative compound found in A. excelsa leaves and assessing its mechanism of action. The active compound was isolated using column chromatography and identified by the spectroscopic method and was also tested for its anti-proliferative properties and the cell cycle ~~analisis~~ analysis in SP-C1 cells by flowcytometry analysis. This work resulted in the isolation of a flavonoid, which was identified to be kaempferol and ~~quercetine~~ quercetin. The compounds inhibited SP-C1 cell proliferation in a time- and dose-dependent manner with IC_{50} values of 0.72 µg/mL and 0. 70 µg/mL for the 24 hours treatments, respectively. Furthermore, the flowcytometry analysis suggested that the compounds exerted its anticancer activities by ~~inhibited~~ inhibiting cell cycle. These results suggested that compounds found in A. excelsa provides a basis for its potential use in cancer disease management.

***Keywords*:** Altingia excelsa, cancer, cell cycle, kaempferol, ~~quercetine~~ quercetin

INTRODUCTION

More than 95% of the carcinomas of the oral cavity are of squamous cell type, in nature. They constitute a major health problem in developing countries, representing a leading cause of death. The survival index continues to be small (50%), as compared to the progress in diagnosis and treatment of other malignant tumors. According to World Health Organization, carcinoma of the oral cavity in males in developing countries, is the sixth commonest cancer after lung, ~~prostrate~~prostate, colorectal, stomach and bladder cancer, while in females, it is the tenth commonest site of cancer after breast, colorectal, lung, stomach, uterus, cervix, ovary, bladder and liver.[1] Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of this anatomic site, and in approximately 80% of cases, it is associated with extrinsic factors such as the use of tobacco, alcohol or both.[2] OSCC is a very 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.[3] The poor outcome of chemotherapy to OSCC contributes to the poor prognosis for OSCC.[4] 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. Of the 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.[5] ~~In an effort~~ To identify novel agents that may inhibit cancer development, we have focused our investigations on discovering bioactive compounds from plants.[6]

In our previous study, we found that the leaves of the Hammamelidaceae family, demonstrated anti-tumor properties.[7,8] These preliminary studies suggest that *A. excelsa* may be

further developed as a source of anti-cancer agents. Thus, in this study, we investigated and characterized the inhibited cell cycle activities of *A. excelsa* leaf extracts.

Commented [WU1]: Alasan pemilihan konsentrasi

MATERIALS AND METHODS

Plant materials. A. Excelsa leaves were collected from Wayang Windu Mountain, Pangalengan, West Java, Indonesia. The plant species ~~was~~ were identified by the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Indonesia.

Extraction and isolation. The dried leaves of *A. excelsa* (2.5 kg) were extracted with methanol (3x24 h) at room temperature. The solvent was subsequently evaporated under reduced pressure at 50˚C to yield a concentrated extract. The methanol extract (280 g) was fractionated *n*-hexane and water to obtain an *n*-hexane extract (86 g) and a water layer. The water layer was then extracted with ethyl acetate to obtain an ethyl acetate fraction (120 g) and a water fraction (90 g). The cytotoxicity of the fractions was assessed on SP-C1 tongue cancer cells using the methyl thiazolyl tetrazolium (MTT) assay. The ethyl acetate fraction, which was the most active fraction, was chromatographed on Wakogel C-200 (Wako Pure Chemical, Japan) with a mixture of *n*-hexane, ethyl acetate and methanol with increased polarity. The major compounds were then isolated and purified using silica G-60 with sulfuric acid-ethanol (1:9) and were identified by spectroscopic methods including ~~ultra violet~~ ultraviolet and infrared spectrometry (UV-IR), and nuclear magnetic resonance (NMR).

Commented [WU2]: Reference method?

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.

Commented [WU3]: Reference?

Cell cycle analysis using flow cytometry. The cells were grown in 24-well plates at 37 ºC under 5% CO2 until 80% confluence ~~were~~ was reached. The medium was subsequently changed, and the flavonoids were added to the indicated concentrations. Next, the cells were incubated at 37ºC for 48 h. After incubation, the cells were harvested and washed three times with ice-cold PBS (pH 7.4). The supernatant was removed, and the cells were washed with 1 mL of PBS and centrifuged at 4 °C. Finally, the supernatant was removed, and 200 µL of 70% ice-cold ethanol and 200µL of PBS was added to the cells and stored at –20 ºC until further use. For use in the flow cytometry experiments, the cell pellet was washed two more times with PBS. The cell pellet was suspended in 0.5 mL of staining reagent (50µg/mL PI, 50 U/mL RNase, 0.1 mM EDTA,0.1% Triton X-100, and PBS) and incubated for 30 min at 37 ºC in the dark. The DNA fluorescence was measured using a Becton Dickinson (Franklin Lakes, USA) FACScanto II flow cytometer with an excitation wavelength of 488 nm and emission wavelength of 585 nm. Pulse width area signals were used to discriminate between G2 cells and cell doublets. The data were analyzed using FACSDiva Software (Beckton Dickinson). The relative distribution of 10^4 events for each sample was analysed for background aggregates and debris, an indicatorof apoptosis and the G0/G1-, S-, and G2/M-phases of the cell cycle. The control treatments consisted of a culture medium supplemented with FBS. Serum-deprivation treatment was used as an inducer of G0/G1 cell cycle arrest.

Commented [WU4]: Reference?

RESULTS

Ethyl acetate fraction of *A. excelsa* leaves inhibits SP-C1 cell proliferation. Treatment MTT assay with the *A. excelsa* extract methanol was found to inhibit the proliferation of SP-C1 human tongue cancer cells (IC_{50} 75.41 µg/mL) (Fig. 1). The methanol extract was then fractionated based

on polarity, using *n*-hexane, ethyl acetate and water. The fractions were then individually applied to SP-C1 cells and were found to inhibit cell proliferation with an IC_{50} value of 44.85 µg/mL for the *n*-hexane fraction, 12.85 µg/mL for the ethyl acetate fraction and 18.02 µg/mL for the water fraction. Due to its low IC_{50} value, we then explored the ethyl acetate fraction for its anti-cancer potential.

Commented [WU5]: Tabel data mean + SD?

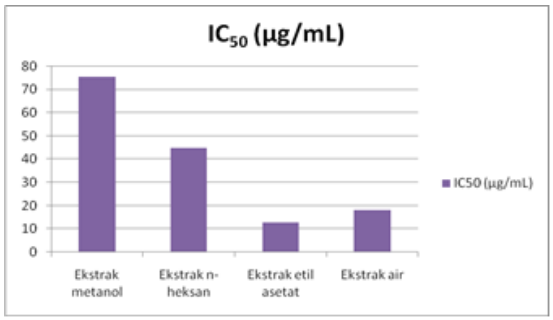
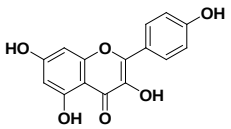


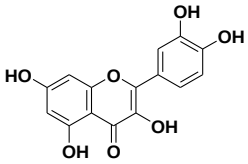
Figure 1. IC_{50} value some extracts *A. excelsa* Nornha

Kaempferol and quercetin are major compounds of the ethyl acetate fraction. The principle active compounds of the ethyl acetate fraction of *A. excelsa* extract were isolated and purified. The compound exhibited a melting point of 152.7-153˚C and a molecular ion peak at *m/z* 432 in the LC-MS spectrum. Based on hydrogen and carbon content, the molecular ion peak and ^{1}H and ^{13}C NMR profiles indicated that the compound had a molecular formula of $C_{15}H_{10}O_7$. The UV spectrum produced maximal absorbance peaks at λ_{max} 265 and 342 nm, which were characteristic of a flavonoid with a flavone skeleton. The addition of NaOH produced a bathochromic shift in the absorption bands, indicating the presence of hydroxyl groups in the skeleton, one of which was attached to C-5, as indicated by a further bathochromic shift following the addition of H_3BO_3. Absorption bands at 1,675 and 3,197 nm of the IR spectrum indicated where the molecule harbors conjugated carbonyl and hydroxyl groups, respectively.

