Open Science Publishers LLP
(Registered under Section 12(1)(b) of the LLP Act, 2008. Reg. No. AAB-0678)

OSP Health
Open Science Publishers

December 23, 2020

Dear herni kusriani,

I am pleased to inform you that your manuscript titled "Cytotoxicity of Quercetin and Quercetin-3-O-Rhamnoside of Etlingera elatior (Jack) R.M. Sm. Leaves Against HeLa Cervical Cancer Cells" (Manuscript Number: JAPS-2020-09-970) is accepted for publication in the Journal of Applied Pharmaceutical Science. You are required to submit article processing charges within the next three days of receiving this mail through Banking/Paypal. Payment details will be sent in a separate email.

We encourage the authors to host the accepted version of the article to their research gate/google scholar. You could check your possible publication date on your author page.

You may log in to your author account page, and visit the accepted articles section in order to get an official/formal acceptance letter as a PDF.

I would like to remind you that you could send your future manuscripts to Journal of Applied Pharmaceutical Science.

Sincerely yours,

Paras Sharma
Associate Editor
Journal of Applied Pharmaceutical Science
editor@japsonline.com
http://japsonline.com/index.php

Cytotoxicity of Quercetin and Quercetin-3-O-Rhamnoside of *Etlingera elatior* (Jack) R.M. Sm. Leaves Against HeLa Cervical Cancer Cells

Kusriani Herni[1,2], Iskandar Yoppi.[1] Diantini Ajeng [1], Subarnas Anas[1]*

[1] Faculty of Pharmacy, Padjadjaran University, Sumedang, Indonesia
[2]Faculty of Pharmacy, Bhakti Kencana University, Bandung, Indonesia

*corresponding author : aasubarnas@yahoo.co.id

ABSTRACT

Introduction: *Etlingera elatior* traditionally used for therapeutic purposes. Pharmacological activities of this plant have been reported, one of which is cytotoxic activity. This study aims to isolate cytotoxic compounds against HeLa cervical cancer cell lines from an ethanol extract of the *E. elatior leaves.*

Methodology: The isolation work was conducted by means of column chromatography and determination of the molecular structure was by spectroscopic data analysis. The cytotoxicity was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) method.

Result: The results of this study were two isolated compounds identified as quercetin and quercetin-3-O-rhamnoside from the ethyl acetate fraction. These two compounds had cytotoxic activity on HeLa cervical cancer cells which was indicated by the inhibition of cell proliferation with IC_{50} values were 29.49 and 46.67 µg/mL, respectively. In the migration assay, quercetin and quercetin-3-O-rhamnoside inhibited migration of the HeLa cells significantly at 10 µg/mL as compared with the control, in 24 and 48 hr experiments.

Conclusion: This study reinforces the previous evidence of the potential of quercetin and quercetin-3-O-rhamnoside as an anticancer drug candidate.

Keywords *: Etlingera elatior* (Jack) R.M.Sm., *HeLa cell, quercetin , quercetin-3-O-rhamnoside*

INTRODUCTION

Etlingera elatior (torch ginger) is a species of the Zingiberaceae, and is an edible native Indonesian plant, Malaysia, and other countries in Southeast Asia (Chan *et al.*, 2011; Lachumy *et al.*, 2010). Its flowers are commonly used as spices for food and vegetable, and also used for medicinal purposes (Chan *et al.*, 2007; Lachumy *et al*., 2010; Shahid and Basher, 2019). Its leaves are usually applied topically as antiseptics to clean wounds (Ibrahim and Setyowati, 1999) and used in a mixture with other fragrant herbs to deal with body odor (Chan *et al.*, 2007). Torch ginger leaves also has been reported to contain flavonoid, tannin, triterpenoid, saponin, and carbohydrates (Lachumy *et al.*, 2010). The flavonoid compounds in *E. elatior* were identified as quercetin, apigenin, kaempferol, luteolin, and myrcetin (Ghasemzadeh *et al*., 2015) and the main

content of essential oil consist of β-pinene, dodecene , and sesquiterpenes of β-farnesene, and caryophylelene (Abdelwahab *et al.*, 2010; Chan *et al.*, 2010)

Several studies on pharmacological activities of *E. elatior* have reported that this species has various kinds of activities including antibacterial, antioxidant, antifungal, tyrosinase inhibition, hepatoprotector, and cytotoxic activity (Chan *et al.*, 2011; Ghasemzadeh *et al*., 2015, Kusriani *et al*., 2017). The rhizome of this plant can inhibit tumor growth in the EBV-EA (Epstein Barr virus early antigens) test, flower extracts showed antiproliferative activity against breast cancer cells, and leaf extracts showed an antioxidant and cytotoxic activity on HeLa cells (Lachumy *et al.*, 2010; Habsah *et al.*, 2005; Shaikh *et al.*, 2016)

Our study on *E. elatior* has successfully isolated and identified quercetin and quercetin-3-O-rhamnoside (quercitrin). Evidence is available concerning the cytotoxicity of quercetin on various kinds of cancer cells, such as cervical, breast, lung, colorectal, prostate, and nasopharyngeal cancer (Shafabakhsh and Asemi, 2019). However, reports on their cytotoxicity on cervical cancer cells are limited. In this study, we evaluated quercetin and quercetin-3-O-rhamnoside for their cytotoxicity against cervical cancer cells and their effect on cell migration.