The ^{1}H NMR spectrum of the compound showed two aromatic hydrogen signals with 'meta coupling' at δ 6.35 (1H, *d*, *J* = 2.2 Hz) and 6.18 (1H, *d*, *J*=2.2 Hz), which was predicted by the hydrogens at C-6 and C-8 of the A ring of the flavone skeleton. Accordingly, this compound was suggested to have a hydroxyl group at C-5 and C-7. Furthermore, its 1H NMR spectrum revealed two signals with 'ortho coupling' at δ 6.92 (2H, *d*, *J* = 6.7 Hz) and 7.74 (2H, *d*, *J* = 6.7 Hz), the signals of which were approximated from the hydrogens at C-2', C-3', C-5' and C-6' of the B ring. The absence of a specific signal for an olefinic hydrogen at C-3 and the presence of an anomeric hydrogen signal at δ 5.37 (1H, *d*, *J* = 7.2 Hz) suggested that the compound was a flavonol glycoside. The appearance of an anomeric carbon signal at δ 94.9 in the ^{13}C NMR spectrum indicated the presence of a sugar moiety. Due to a correlation between the anomeric hydrogen signal (δ 5.37) and the anomeric carbon signal (δ 94.9) that was revealed by analysis of the HMBC spectral data, the position of the sugar moiety was assigned to the C-3 hydroxyl group, The methyl signal.



Chemical structure of Kaempferol



Chemical structure of Quercetin

Kaempferol and quercetin inhibits SP-C1cell proliferation in a dose-dependent manner. The effects of kaempferol and quercetin on the viability of SP-C1 cells were evaluated. The treatment of cancer SP-C1 cell lines with kaempferol and quercetin resulted in a dose-dependent inhibition of cell growth, as demonstrated by the MTT assay. Twenty-four hours of treatment with kaempferol and q~~uercetine~~ quercetin inhibited the proliferation of SP-C1 cells with IC_{50} values of 0.72 and 0.70 µg/mL, respectively. Subsequent cell cycle analysis, based investigation applying the IC_{50} dose of 0.39, 0.78, and 1.56 µg/mL kaempferol and quercetin was performed on SP-C1 cells.

Kaempferol and quercetin inhibited the SP-C1 cell cycle in the G0/G1 phase. To determinate the percentage of SP-C1 cells present in different phases of the cell cycles G0/G1, S, and G2/M, the cells were first synchronized with serum deprivation ~~prior to~~ before the treatment with or without quercetin and kaempferol at various concentrations (C, K 0.39, K 0.78, K 1.56, Q 0.39, Q 0.78, and Q 1.56 µg/mL) for 24 h period, serum-deprived cells were progressively accumulated in the G0/G1-phase, ~~where as~~ whereas serum-supplied cells were present in the S- and G2/M-phases (Figure 2). Treatment with K (all concentrations) and Q (all concentrations) significantly increased the percentage of cells in the G0/G1-phase and decrease in S and G2/M-phase as compared with the control. Notably, the percentage of cells in the G0/G1-phase increased ~~a~~ an apropiate increase concentration compared to the control after K treatment. Percentage of cells in the G0/G1 – phase increase compared to the control after Q treatment but the percentage of cell decrease a long increase in concentration.

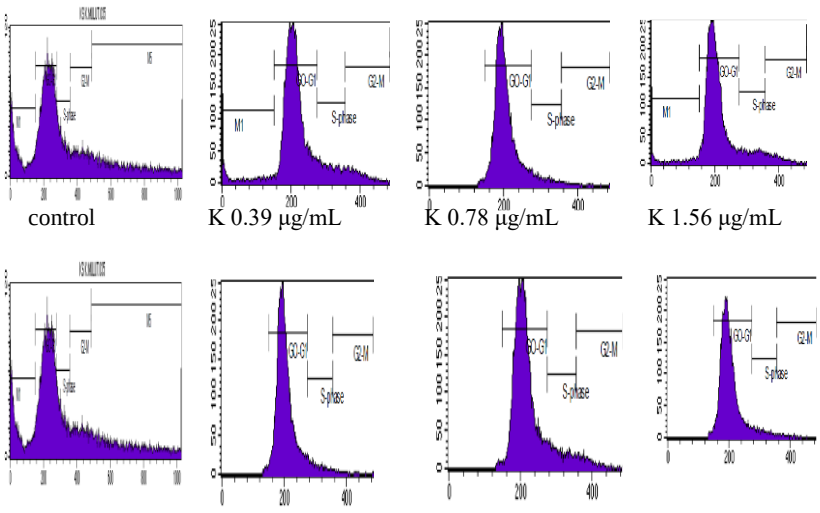


Figure 2. Flowcytometry analysis SP-C1 cancer cell using treated some concentration of kaemperol and quercetin.

DISCUSSION

The ^{1}H- and ^{13}C-NMR spectra of compounds from *A. Excelsa* exhibited resonances due to aromatic systems. The ^{1}H-NMR spectrum of them showed the presence of two doublet signals corresponds to four aromatic protons in ring B, characteristics for the 1′,4′-disubstituted flavone. The ^{13}C-NMR signals of them were assigned with the help of a DEPT experiment. A total of fifteen carbon signals were observed in the ^{13}C-NMR spectrum. The degree of unsaturation was accounted for eight out of the total eleven double bond equivalents. The ^{13}C-NMR spectrum of them showed the presence of 15 aromatic carbon signals. So, from ^{1}H and ^{13}C NMR profiles indicated that the compound had a molecular formula of $C_{15}H_{10}O_7$.

Traditional medicinal plants traditionally have long been regarded as a source of potential therapeutic agents, and the search for new drugs or leads are usually based on that approach.[9,10] In drug discovery, we have recently applied a new approach ~~of~~ to selecting plants based on the Hammamelidaceae family.[7,8] In our previous study, we found that the extracts of the *A. excelsa* leaves were strongly cytotoxic to the SP-C1 Human tongue cancer cell lines. Thus, these extracts had the potential for further investigation. The present study was focused on identifying an anti-proliferative compound from the *A. excelsa* leaves. This work resulted in the isolation of two flavonoids, kaempferol and quercetin, which strongly inhibited the SP-C1 cell lines proliferation in a time- and dose-dependent manner. This compound has not been reported before in connection with its cytotoxicity in these cancer cell lines

In this study, kaempferol and quercetin with some concentration to arrest G0/G1 phase cell cycle. Cell growth is an ~~addition~~ additional amount of cells from the cell cycle process. The cell cycle consists of 4 phases, that is G_1, S, G_2, and M. G_1 (Gap 1) is a phase when the cell will synthesis the DNA or go out from cell cycle reversibly or irreversibly to differentiation. The cell that in the G_1 phase will easily controlling cell ~~cyle~~ cycle at a point, that is in the restriction point (R) that will determine the cell to come in back to the cell cycle, go out from cell cycle entering G_0 phase or differentiating.[11]

When the cell cycle phase flow in restriction point dan enter S phase controlled by cyclin-dependent kinase (Cdks) and D, E, and A cyclin. D cyclin function as a growth factor that the expression more depend on the extracellular signal than the cell position on the cycle.[12] When the cell entering the G_0 cycle, on or more of the D cyclin (D1, D2 and D3) were inducted as ~~a part~~ apart from the first response of a growth factor stimulation, protein synthesis and ~~fomer~~ former complex with catalytic subunit (Cdk4, Cdk6) depend on the mitogenic stimulation.[13] In reverse, if the mitogenic substance were removed, so the D Cyclin synthesis will stop. D cyclin is an unstable protein and the enzymatic activity quickly disappear, so that the cell quickly go out from the cell cycle. A specific inhibitor of Cdk4 and Cdk6 known as Ink4 can directly stop the activity of D cyclin/cdk 4/6 and causing G_1 phase arrest (rest of G_1 phase).[13]

Cell cycle inhibition of tongue cancer (SP-C1 after kaempferol and ~~kuersetin~~ quercetin intervention as the results of Rasmala leaves isolation. There is a system that controlled the cell cycle. The controlling system known as a checkpoint. There are 4 checkpoints that found on cycle cell, that is G_1, S, G_2, and M. Checkpoint in a cell cycle ~~onvolving~~ involving protein cyclin groups and cyclin-dependent kinase (cdk).[14] Protein groups that involved on the cell cycle checkpoint determine cell cycle to stop, DNA repair, or apoptosis if the DNA repair doesn't occur.[15] Kaempferol (4) can inhibit cell cycle on G_0-G_1 phase at 0.39 µg/mL, 0,78 µg/mL and 1,56 µg/mL concentration. This cell cycle inhibition suspected because of kaempferol (4)/~~kuersetin~~ quercetin (5) that can inhibit the work of c-myc. We know that the function from c-myc transcription factor is increasing D cyclin and E cyclin, so that there are increasing activity of G1-CDK (D1-CDK cyclin) and G1/S-CDK (E-CDK6 cyclin). Thus, the ~~kuersetin~~ quercetin in this research can inhibit tongue cancer SP-C1 cell cycle on G_0-G_1 phase at 0,39 µg/mL, 0,78 µg/mL dan 1,56 µg/mL concentration. This results ~~analogue~~analog with the research who is conducted by Mocanu *et al*, in 2013 years that shows ~~kuersetin~~ quercetin can inhibit Epidermoid Cancer Cell line A-431 cell cycle at 5 and 10 µM concentration in 24 hours on G_0/G_1 phase and inhibit Mamary SK-BR-3 cell cycle at 50 and 75 µM concentration in 24 hours on G_0-G_1 phase. Research belonging to Chen *et al*, in 2011 years shows

that ~~kuersetin~~ quercetin can inhibit oral cancer OSCC SCC-25 cell cycle at 50 and 75 µM concentration in 12 hours incubation on the G_1 phase. Cell cycle inhibition which is conducted by ~~kuersetin~~ quercetin on G_0-G_1 phase possibly because ~~kuersetin~~ quercetin will synergize with cyclin-dependent kinase inhibitor (CDK1) p21 that that function as inhibitor D-CDK4/6 cyclin activity.[16] With inhibition of D-CDK4/6 cyclin activity so the cell will be obstructed to enter S phase, so that the cell will stop on the G_0/G_1 phase or p21 beside binding cdk and PCNA (proliferating cell nuclear antigen), that is a DNA polymerase α subunit, which needed for replication or DNA reparation. P21 will inhibit cell cycle on the G_1 phase as a result of cell-contact inhibition and because of TGF β.[17]

CONCLUSION

In conclusion, our results suggest that kaempferol and quercetine inhibited the growth of SP-C1 cells through inhibited cell cycle in the G0/G1 phase.

REFERENCES

Commented [WU6]: Gaya penulisan belum sesuai dan Masih ada referensi yg lebih dari 10 tahun

1. Mehrotra R, and Yadav S. Oral squamous cell carcinoma: Etiology, pathogenesis and prognostic value of genomic alterations. *Indian journal of cancer*. 2006; 43(2).
2. Juliana HG, Scheidt LS, Yurgel KC, Maria AZ, Figueiredo FGS. Characteristics of oral squamous cell carcinoma in users or non users of tobacco and alcohol. *Rev Odonto Cience*. 2012; 27(1): p. 69-73.
3. Shah JP, and Gil Z. Current concepts in management of oral cancer– surgery. *Oral Oncol*. 2009; 45: p. 394–401.
4. Scully C, and Bagan J. Oral squamous cell carcinoma overview. *Oral Oncol*. 2009; 45: p. 301–308.
5. Tan W, Lu J, Huang M, Li Y, Chen M, Wu G, Gong J, Zhong Z, Xu Z, Dang Y, Guo J, Chen X, and Wang Y. Anti-cancer natural products isolated from chinese medicinal herbs. *Chinese Medicine*. 2011; 6: p. 27-37.
6. Dewick PM. *Medicinal Natural Product a Biosyntesis Approach*. John Willey and Sons. London; 2009.
7. Kim HH, Yi HS, Hwan MO, Hyuk KH, Ra KS, and Lee MW. Anti oxidative and anti-proliferative activity on Human Prostate Cancer Lines of the phenolic compounds from *Corylopsis coreana* Uyeki. *Molecules*. 2013; 18: p. 4876-4888.
8. Yang YN, Chen JH, Zhou GS., and Tan YP. Pentacyclic triterpenes from the resin of *Liquidambar formosana*. *Fitoterapia*. 2011; 82: p. 927-931.
9. Nguyen TT, Tran E, Nguyen TH, Do PT, Huynh TH, Huynh H. The role of avtivated MEK-ERK pathway in quercetin-induced growth inhibition and apoptosis in A549 lung cancer cells. Oxford University Press : *Carcinogenesis* . 2004; 25 (5): p. 647-659.
10. Zhang D, Liu J, Mi X, Liang Y, Li J, and Huang C. The N-terminal region of p27 inhibits HIF-1α protein translation in ribosomal protein s6-dependent manner by regulating PHLPP-ras-ERK-p90RSK axis. *Macmillan Publisher Limited: Cell Death and Disease*. 2014: 5: p.1535.
11. Liu. J. D, Wang. Y. J, Yu. C. F, Chen. L. C, Lin. J. K, Liang. Y. C, Lin. S. Y, and Ho.Y. S. 2013. Molecular mechanisms of G_0/G_1 cell-cycle arrest and apoptosis induced by terfenadine in human cancer cells. *Molecular carcinogenesis*.8 (3).
12. Denkert. C, Furstenberg. A , Daniel. P. T , Koch. I , Kobel. M , Weichert. W , Siegert. A, and Hauptmann.S. 2013. Induction of G0/G1 cell cycle arrest in ovarian carcinoma cells by the anti-inflammatory drug NS-398,but not by COX-2-specific RNA interference. *Oncogene* . 22, 8653–8661.

13. Xiao. L. S, Yun. S. Z, Xue. F. W , Wen. J. Z , Zheng. W, Fei. Z, Yi. J. Z, Jian. H. L , Jia. W. M , Yun. P. H, Lei. C , Huai. P. L, Yuan. Y. Y, Ying. B. L and Jun. G. 2017. Casticin induces apoptosis and G0/G1 cell cycle arrest in gallbladder cancer cells. *Cancer Cell Int* 17:9.

14. Marcella. L. B. C, Elaine. P. P, Andréia. H. O, Roger. C, Sônia. N. B, and Lidia. A. G. 2010. Morphological alterations and G0/G1 cell cycle arrest induced by curcumin in human SK-MEL-37 melanoma cells. *Braz. arch. biol. technol*. 53 (2)

15. Cai. X, Hu. X, Tan. X, Cheng. W, Wang. Q, and Chen. X. 2015. Metformin Induced AMPK Activation, G0/G1 Phase Cell Cycle Arrest and the Inhibition of Growth of Esophageal Squamous Cell Carcinomas In Vitro and In Vivo. *PLoS ONE*. 10(7): e0133349. doi:10.1371/journal.pone.0133349

16. Xinying. L, Hongxia. S, Feng. K, Yanxia. G, Yuan. C, Lu. Z, Dongfang. G, Xiaofei. Z and Han. Z. 2018. Pemetrexed exerts anticancer effects by inducing G0/G1-phase cell cycle arrest and activating the NOXA/Mcl-1 axis in human esophageal squamous cell carcinoma cells. *oncology letters*. 17 (2). 1851-1858.