MATERIALS AND METHODS

General procedures

UV spectra were measured using Shimadzu spectrophotometer UV 1800 (Kyoto, Japan). The IR spectrum was analyzed with the IR Spectrometer instrument by PerkinElmer® (Waltham, MA, USA). (Waltham, MA, USA). NMR (Nuclear Magnetic Resonance) specra was analyzed with the Agilent® DD2 series console spectrophotometer at 500 (1H) and 125 (13C) MHz using acetone as the solvent. The column chromatography process was carried out with silica gel 60 (70-230 Mesh ASTM; Merck, Darmstadt, Germany) as stationary phase. Thin layer chromatography was performed on pre-coated silica gel 60 PF_{254} plates (Merck, Darmstadt, Germany). A universal spray reagent, sulfuric acid 10% was used to detect the chromatogram. All solvents for the separation process are technical solvents which have been previously distilled off.

Plant materials

Etlingera elatior (Jack) R.M.Sm. leaves collected from local gardens in Bandung, West Java, Indonesia. The plant was authenticated in the Herbarium of the Department of Biology,

Padjadjaran University, Indonesia. Leaves were cleaned under running water, sliced to reduce sample size, and oven-dried at 40°C until the constant weight.

Chemicals and Reagents

RPMI (Roswell Park Memorial Institute) 1640 medium (cat no. 11875093), FBS (foetal bovine serum) (cat no. 10270106). DMSO (Dimethyl sulfoxide) (cat no. D8418), and MTT (3- (4,5-dimethylthiazol-2-yl) -2,5 diphenyl tetrazolium bromide) (cat. no. M2128), were purchased from Sigma-Aldrich, Missouri, USA. Doxorubicin was provided by the Laboratory of Cell Culture and Cytogenetic, Faculty of Medicine, University of Padjadjaran, Indonesia. All chemical reagents were analytical grade.

Cell culture and Conditions

HeLa cell lines (human cervix carcinoma) were used to to investigate cytotoxic activity of leaves extract, fractions and isolate of the *E. elatior* leaves. HeLa cells were cultured in RPMI as a medium (pH 7.4), which was added with 10% FBS, penicillin (100 U / mL), and streptomycin sulfate (100 mg / mL). All experiments were conducted triplicate at Laboratory of Cell Culture and Cytogenetic of the Faculty of Medicine, University of Padjadjaran, Indonesia.

Extraction and Isolation

The powders of the *E. elatior* leaves (2000 g) were extracted with ethanol 70% (3 x, each 24 h) by the maceration method. The extract was concentrated using a rotary vaporator at 60˚C to produce a concentrated extract (570 g), and the concentrated extract was fractionated with a mixture of water: hexane (3: 1) to produce a layer of hexane and water. The water layer is then re-partitioned with etOAc to produce an etOAc and H_20 layer, and finally the H_20 layer is partitioned with buOH to produce buOH and aqueous layers. The four layers evaporated to produce n hexane fraction (3.16 g), etOAc fraction (106.6 g), buOH fraction (116.83 g) and H_20 fraction (248.92 g). Extract and all fractions tested for cytotoxic activity against HeLa cell lines by MTT test. Among four fractions, the ethyl acetate exhibits the highest cytotoxicity, so further work was focused on the ethyl acetate fraction to isolate active compounds. Subsequently, the ethyl acetate fraction was then separated again by column chromatography with silica gel and eluted with a mixture of n hexane and ethyl acetate with various ratios to increase polarity (n hexane to ethyl acetate, 9: 1, 8: 2, 7: 3, 6: 4 and 5: 5) resulting in several subfractions. The fractions 2 of the nine fractions was repeatedly column chromatographed with silica gel and the eluent of the chloroform metahanol water mixtures to result in a pure compound **1 (** 103 mg). Fraction 6 was carried out by column

chromatography with silica gel and eluted with $CHCl_3$:MeOH (9: 1) to produce one isolate, which was purified into pure compound 2 (30 mg). The two compounds were identified by spectral (UV), (IR), (MS), and (1H and 13C NMR) spectral data.

Cytotoxicity Examination in MTT Assay

The cytotoxicity of the extract, fractions, and compounds that have been isolated against cell of HeLa cervical cancer were tested by MTT assay (Mosmann, 1983). The 3000 cells per well were seeded in 96-well microtiter with 100 mL RPMI medium. Next, cells were incubated for 24 hours at 37°C under 5% CO_2 in a humid atmosphere. Then the RPMI media was discarded and fresh media containing extracts and fractions with different doses was added. After an incubation period, the media was removed. To each well, 20 µL of MTT (5 mg / mL pH 4.7) was added then incubated again for 4 hours. The supernatan liquid was removed. 100 µL of DMSO as stopper reaction was added and shaken for 15 minutes. The absorbance was measured at 550 nm using a plate reader (Thermo Scientific® Multiscan EX, Singapore). All experiments were carried out in triplo. The absorbance of cells without sample is considered to be 100%. The 50% inhibition concentration (IC50) was determined by graph.

Migration Assay

Cell migration was observed with a 24-well Transwell plate system (Costar, Corning, USA) in the scratch/wound healing assay in HeLa cell lines. HeLa cells were grown in 24-well plates, and were treated or untreated with quercetin, quercetin 3-O rhamnoside, and doxorubicine (a positif control) in the complete medium then placed in the incubator. After 24 hours of incubation, gaps were introduced with a P10 pipette tip by gently scraping monolayer. After scrapped, to remove debris, cells were washed with PBS (Phosphate Buffered Saline) three times. Cells that have been given the gap, were incubated for 0 h, 24 h and 48 hour at 37°C (5% CO2) (Cory, 2011). The treatments were observed with a microscope linked to a computer and Toupview software (version x64, 3.7.7892) and documented as TIFF, and gap area were measured with ImageJ (NIH). The experiments was carried out in triplo. (Schneider, Rasband and Eliceiri, 2012)

RESULTS AND DISCUSSION

Cytotoxicity of *E. elatio*r Leaves

The extract and fractions of the *E. elatior* leaves were tested for their 24 h effects against HeLa cancer cell lines using the MTT bioassay; result are shown in Figure 1. IC_{50} values of all samples are presented in Table 1. Among all the samples tested, the extract had the strongest inhibition against the HeLa cell lines proliferation followed by ethyl acetate, butanol, n-hexane, and water fractions with IC_{50} of 127.98, 156.47, 225.68, 312.47, and 561.58 µg/ml, respectively. A further study was conducted on the ethyl acetate fraction to explore active compounds responsible for their cytotoxicity.

Identification of Compound 1 and Compound2

The ethyl acetate fraction which had cytotoxicity against the HeLa cell lines in the MTT assay was subjected to repeated column chromatography and two compounds, compounds **1** and **2**, were isolated. Both compounds were identified their spectral data.

Compound 1

Compound **1** was the crystal of yellow colour with a melting point variously reported of 309-314^{0}C and showed molecular ion peak at m / z 303.05 with EI-MS (Electron Ionization-Mass Spectrometry). From the MS and NMR datas indicated molecular formula of compound 1 is $C_{15}H_{10}O_7$.

Two maximum absorption peaks of UV spectrum showed at a wavelength of 363 nm and 282 nm. It is specific for flavonol spectrum from flavonoids (Mabry, Markham, Thomas, 1970). The IR spectrum showed the presence of a hydroxyl (3450, 3255, 1400, and 1320 cm^{-1}), carbonyl (1606 cm^{-1}), alkene (1562 cm^{-1}), and aromatic (1458 cm^{-1}) groups in the molecule.

The ^{1}H NMR data of the compound **1** revealed five aromatic proton signals at δ 6.26 (1H, d, J=2.0 Hz), 6.51 (1H, d, J=2.0 Hz), δ 7.00 (1H, d, J=8.5 Hz), 7.70 (1H, dd, J=8.5; 2.0 Hz), and 7.82 (1H, d, J=2.0 Hz), which showed that this compound has 1,3,4-tri- and 1,2,3,5-tetrasubstitutes in benzene. There are two doublet signals at δ 6.26 and 6.51 were characteristic derived from H-6 and H-8 of a flavonol containing a 5,7-dihydroxy substitution pattern of the A ring, whereas the signals appearing at δ 7.00, 7.70, and 7.82 were assumed attributed to H-5', H-6', and H-2', respectively, of the B-ring of flavonol. Furthermore, a downfield peak at δ 12.16 (1H, *s*) was a characteristically signal of the hydroxyl proton of C-5. These ^{1}H NMR spectral data suggested the compound **1** was quercetin. This assumption was supported by the ^{13}C NMR spectra which indicates the presence of 15 signals consisting of 14 signals at δ 94.45-164.98 ppm of aromatic

carbons of benzene rings and one signal derived from a carbonyl carbon at δ 176.55 ppm. Comparison of all NMR spectral data with the reported evidence (Aisyah *et al.,* 2017) confirmed that compound **1** is quercetin (Figure 2).