17. Che. J. H, George. H, Wei. L. N, Shih. W. W, Chun. P. C, Li. Y. L, Jih. H. G, Tzong. W. L and Chi. L. C. 2014Cephalochromin Induces G0/G1 Cell Cycle Arrest and Apoptosis in A549 Human Non-Small-Cell Lung Cancer Cells by Inflicting Mitochondrial Disruption. *J. Nat. Prod.* 77 (4). 758-765.

denta
Jurnal Kedokteran Gigi

REVIEWER FORM

Manuscript Title :**Kaempferol And Quercetin Isolated From The Leaves Of Atingia Excelsa To Arrest Cell Cycle In G0/G1 Phase Human Tongue Cancer Sp-C1 Cell Lines**

Send Date :..... Back Date:.....

FORM

1. Has the script been published on other media? ~~Yes~~/No
   How is the contents of the script? Proportional manuscripts according to guidelines Yes/~~No~~
   (introduction, material and methods, results, discussion, conclusions, bibliography)
2. Title appropriate the problem, goals with detailed variables Yes/~~No~~
   Title 10-15 words Yes/~~No~~
   The title is precise, concise, clear, and describes the contribution scientific development Yes/~~No~~
3. Abstract and keywords in English; Indonesian abstracts and keywords are in accordance Yes/~~No~~
   with the script reference guidelines
4. Reference to the last 5-10 years ~~Yes~~/No
   The number, volume, and page are already listed Yes/~~No~~
   In the manuscript, sort by appearance Yes/~~No~~
   Abbreviation of author's name appropriate with the guidelines Yes/~~No~~
   (no limit on the number of authors)
   Download from the internet, specify the website address and time Yes/~~No~~
   Abbreviation of journal-title following the medical/dental index Yes/~~No~~
   Adequacy of library Yes/~~No~~
5. The table and the image represent the article ~~Yes~~/No

REVIEW RESULTS

1. Subtitles must be in order, they must be related to one another Yes/~~No~~
2. Empirical background There/~~Not~~
3. Theoritical background There/~~Not~~

4. Background There/~~Not~~
5. Materials and Methods, types research, kind of research, research design
   There/~~Not~~(kind, time, research site)
6. Sampling technique There/~~Not~~
7. Procedure research There/~~Not~~
8. Data analysis method (frequency distribution, reliability dan validity ~~There~~/Not
9. Result : data esposure, data analysis There/~~Not~~
10. The discussion does not repeat the results of the study (do not repeat the subtitles literature review, there is the latest novelty compared to similar libraries, answer the problem) There/~~Not~~
11. The discussion is in accordance with the research and compared with the results of other studies There/~~Not~~
12. Conclusions (explaining the results of research in line with the title, answer the problem according to the results of the study) There/~~Not~~
13. bibliography minimum of 20 sources of literature and vancouver style There/~~Not~~
14. A new problem/newness of development dentistry
    ~~There~~/NotContribute to the development of dentistry
    There/~~Not~~

REVIEWER NOTE'S

1. The manuscript is processed according to the author Yes/No
2. Manuscripts are processed with the authors' corrections (suggestions for improvements written directly on the manuscript) Yes/~~No~~
3. The manuscript was rejected with a thorough author overhaul Yes/No

   Cause.....

Surabaya, 8 April 2020

Reviewer

Denta Jurnal Kedokteran Gigi
Fakutas Kedokteran Gigi Universitas Hang Tuah
Jalan Arif Rahman Hakim No. 150, Surabaya. Telp. 031-5912191
Email: journal.denta@hangtuah.ac.idWebsite: http://journal-denta.hangtuah.ac.id

BIAYA PEMROSESAN ARTIKEL

Dengan Hormat,

Dengan ini menerangkan dan menyatakan bahwa naskah akan diterbitkan dalam DENTA Jurnal Kedokteran Gigi Volume 14 Nomer 1 tahun 2020 dengan p-ISSN: 1907-5987 e-ISSN: 2615-1790. Berikut rincian biaya yang dikenakan dalam publikasi:

Biaya pemrosesan artikel	Penulis / koresponden penulis dari luar Fakultas Kedokteran Gigi, Universitas Hang Tuah Surabaya.	**IDR 300,000**

Mohon lakukan pembayaran dengan transfer ke rekening bank:
Bank : BNI
Atas nama : Dian W. Damaiyanti
Nomor rekening : 0908125805

Bukti pembayaran bisa dikirim melalui email: journal.denta@hangtuah.ac.id
atau melalui Telp. +62856-3385-805 (Editor).

Hormat Kami,
An.
Pimpinan Denta Jurnal Kedokteran Gigi

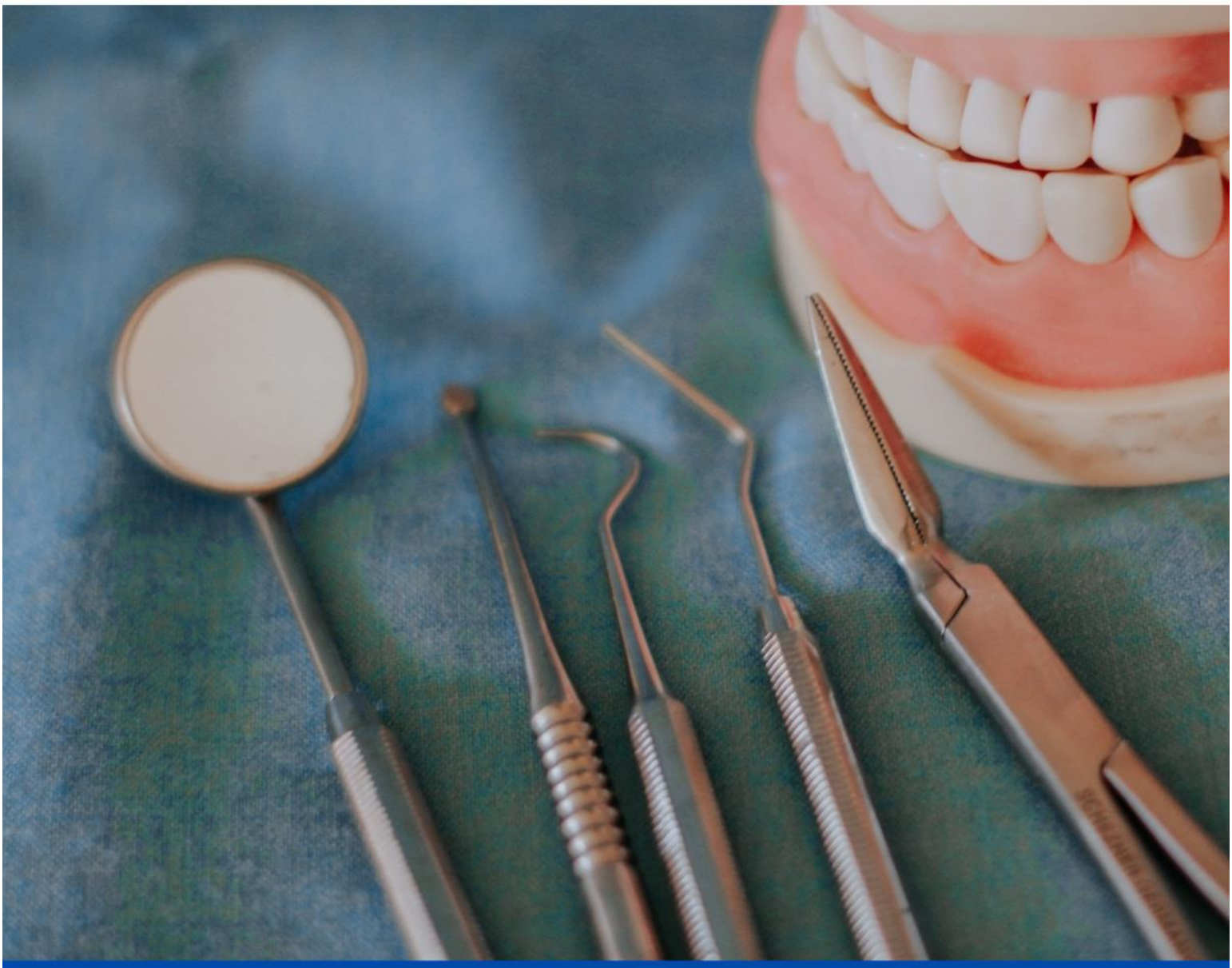
Dian Widya Damaiyanti, drg., M.Kes
(Duty Editor Denta Jurnal Kedokteran Gigi)

DENTA JURNAL

KEDOKTERAN GIGI

P-ISSN: 1907-5987
E-ISSN: 2615-1790

VOLUME 14 NO.1 FEBRUARI 2020



HTTP://JOURNAL-DENTA.HANGTUAH.AC.ID

Denta
Jurnal Kedokteran Gigi

SUSUNAN REDAKSI

Jurnal Kedokteran Gigi diterbitkan setiap bulan Februari dan Agustus oleh Fakultas Kedokteran Gigi Universitas Hang Tuah.