Compound 2

Compound 2 was a yellow amorphous powder.The molecular ion peak at *m/z* 471.08 [M+Na]. This molecular ion peak together with the number of hydrogens and carbons in ^{1}H and ^{13}C NMR data indicated that molecular formula of this compound was $C_{21}H_{20}O_{11}$. Its UV spectrum showed strong absorbance at 350 nm and 256 nm. These absorbance values are characteristic for a flavonol skeleton glycosylated at 3-hydroxyl group. The infrared spectrum showed the presence of the functional groups of hydroxyl (3271 cm^{-1}), carbonyl (1652 cm^{-1}), alkene (1556 cm^{-1}), and aromatic ring (1456 cm^{-1}).

^{1}H NMR spectra of compound **2** showed coupling patterns similar to compound 1. Aromatic proton signals appearing at δ 6.40 (1H, *d*, *J*=2.0 Hz) and 6.50 (1H, *d*, *J*=2.0 Hz) were derived from H-6 and H-8 of a flavonol with the 5,7-dihydroxy substitution, whereas those at δ 6.9 (1H, d), 7.30 (1H, *dd*, *J*= 8.4; 2.1 Hz), and 7.48 (1H, *d*, *J*=2.1 Hz) were respectevely generated from H-5', H-6', and H-2' of the B-ring. The characteristic signal of hydroxyl proton at C-5 is a downfield peak at δ 12.68 (1H, *s*). In this ^{1}H NMR spectrum, the anomeric proton signal showed at δ 5.47 (1H, *d*, *J* = 1.2) along with four proton signals of oxygenated methines at δ 3.00 – 4.35 ppm, and one methyl proton signal at δ 0.90 (3H, *s*). These proton signals were derived from a sugar moiety, the sugar of which was supposed to be rhamnose, and this assumption was supported by the ^{13}C NMR data which showed five oxygenated methine carbons at δ 102.80, 72.94, 72.02, 71.44, and 71.32 and one methyl carbon at δ 17.73. In addition, the ^{13}C NMR spectrum showed 14 signals at δ 99.50-163.07 ppm of aromatic carbons of benzene rings and one signal derived from a carbonyl carbon at δ 179.20 ppm. Hence, this compound was assumed to be quercetin-3-O-rhamnoside or quercitrin (Figure 3), confirmed by comparing with previously reported NMR spectral data (Utari *et al*., 2019)

Cytotoxicity of Quercetin and Quercetin-3-0-rhamnoside Against HeLa Cancer Cell Lines

Quercetin and quercetin-3-0-rhamnoside (quercitrin) examined for cytotoxicity effects on HeLa cancer cells with MTT bioassay in a 24-hour examination (Figure 4) and the IC50 value is shown

in Table 2. In Figure 4, it showed, both quercetin and quercitrin can inhibited the growth of HeLa cells at certain doses., which demonstrated with IC_{50} values respectively were 29.49 and 46.67 µg/mL.

Inhibitory Effect of Quercetin and Quercetin-3-0-rhamnoside on Cell Migration

Quercetin (2.5, 5.0, and 10.0 µg/mL), quercetin 3-O-rhamnoside (10.0, 15.0, and 20.0 µg/mL), and doxorubicin (1.0 µg/mL) were evaluated after 24 and 48 h incubations for their effects on cell migration of the HeLa cell lines by a wound-healing assay (Figure 5). All samples tested showed the inhibition of cell migration at 24 and 48 hours experiments, but in the 48 hours, their effects were decreased. Quercetin and quercetin 3-O-rhamnoside showed concentration-dependent inhibitory effects, and the significant effect ($p<0.05$) was shown by the concentration of 10.0 µg/mL.

DISCUSSION

Quercetin and quercetin-3-O-rhamnoside, are flavonoid compounds derived from nature and are widely found in the daily foods. In this study, these isolated compound from the *E. elatior* leaves by a means of cytotoxicity bioassay-guided fractionation and their molecular structures were determined from their spectroscopic data. Their cytotoxicity in HeLa cervical cancer cells was evaluated along with its inhibitory effect on cell migration. The isolation of quercetin and quercetin-3-O-rhamnoside from the *E. elatior* leaves and evaluation of their cytotoxic activity were reported for the first time.

Quercetin and quercetin-3-O-rhamnoside showed cytotoxic activity in a concentration-dependent manner against HeLa cell lines, which was indicated by their inhibition of cell proliferation as shown in Table 2. These two compounds had higher inhibitory activity against cell proliferation as compared to the active extract and ethyl acetate fraction (Table 1). This may be due to the fact that the extract as well as the ethyl acetate fraction contain diverse compounds of non-specific chemical substances, that may interact with each other and decreased the activity. Quercetin decreased cell viability with the IC_{50} value of 29.49 µg/mL lower than that of quercetin-3-O-rhamnoside (46.67 µg/mL), revealing that quercetin had more potent cytotoxicity against cervical cancer cells. Quercetin as an aglycone which has higher lipophilic properties than quercetin-3-O-

rhamnoside would be easier to penetrate the cell membrane, causing it higher cytotoxicity against cells.

It has been reported that quercetin can inhibit HeLa cell growth as well as induce apoptosis by influencing the cell cycle in the G0 / G1 phase and down - regulating the expression of bcl-2, PI3K, p-Akt, and up regulating Bax and increasing the action of caspases (Rauf et al. , 2018), suppresses cell viability with the initiation of the cell cycle arrest G2 / M phase and apoptosis through a p53-dependent mechanism (Priyadarsini et al., 2010).

Anticancer agents generally not only supress the cancer cell growth, but may also inhibit cell metastases. The inhibition activity of cancer cells in migration plays an important role in cancer development and progession. The inhibition of cell migration to the formation of wound or gap was examined with scratch wound assay (Cory, 2011). In this study, quercetin and quercetin-3-O-rhamnoside evaluated for their inhibition on the migration of the HeLa cancer cell lines inhibited migration of the cells significantly at the concentration of 10 µg/mL when compared to controls, in 24 and 48 hours experiments. The increasing concentrations increased their inhibitory effects, while the longer the incubation time, their effects were decreased. Quercetin has been reported to inhibit cell migration and invasion of several cancer cells, such as glioblastoma cells (Levesque et al., 2012), oral cancer cells (Lai et al., 2013), melanoma cells (Cao et al., 2015), breast cancer (Li et al., 2017) and hepatocellular carcinoma (Lu et al., 2018). Inhibition of cell migration and invasion is caused by inhibition of MMP-2 / MMP-9 (Lai et al., 2013) through the signal transduction pathway on PI3K / Akt (Lu et al., 2018). One of the matrix metalloproteinase (MMP) families is MMP-2 / MMP-9 metalloproteinase (MMP), which plays a role in migration, invasion, and metastasis in various types of cancer. (Lu *et al*.,2018). Hence, quercetin and quercetin-3-O-rhamnoside may have potential as cancer drug candidates that could be able to prevent the spread of cancer cells in the body.

CONCLUSION

Our research provided findings that quercetin and quercetin-3-O-rhamnoside isolated from the *E. elatior* leaves showed their cytotoxic activity and inhibited cell migration against HeLa cells line. These findings support previous evidence that quercetin and its derivative have the potential for therapeutic application in cancer treatment, including cervical cancer treatment.

ACKNOWLEDGEMENT

Authors are thank to Bhakti Kencana University for providing financial assistance and Padjadjaran University for supporting this reseach. And thanks to Dr. Ahmad Faried, MD., SPBS., PhD., Dr. med. Hasan Bashari, dr., M.Kes., Tenny Putri Wikayani, S.ST and Nurul Qomarilla, SKM. for providing and handling HeLa cell lines in the Cell Culture and Cytogenic Laboratory, Faculty of Medicine, Padjadjaran University

REFERENCES

Abdelwahab SI, Zaman FQ, Mariod AA, Yaacob M, et.al. Chemical composition, antioxidant and antibacterial properties of the essential oils of *Etlingera elatior* and *Cinnamomum pubescens Kochummen*. J Sci Food Agric 2010;90(15):2682–8.

Aisyah LS, Yun YF, Herlina T, Julaeha E, Zainuddin A, I. Nurfarida I, et al. Flavonoid compounds from the leaves of *Kalanchoe prolifera* and their cytotoxic activity against P-388 Murine Leukaemia Cells. Natural Product Sciences 2017;23(2):139–145

Cao HH, Cheng CY, Su T, Fu XQ, Guo H, Li T, et al. Quercetin inhibits HGF/c-Met signaling and HGF-stimulated melanoma cell migration and invasion. Mol Cancer 2015;14:103.

Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia. Food Chem 2007;104:1586–93.

Chan EWC, Lim YY, Nor Azah MA. Composition and antibacterial activity of essential oils from leaves of Etlingera species (Zingiberaceae). Inter J Adv Sci Art 2010;1(2):1–12.

Chan EWC, Lim YY, Wong SK. Phytochemistry and pharmacological properties of *Etlingera elatior*: A Review. Pharmacognosy Journal 2011;3(22),6–10.

Cory G. Scratch-Wound Assay. In: Wells C, Parsons M. (eds) Cell Migration. Methods in Molecular Biology (Methods and Protocols). Humana Press 2011.p.769.