ALAMAT REDAKSI
Cp. Carissa Endianasari
Fakultas Kedokteran Gigi-Universitas Hang Tuah
Jl. Arief Rahman Hakim 150 Surabaya
Telp. 031-5945864, 5945894 psw 219/220 Fax. 031-5946261
E-mail: journal.denta@hangtuah.ac.id
http://journal-denta.hangtuah.ac.id

Denta
Jurnal Kedokteran Gigi

Vol. 14 No.1 Februari 2020

ISSN : 1907-5987

DAFTAR ISI

Accredited No. 28/E/KPT/2019
p-ISSN: 1907-5987 e-ISSN: 2615-1790

RESEARCH ARTICLE

Kaempferol and Quercetin Isolated from The Leaves of Atingia Excelsa to Arrest Cell Cycle in G0/G1 Phase Human Tongue Cancer SP-C1 Cell Lines

Risyandi Anwar[*], Arlette Setiawan[**], Supriatno[***], and Unang Supratman[****]
[*]Departement of Pediatric Dentistry, Faculty of Dentistry, University of Muhammadiyah Semarang.
[**]Departement of Pediatric Dentistry, Faculty of Dentistry, University of Padjadjaran.
[***]Departement of Oral Medicine, Faculty of Dentistry, University of Gajah Mada, Yogyakarta.
[****]Departement of Chemistry, Faculty of Mathematics and Natural Sciences, University of Padjadjaran.

Online submission : 23 January 2020
Accept Submission : 10 March 2020

ABSTRACT

The leaves of Altingia excelsa were found to strongly inhibit SP-C1 human tongue cancer cell lines. This study was focused on identifying the antiproliferative compound found in A. excelsa leaves and assessing its mechanism of action. The active compound was isolated using column chromatography and identified by the spectroscopic method and was also tested for its anti-proliferative properties and the cell cycle analysis in SP-C1 cells by flowcytometry analysis. This work resulted in the isolation of a flavonoid, which was identified to be kaempferol and quercetin. The compounds inhibited SP-C1 cell proliferation in a time- and dose-dependent manner with IC_{50} values of 0.72 µg/mL and 0. 70 µg/mL for the 24 hours treatments, respectively. Furthermore, the flowcytometry analysis suggested that the compounds exerted its anticancer activities by inhibiting cell cycle. These results suggested that compounds found in A. excelsa providies a basis for its potential use in cancer disease management.

Keywords: *Altingia excelsa, cancer, cell cycle, kaempferol, quercetin.*

Correspondence: *Risyandi Anwar, Paediatrics Dentistry Departement Muhammadiyah Semarang University, Kedungmundu Raya Street No. 22 Semarang. E-mail: riezdrgms@gmail.com.*

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 21-28
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

Accredited No. 28/E/KPT/2019
p-ISSN: 1907-5987 e-ISSN: 2615-1790

INTRODUCTION

Approximately 300.373 new cases of oral squamous cell carcinoma (OSCC) are annually reported around the world, what makes oral cancer the sixth most common cancer worldwide. The term oral cancer is referred to as a subgroup of head and neck malignant neoplasms affecting the lips, the anterior two-thirds of tongue, the salivary glands, the gingiva, the floor of the mouth, the oral mucosal surface and the palate, with the tongue being the most common location.[1] Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of this anatomic site, and in approximately 80% of cases, it is associated with extrinsic factors such as the use of tobacco, alcohol or both.[2] OSCC is a very 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. Management of OSCC varies consider-ably; small cancers of the oral cavity are usually managed by surgery alone, whereas advanced oral cancers are usu-ally treated with primary radical surgery followed by ra-diation or chemoradiation and targeted therapy.[3] The poor outcome of chemotherapy to OSCC contributes to the poor prognosis for OSCC.[4] 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. Of the 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.[5] To identify novel agents that may inhibit cancer development, we have focused our investigations on discovering bioactive compounds from high plants level. [6]

In our previous study, we found that the leaves of the Hammamelidaceae family, demonstrated anti-tumor properties.[7,8] These preliminary studies suggest that *A. excelsa* may be further developed as a source of anti-cancer agents. Thus, in this study, we investigated and characterized the inhibited cell cycle activities of *A. excelsa* leaf extracts.

MATERIALS AND METHODS

Plant materials. A. Excelsa leaves were gatted from Wayang Windu Mountain, Pangalengan, West Java, Indonesia. The plant species were identified by plant taxonomy laboratorium of Biology Departement, FMIPA, Padjadjaran University, Indonesia.

Extraction and isolation. Simplicia leaves of *A. excelsa* (2.5 kg) were extracted with methanol (3x24 h) at 20-25^{0}C (Room temperature). The solvent was subsequently evaporated under reduced pressure at 50˚C to produce a concentrated extract. 280 g of methanol extract was fractionated by *n*-hexane and water to gained an 86 g *n*-hexane extract and the layer of water. The water layer then extracted with ethyl acetate to gained an 120 g ethyl acetate fraction and 90 g water fraction. Fractions cytotoxicity was assessed on SP-C1 tongue cancer cells using methyl thiazolyl tetrazolium (MTT) assay. Ethyl acetate fraction, which was the most active fraction, was chromatographed by Wakogel C_{200} (Wako Pure Chemical, Japan) with a mixture of *n*-hexane, ethyl acetate and methanol with raised the polarity. Main compounds were then isolated and purified by silica G_{60} with sulfuric acid-ethanol (1:9) and were identified by spectroscopic methods consist of mass spectroscopy (MS), ultraviolet (UV), infrared spectrometry (IR), and nuclear magnetic resonance (NMR).[9]

Cell culture and treatment. SP-C1 human tongue cancer cell line was used in this research were used RPMI-1640 as cultured medium (Sigma, St. Louis, MO, USA) added with 10% FBS and antibiotics among them 100 U/mL penicillin and 100 µg/mL streptomycin. Cell's treatments, several 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 gathered at the indicated times.[10]

Cell cycle analysis using flow cytometry. The cells were grown in 24-well plates at 37ºC with CO_2 from 5% until 80% encounter was

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 21-28
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