Ghasemzadeh A, Jaafar HZE, Rahmat A, Ashkani S. Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of *Etlingera elatior* (Jack) R.M.Sm grown in different locations of Malaysia. International Society for Complementary Medicine Research (ISCMR) 2015;15:335.

Habsah, M, Lajis, NH, Sukari, MA, Yap, YH, Kikuzaki, H, et al. Antitumor-promoting and cytotoxic constituents of *Etlingera elatior*. Malays J Med Sci 2005;12(1) 6–12.

Ibrahim H, Setyowati FM. Etlingera. In: de Guzman CC, Siemonsma JS. Plant resources of South-East Asia. Pudoc Wageningen;1999.p.123–126.

Kusriani H, Subarnas A, Diantini A, Iskandar Y, Marpaung S, Juliana M, Silalahi F. Antioxidant and cytotoxic activities, total phenolic content of leaf, flower, and rhizome extract of thorch ginger (*Etlingera elatior*). Pharmacy 2017;14(01); 51-63

Lachumy SJT, Sasidharan S, Sumathy V, Zuraini Z. Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etlingera elatior* (torch ginger) flowers. Asian Pacific Journal of Tropical Medicine 2010;3(10):769–774.

Lai WW, Hsu SC, Chueh FS, Chen YY, Lin JP, Lien JC, et al. Quercetin inhibits migration and invasion of SAS human oral cancer cells through inhibition of NF-kappaB and matrix metalloproteinase-2/-9 signaling pathways. Anticancer Res 2013;33:1941–50.

Levesque JM, Bousquet-Gagnon N, Beliveau R. Quercetin abrogates IL-6/STAT3 signaling and inhibits glioblastoma cell line growth and migration. Exp Cell Res 2012;318:925–35.

Li J, Zhang J, Wang Y, Liang X, Wusiman Z, Yin Y, Shen Q. Synergistic inhibition of migration and invasion of breast cancer cells by dual docetaxel/quercetin-loaded nanoparticles via Akt/MMP-9 pathway. Int J Pharm 2017;523:300–9.

Lu J, Wang Z. Li S, Xin Q, Yuan M, Li H, Song X, Gao H, Pervaiz N, Sun X, Lu W, Jing T, Zhu Y. Quercetin Inhibits the Migration and Invasion of HCCLM3 Cells by Suppressing the Expression of p-Akt1, Matrix Metalloproteinase (MMP) MMP-2, and MMP-9. Med Sci Monit 2018:24:2583–2589.

Mabry T, Markham KR, Thomas MB. The Systematic Identification of Flavonoids. New York: Springer-Verlag Berlin Heidelberg. 1970. DOI: 10.1007/978-3-642-88458-0

Mosmann T. Rapid Colorimetric Assay for Cellular Grow and Survival 1983;65,55–63.

Priyadarsini RV, Murugan RS, Maitreyi S, Ramalingam K, Karunagaran D, Nagini S. The flavonoid quercetin induces cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells through p53 induction and NF-κB inhibition, European Journal of Pharmacology 2010;649:84–91.

Rauf A, Imran M, Khan IA, Rehman MU, Gilani SA, Mehmood Z, Mubarak MS. Review: Anticancer potential of quercetin: A comprehensive review. Phytotherapy Research 2018; 32(11):2109-2130.

Shafabakhsh R and Asemi Z. Quercetin: A natural compound for ovarian cancer treatment. Journal of Ovarian Research 2019;12(55); 1-9

Shaikh AM, Shrivastava B, Apte KG, Navale SD. Medicinal Plants as Potential Source of Anticancer Agents: A Review, Journal of Pharmacognosy and Phytochemistry 2016;5(2):291–295.

Shahid Ud Daula AFM and Basher MA. Genus Etlingera - A review on chemical composition and antimicrobial activity of essential oil. Academic Journals 2019;13(7) 135–156.

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nature methods. 2012;9(7):671–5. Epub 2012/08/30.

Utari F, Itam A, Syafrizayanti S, Putri WH, Ninomiya M, Koketsu M, et al. Isolation of flavonol rhamnosides from *Pometia pinnata* leaves and investigation of α-glucosidase inhibitory activity of flavonol derivatives. Journal of Applied Pharmaceutical Science 2019;9(08): 053–065.

FIGURES AND TABLE

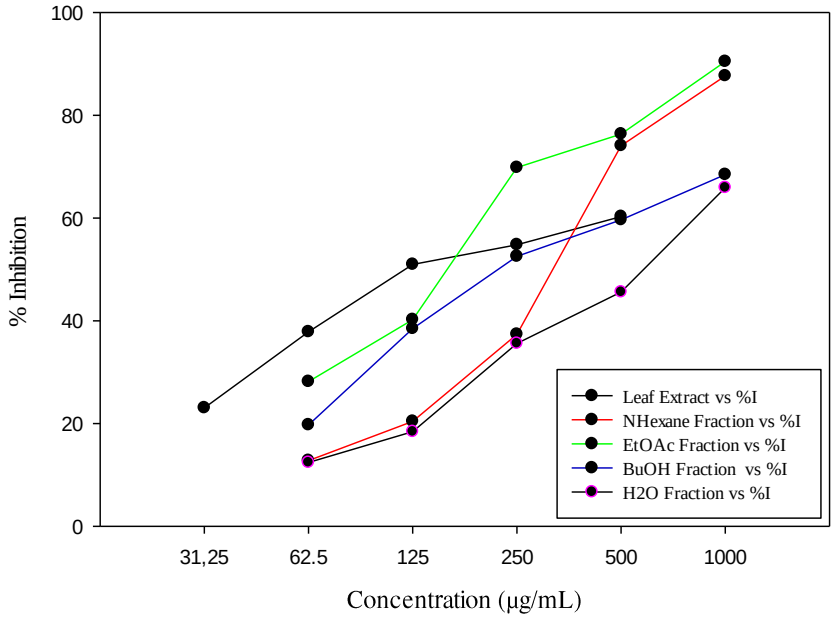

Figure 1. Inhibition of ethanol extract and fractions of *Etlingera elatior* leaves against HeLa cancer cells

Figure 2. Quercetin

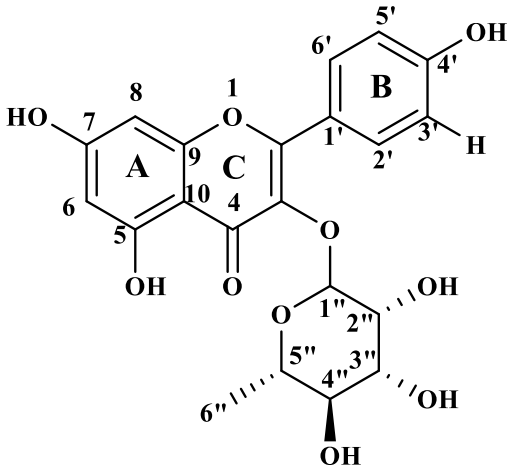

Figure 3. Quercetin-3-0-rhamnoside

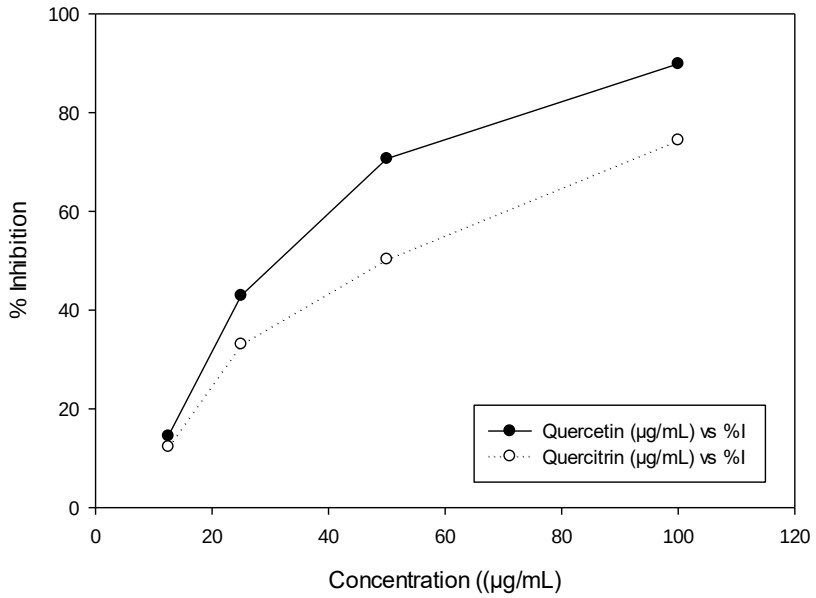

Figure 4. Inhibition of quercetin and quercetin-3-O-rhamnoside of *E. elatior* against HeLa cancer cells.