Accredited No. 28/E/KPT/2019
p-ISSN: 1907-5987 e-ISSN: 2615-1790

reached. Afterwards, medium was changed, and flavonoids were added to the indicated concentrations. Furthermore, cells were incubated for 48 h at 37ºC. After incubation, the cells were harvested and rinsed three times with ice-cold PBS (pH 7.4). The supernatant was eliminated, and the cells were rinsed with 1 mL of PBS and centrifuged at 4 °C. Finally, the supernatant was eliminated, and 200 µL of 70% ice-cold ethanol and 200µL of PBS was added to the cells and stored at –20 ºC for further use. Utilization in the flow cytometry experiments, the cell pellet was rinsed twice with PBS. Cell pellet was suspended in 0.5 mL of staining reagent (50µg/mL PI, 50 U/mL RNase, 0.1 mM EDTA,0.1% Triton X-100, and PBS) and incubated for 30 min at 37 ºC in the dark. Measuring DNA fluorescence was used Becton Dickinson (Franklin Lakes, USA) FACScanto II flow cytometer with 488 nm excitation wavelength and 585 nm emission wavelength. Pulse width area signals were used to discriminate between G2 cells and cell doublets. Data analyzed was used FACSDiva Software (Beckton Dickinson). Relative distribution of 10^4 events for each sample was analyzed for background aggregates and debris, apoptosis indicator and the G0/G1-, S-, and G2/M-phases of the cell cycle. Control treatments consisted of a culture medium supplemented with FBS. Serum-deprivation treatment was used as an inducer of G0/G1 cell cycle arrest.[11]

RESULTS

Fraction of Ethyl acetate *A. excelsa* leaves inhibits SP-C1 cell proliferation. Treatment MTT assay shown that *A. excelsa* methanol extract was inhibit the proliferation of SP-C1 human tongue cancer cells (IC_{50} 75.41 µg/mL) (Fig. 1). Methanol extract then fractionated based on polarity, using *n*-hexane, ethyl acetate and water. Afterward the fractions individually applied to SP-C1 cells and inhibit cell proliferation with an IC_{50} value of 44.85 µg/mL for the *n*-hexane fraction, 12.85 µg/mL for the ethyl acetate fraction and 18.02 µg/mL for the water fraction. Due to its low IC_{50} value, we then explored the ethyl acetate fraction for its anti-cancer potential.

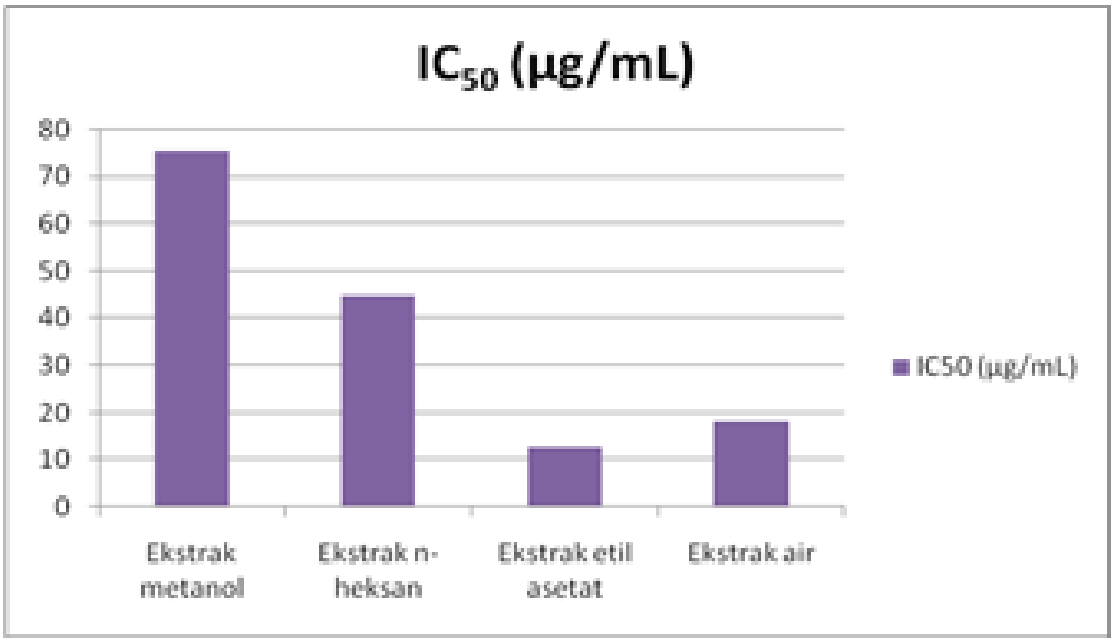
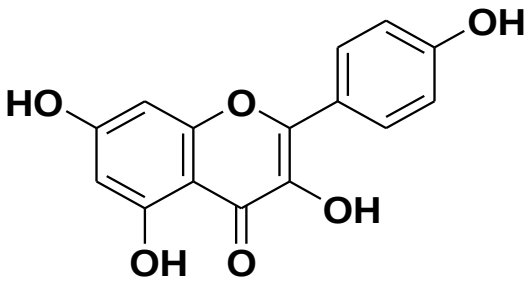


Figure 1. IC_{50} value some extracts *A. excelsa* Nornha

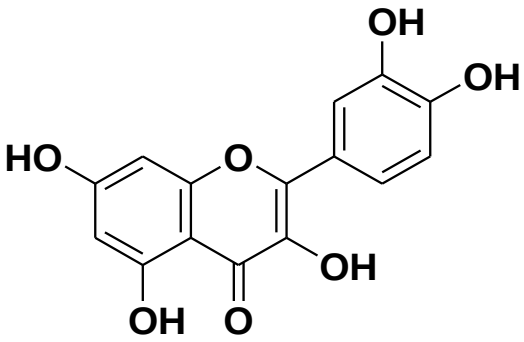
Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 21-28
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

Main compounds of the ethyl acetate fraction are Kaempferol and quercetin. Isolation and purification of the ethyl acetate fraction *A. excelsa* extract to gathered bioactive compounds. The compound exhibited a melting point at 152.7-153˚C and a molecular ion peak at *m/z* 432 in the LC-MS spectrum. According to hydrogen and carbon content, the molecular ion peak and ^{1}H and ^{13}C NMR profiles indicated that the compound has a molecular formula of $C_{15}H_{10}O_7$. Ultraviolet spectrum provides maximal absorbance peaks at λ_{max} 265 and 342 nm, which were characteristic of a flavonoid with flavone skeleton. The addition of NaOH produced bathochromic shift in the absorption bands, indicating the presence of hydroxyl groups in the skeleton, one of which was attached to C-5, as indicated by further bathochromic shift following the addition of H_3BO_3. Absorption bands at 1,675 and 3,197 nm of the IR spectrum indicated where the molecule harbors conjugated carbonyl and hydroxyl groups, respectively.

^{1}H NMR spectrum of the compound shown two hydrogen of aromatic signals with 'meta coupling' at δ 6.35 (1H, *d*, *J* = 2.2 Hertz) and 6.18 (1H, *d*, *J*=2.2 Hertz), which was predicted by hydrogens at C-6 and C-8 of the A ring of the flavone skeleton. Hence, this compound was suggested to have a group of hydroxyl in C-5 and C-7. Furthermore, ^{1}H NMR spectrum revealed two signals with 'ortho coupling' at δ 6.92 (2H, *d*, *J* = 6.7 Hz) and 7.74 (2H, *d*, *J* = 6.7 Hz), the approximation signals from the hydrogens at C-2', C-3', C-5' and C-6' of the B ring. Lack of a specific signal for olefinic hydrogen at C-3 and existence of an anomeric hydrogen signal at δ 5.37 (1H, *d*, *J* = 7.2 Hz) suggested that the compound was a flavonol glycoside. Appearance of anomeric carbon signal at δ 94.9 in the ^{13}C NMR spectrum indicated the availability of a sugar moiety. In consequence to a correlation between the anomeric hydrogen signal (δ 5.37) and the anomeric carbon signal (δ 94.9) that revealed by analysis of the HMBC spectral data, sugar moiety position was established to the C-3 hydroxyl group, signal of methyl.



Chemical structure of Kaempferol



Chemical structure of Quercetin

Quercetin and Kaempferol inhibits SP-C1 cell proliferation in a concentration-dependent manner. Kaempferol and quercetin effects on the viability of SP-C1 cells were evaluated. Treatment of cancer SP-C1 cell lines with kaempferol and quercetin shown that concentration-dependent inhibit of cell growth, as demonstrated by the MTT assay. 24 hours of treatment with kaempferol and quercetin inhibited the proliferation of SP-C1 cells with IC_{50} values 0.72 and 0.70 µg/mL, respectively. Subsequent cell cycle analysis, based investigation applying the IC_{50} concentration of 0.39, 0.78, and 1.56 µg/mL kaempferol and quercetin was applied on SP-C1 cells.

Kaempferol and quercetin inhibited the SP-C1 cell cycle in the G0/G1 phase. To determinate the percentage of SP-C1 cells present in different phases of the cell cycles G0/G1, S, and G2/M, first cells were synchronized with serum deprivation before

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 21-28
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

treatment with or without quercetin and kaempferol at several concentrations (C, K 0.39, K 0.78, K 1.56, Q 0.39, Q 0.78, and Q 1.56 µg/mL) for 24 h period, serum-deprived cells were progressively accumulated in the G0/G1-phase, whereas serum-supplied cells were present in the S- and G2/M-phases (Figure 2). Treatment with K (all concentrations) and Q (all concentrations) significantly increased the percentage of cells in the G0/G1-phase and decrease in S and G2/M-phase as compared with the control. Particularly, Cell percentage in G0/G1-phase increased an appropriate increase concentration compared to the control after K treatment. Percentage of cells in the G0/G1 – phase increase compared to the control after Q treatment but the percentage of cell decrease a long increase in concentration.

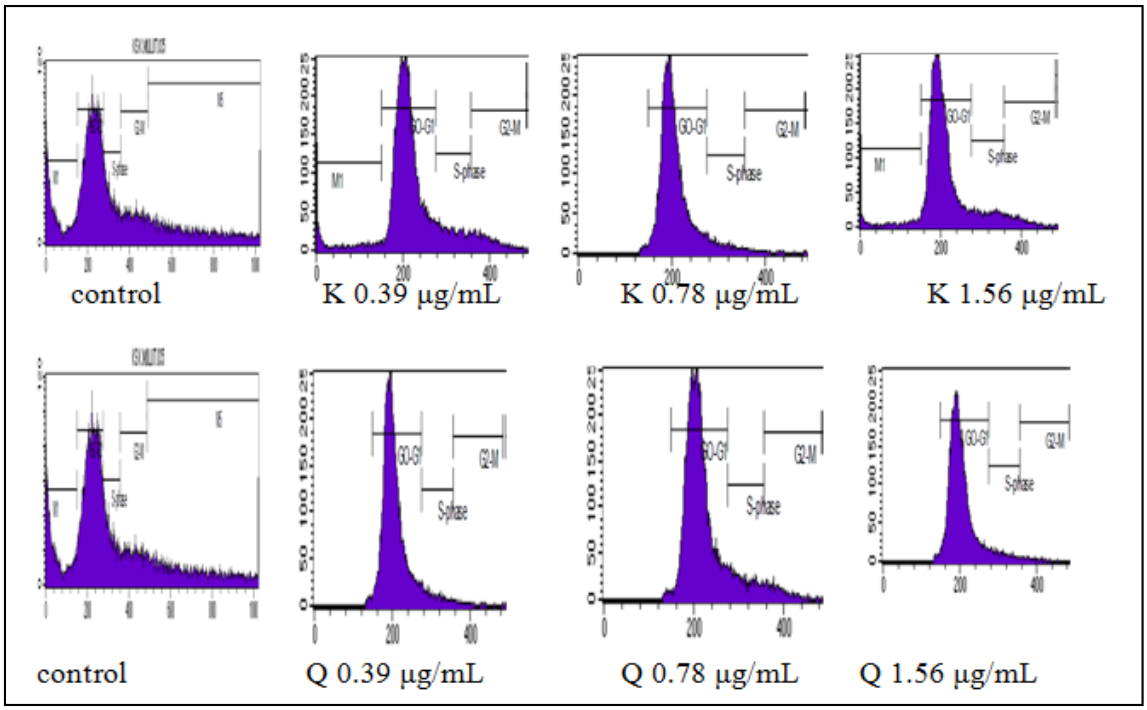


Figure 2. Flowcytometry analysis SP-C1 cancer cell using treated some concentration of kaemperol and quercetin.

DISCUSSION

The ^{1}H- and ^{13}C-NMR spectra of compounds from *A. Excelsa* exhibited resonances due to aromatic systems. The ^{1}H-NMR spectrum of them showed the presence of two doublet signals corresponds to four aromatic protons in ring B, characteristics for the 1′,4′-disubstituted flavone. The ^{13}C-NMR signals of them were assigned with the help of a DEPT experiment. A total of fifteen carbon signals were observed in the ^{13}C-NMR spectrum. The degree of unsaturation was accounted for eight out of the total eleven double bond equivalents. The ^{13}C-NMR spectrum of them showed the presence of 15 aromatic carbon signals. So, from ^{1}H and ^{13}C NMR profiles indicated that the compound had a molecular formula of $C_{15}H_{10}O_7$.

Rural medicinal plants since a long time as sources of potential therapeutic medication, and find out of novel medication or leads compound are usually based on that approach.[12,13] In drug invention, researcher have recently applied a new approach to selecting plants based on the Hammamelidaceae family.[7,8] In previous research, we found that the *A. excelsa* leaves extracts were strongly cytotoxic to the SP-C1 Human tongue cancer cell lines. Therefore, these extracts potential for further investigation. Present study focused on identifying anti-

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 21-28
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

proliferative compound from the *A. excelsa* leaves. This study was isolated of two flavonoids, kaempferol and quercetin, which strongly inhibited proliferation of SP-C1 cell lines in a time- and concentration-dependent manner. There is no compound that reported before in relation with its cytotoxicity in these cancer cell lines.

In this study, kaempferol and quercetin with some concentration to arrest G0/G1 phase cell cycle. Cell growth is an additional amount of cells from the cell cycle process. The cell cycle consists of 4 phases, that is G_1, S, G_2, and M. G_1 (Gap 1) is a phase when the cell will synthesis the DNA or go out from cell cycle reversibly or irreversibly to differentiation. The cell that in the G_1 phase will easily controlling cell cycle at a point, that is in the restriction point (R) that will determine the cell to come in back to the cell cycle, go out from cell cycle entering G_0 phase or differentiating.[14]

When the cell cycle phase flow in restriction point dan enter S phase controlled by cyclin-dependent kinase (Cdks) and D, E, and A cyclin. D cyclin function as a growth factor that the expression more depend on the extracellular signal than the cell position on the cycle.[15] When the cell entering the G_0 cycle, on or more of the D cyclin (D1, D2 and D3) were inducted as apart from the first response of a growth factor stimulation, protein synthesis and former complex with catalytic subunit (Cdk4, Cdk6) depend on the mitogenic stimulation.[16] In reverse, if the mitogenic substance were removed, so the D Cyclin synthesis will stop. D cyclin is an unstable protein and the enzymatic activity quickly disappear, so that the cell quickly go out from the cell cycle. A specific inhibitor of Cdk4 and Cdk6 known as Ink4 can directly stop the activity of D cyclin/cdk 4/6 and causing G_1 phase arrest (rest of G_1 phase).[16]