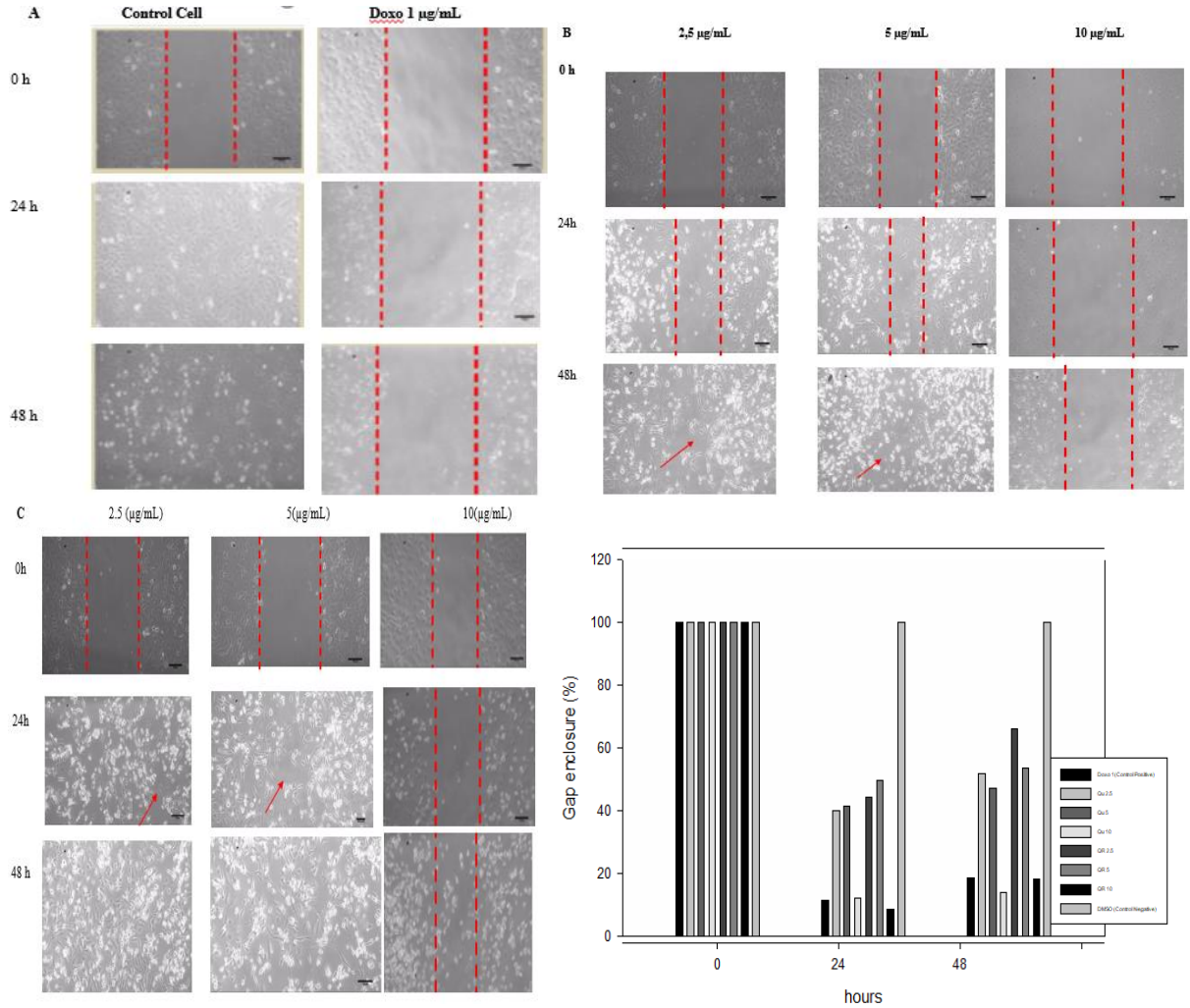

Figure 5 Effect of doxorubicin (A), quercetin (B) and quercetin 3-O-rhamnoside (C) on cell migration in HeLa cell lines.

Note: Figures A , B and C are representative migration photographs of HeLa cell with doxorubicin (A), quercetin (B) and quercetin 3-O-rhamnoside (C). HeLa cells were untreated and treated sample for 24 and 48 h. Quantification gap enclosure (% area) of HeLa cells (D). Value represent mean ± SD (n=3), $p < 0.05$ to control

Table 1. IC_{50} of extract and fractions of *E.elatior* leaves against HeLa cell lines.

Samples	**IC_{50} (μgmL^{-1})***
Extract	127.98 ± 2.83
n-Hexane fraction	312.47 ± 4.01
EtOAc fraction	156.47 ± 4.12
Butanol fraction	225.68 ± 2.74
Water fraction	561.58 ± 3.08

* Data are presented as mean and SD of triplicate. IC_{50} value were analyzed with Sigmaplot ver.12. four parametric logistic model

Table 2. IC_{50} of quercetin and quercitrin of *E.elatior* leaves against HeLa cell lines.

Samples	**IC_{50} (μgmL^{-1})***
Quercetin	29.49 ±1.35
Quercitrin	46.67 ±1.97

* Data are presented as mean and SD of triplicate. IC_{50} of each sample were analyzed Sigmaplot ver.12. four parametric logistic model