Cell cycle inhibition of tongue cancer (SP-C1 after kaempferol and quercetin intervention as the results of Rasmala leaves isolation. There is a system that controlled the cell cycle. The controlling system known as a checkpoint. There are 4 checkpoints that found on cycle cell, that is G_1, S, G_2, and M. Checkpoint in a cell cycle involving protein cyclin groups and cyclin-dependent kinase (cdk).[17] Protein groups that involved on the cell cycle checkpoint determine cell cycle to stop, DNA repair, or apoptosis if the DNA repair doesn't occur.[18] Kaempferol (4) can inhibit cell cycle on G_0-G_1 phase at 0.39 µg/mL, 0,78 µg/mL and 1,56 µg/mL concentration. This cell cycle inhibition suspected because of kaempferol (4)/ quercetin (5) that can inhibit the work of c-myc. We know that the function from c-myc transcription factor is increasing D cyclin and E cyclin, so that there are increasing activity of G1-CDK (D1-CDK cyclin) and G1/S-CDK (E-CDK6 cyclin). Thus, the quercetin in this research can inhibit tongue cancer SP-C1 cell cycle on G_0-G_1 phase at 0,39 µg/mL, 0,78 µg/mL dan 1,56 µg/mL concentration. This results analog with the research who is conducted by Mocanu *et al,* in 2013 years that shows quercetin can inhibit Epidermoid Cancer Cell line A-431 cell cycle at 5 and 10 µM concentration in 24 hours on G_0/G_1 phase and inhibit Mamary SK-BR-3 cell cycle at 50 and 75 µM concentration in 24 hours on G_0-G_1 phase. Research belonging to Chen *et al*, in 2011 years shows that quercetin can inhibit oral cancer OSCC SCC-25 cell cycle at 50 and 75 µM concentration in 12 hours incubation on the G_1 phase. Cell cycle inhibition which is conducted by quercetin on G_0-G_1 phase possibly because quercetin will synergize with cyclin-dependent kinase inhibitor (CDK1) p21 that that function as inhibitor D-CDK4/6 cyclin activity.[19] With inhibition of D-CDK4/6 cyclin activity so the cell will be obstructed to enter S phase, so that the cell will stop on the G_0/G_1 phase or p21 beside binding cdk and PCNA (proliferating cell nuclear antigen), that is a DNA polymerase α subunit, which needed for replication or DNA reparation. P21 will inhibit cell cycle on the G_1 phase as a result of cell-contact inhibition and because of TGF β.[20]

CONCLUSION

In conclusion, our results suggest that kaempferol and quercetin inhibited the growth of

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 21-28
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

SP-C1 cells through inhibited cell cycle in the G0/G1 phase.

REFERENCES

1. Liviu F and Johan L. Oral Squamous Cell Carcinoma: Epidemiology, Clinical Presentation and Treatment. Journal of Cancer Therapy. 2012; 3:263-268.
2. Juliana HG, Scheidt LS, Yurgel KC, Maria AZ, Figueiredo FGS. Characteristics of oral squamous cell carcinoma in users or non users of tobacco and alcohol. *Rev Odonto Cience*. 2012; 27(1): 69-73.
3. Sankalp A G, Munira M, and Chintan B. Oral Squamous Cell Carcinoma: Current Treatment Strategies and Nanotechnology-Based Approaches for Prevention and Therapy. Critical Review in Therapeutic Drug Carrier Systems. 2016; 33(4): 363–400.
4. Tantry M, Aulia I, Andri H, Endang S, Mantra N, Alwin K, and Harmas Y Y. The incidence of oral squamous cell carcinoma (OSCC) and its relationship with orofacial pain in oral cancer patients in West Java Province, Indonesia. Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. 2017; 29: 29–32
5. Tan W, Lu J, Huang M, Li Y, Chen M, Wu G, Gong J, Zhong Z, Xu Z, Dang Y, Guo J, Chen X, and Wang Y. Anti-cancer natural products isolated from chinese medicinal herbs. *Chinese Medicine.* 2011; 6: 27-37.
6. Anna L and Krzysztof G. Anticancer Activity of Natural Compounds from Plant and Marine Environment. Int. J. Mol. Sci. 2018; 19: 1-38
7. Kim HH, Yi HS, Hwan MO, Hyuk KH, Ra KS, and Lee MW. Anti oxidative and anti-proliferative activity on Human Prostate Cancer Lines of the phenolic compounds from *Corylopsis coreana* Uyeki. *Molecules*. 2013; 18: 4876-4888.
8. Yang YN, Chen JH, Zhou GS., and Tan YP. Pentacyclic triterpenes from the resin of *Liquidambar formosana*. *Fitoterapia*. 2011; 82: 927-931.
9. Ashok A. Structural elucidation of compounds using different types of spectroscopic techniques. JCHPS. 2014; Special Issue 5:22-25.
10. Arlette SS, Roosje RO, Supriatno, Willyanti S, Sidik and Unang S. 8-Hydroxycudraxanthone G Suppresses IL-8 Production in SP-C1 Tongue Cancer Cells. NatProdComm. 2014; 9 (1):75-78.
11. Vanzyl EJ, Rick KRC, Blackmore AB, MacFarlane EM, McKay BC. Flow cytometric analysis identifies changes in S and M phases as novel cell cycle alterations induced by the splicing inhibitor isoginkgetin. PLoS ONE. 2018; 13(1): 171-191.
12. Ritva T and David J N P. Mitogen-Activated Protein Kinases: Functions in Signal Transduction and Human Diseases. Int. J. Mol. Sci. 2019; 20: 2-5
13. Zhang D, Liu J, Mi X, Liang Y, Li J, and Huang C. The N-terminal region of p27 inhibits HIF-1α protein translation in ribosomal protein s6-dependent manner by regulating PHLPP-ras-ERK-p90RSK axis. *Macmillan Publisher Limited: Cell Death and Disease*. 2014; 5:1535-1540.
14. Liu JD, Wang YJ, Yu CF, Chen LC, Lin JK, Liang YC, Lin SY, and Ho YS. . Molecular mechanisms of G_0/G_1 cell-cycle arrest and apoptosis induced by terfenadine in human cancer cells. *Molecular carcinogenesis*. 2013; 8 (3): 26-31
15. Denkert C, Furstenberg A, Daniel PT, Koch I, Kobel M, Weichert W, Siegert A, and Hauptmann S. . Induction of G0/G1 cell cycle arrest in ovarian carcinoma cells by the anti-inflammatory drug NS-398,but not by COX-2-specific RNA interference. *Oncogene* . 2013; 22, 8653–8661.
16. Xiao LS, Yun SZ, Xue FW, Wen JZ, Zheng W, Fei Z, Yi JZ, Jian HL, Jia WM , Yun PH, Lei C, Huai PL, Yuan YY, Ying BL and Jun G.. Casticin induces apoptosis and G0/G1 cell cycle arrest in gallbladder cancer cells. *Cancer Cell Int*. 2017; 17(9):111-116.
17. Marcella. L. B. C, Elaine. P. P, Andréia. H. O, Roger. C, Sônia. N. B, and Lidia. A. G.. Morphological alterations and G0/G1 cell cycle arrest induced by curcumin in human SK-MEL-37 melanoma cells. *Braz. arch. biol. technol*. 2010; 53 (2): 6-11.
18. Cai. X, Hu X, Tan X, Cheng W, Wang Q, and Chen X.. Metformin Induced AMPK Activation, G0/G1 Phase Cell Cycle Arrest and the Inhibition of Growth of Esophageal Squamous Cell Carcinomas In Vitro and In Vivo. *PLoS ONE*. 2015; 10(7):133-139.
19. Xinying L, Hongxia S, Feng K, Yanxia G, Yuan C, Lu Z, Dongfang G, Xiaofei Z and Han Z.. Pemetrexed exerts anticancer effects by inducing G0/G1-phase cell cycle arrest and activating the NOXA/Mcl-1 axis in human

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 21-28
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

Accredited No. 28/E/KPT/2019
p-ISSN: 1907-5987 e-ISSN: 2615-1790

esophageal squamous cell carcinoma cells. *oncology letters*. 2018; 17 (2): 1851-1858.

20. Che. J. H, George. H, Wei. L. N, Shih. W. W, Chun. P. C, Li. Y. L, Jih. H. G, Tzong. W. L and Chi. L. C. Cephalochromin Induces G0/G1 Cell Cycle Arrest and Apoptosis in A549 Human Non-Small-Cell Lung Cancer Cells by Inflicting Mitochondrial Disruption. *J. Nat. Prod.* 2014; 77 (4): 758-765.

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 21-28
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1