Original Article

Anti-proliferative and total ERK1/2 inhibitory effects of plant flavonols on Human cervical cancer (HeLa) cells

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Abstract

Kaempferol, myricetin and quercetin are abundant flavonols in edible fruit and vegetables. Much previous data has shown the beneficial effects of these flavonols in cancer treatments. Thus, the cytotoxic effects on rhesus monkey kidney epithelial cells (LLC-MK2) and the anti-proliferative effects on human cervical cancer (HeLa) cells through total ERK protein expression of kaempferol, myricetin and quercetin have been investigated. The cytotoxic assay at 24h revealed the high safety of three flavonols on LLC-MK2 cells. Kaempferol (1-1000 μM) did not have any significant effect on the viability of these normal epithelial cells. The cytotoxicity of myricetin and quercetin was 5 μM and 50 μM, respectively. No flavonols could suppress total ERK1/2 protein expression in LLC-MK2 cells. In HeLa cells, kaempferol (5 μM) and quercetin (1 μM) significantly inhibited cell proliferations and total ERK1/2 protein expression. Myricetin significantly reduced cancer cell proliferations at 1 μM without any effect on total ERK1/2 protein expression. In conclusion, kaempferol and quercetin had an inhibitory effect on HeLa cells via reduction of total ERK1/2 protein at 24h. But the anti-proliferative effects of myricetin did not exert via total ERK1/2 protein expression. This study affirms the potency of three flavonols as future chemotherapeutic agents and herbal supplements.

Keywords: ERK, HeLa, kaempferol, myricetin, quercetin

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Introduction

Flavonols belong to a class of flavonoids that are abundantly found in fruit and vegetables (Yao et al., 2004; George et al., 2017). The flavonoids with a ketone group and 3'-hydroxyl group on the C ring structure are called flavonols (Kumar and Pandey, 2013). The three important flavonols; kaempferol, myricetin and quercetin are major flavonoids in many edible tropical plant extracts (Miean and Mohamed, 2001). They are mostly found in black and green tea, onions, broccoli, apples, grapes, coffee and beans (Somerset and Johannot, 2008) with beneficial effects in cancer treatment (Panche et al., 2016; George et al., 2017). Kaempferol exhibits anti-tumor properties (Xie et al., 2013; Qiu et al., 2017) in hepatocellular carcinoma (Guo et al., 2016) and breast cancer (Lee et al., 2017). The apoptotic effects of myricetin have been reported in pancreatic (Phillips et al., 2011), colon (Kim et al., 2014) and thyroid cancer (Jo et al., 2017). As well as quercetin, this ubiquitous flavonol may be used as a potent systemic chemo-therapeutic agent (Mendoza and Burd, 2011; Carvalho et al., 2017) in breast (Su et al., 2016), gastric (Li and Chen, 2017), and bladder cancer (Tao et al., 2017).

The extracellular signal-regulated kinase (ERK) signalling pathway plays a crucial role in cancer cell proliferations and apoptosis (Cagnol et al., 2010). The limitation of total ERK1/2 and phosphorylated ERK activities enhance the death of HeLa cells by down-regulation of Bcl-2 expression and the collapse of mitochondrial membrane potential (MMP) (Chang et al., 2017). Most previous studies have revealed the anti-cancer effects of kaempferol (Jeong et al., 2009; Chen et al., 2013) and myricetin (Lee et al., 2007) by reducing the phosphorylated ERK more than total ERK protein expression. The ERK signalling pathway is also the direct molecular target of quercetin (Lee et al., 2008). However, the direct mechanism of these flavonols on HeLa cells through total ERK protein expression has not been investigated. Thus, this study aims to determine the cytotoxic effect on the normal epithelial cells and the anti-proliferative effect on cervical cancer cells via total ERK1/2 expression. This type of cancer is one of the most common malignancies with high mortality among Asian woman (Patra et al., 2017; Zeng et al., 2017). Knowledge about the efficacy and the ERK signalling mechanism of plant flavonols may be useful for developing new alternative anticancer agents from the nature.

Materials and Methods

Cell cultures and treatments: Rhesus monkey (*Macaca mulatta*) kidney epithelial cells (LLC-MK2) were used to evaluate the cytotoxicity of three flavonols: kaempferol, myricetin and quercetin (Sigma, USA). Various concentrations of flavonols (1, 5, 10, 50, 100, 250, 500 and 1000 µM) in 0.001 % Dimethyl sulfoxide (DMSO) were incubated with epithelial cells for 24h. Human cervical adenocarcinoma (HeLa) cells were used to evaluate the effects of flavonols on cancer cell proliferations. LLC-MK2 and HeLa cells were cultured with Dulbecco's modified Eagle's medium (DMEM) at 37°C. The culture media contained 10% fetal bovine serum (FBS), 100 unit/ml penicillin G sodium, and 100 µg/ml streptomycin. After the cells were confluent in T-75 flasks, they were sub-cultured into 96-well plates at a density of 5×10^3 cells/well for 24h before treatment, followed by determination of the epithelial cell cytotoxicity and the anti-proliferative effects on cervical cancer.

Evaluation of cytotoxicity and anti-proliferative activity: After treatment with various concentrations of flavonols for 24h, the epithelial cell cytotoxicity and the cancer cell proliferations were determined by both calorimetric MTT and SRB assays. The MTT assay has been used as the gold standard to evaluate the cytotoxicity but the results had some variation when detected by the variation observed in the linear range. Thus, the SRB assay was also performed because this assay is suitable for screening the effects of novel therapeutic compounds (van Tonder et al., 2015). The anticancer drug (5-Fluorouracil: 5-FU) and the mitogen-activated protein kinase (MEK) inhibitor (PD-98059) were used as positive control. The MTT assay was modified from Mosmann's method (Mosmann, 1983). After 24h treatment with various reagents, 2 mg/ml 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was incubated for 3h at 37°C with the normal epithelial cells and cancer cells in 96-well plates. DMSO was used to solubilize the intracellular MTT-formazan for 5 mins at room temperature. Then a quantity of formazan that was directly proportional to the viable cell number was determined by using a plate reading spectrophotometer: Synergy™ HT Multi-Detection Microplate Reader (BioTek, USA) at 570 nm wavelength. The cell proliferations were confirmed by SRB calorimetric assay (Vichai and Kirtikara, 2006; Orellana and Kasinski, 2016). After the incubation period, 50 µl ice-cold 50% trichloroacetic acid (TCA) in sterile distilled water were incubated with the cells at 4°C for 60 min. The fixed cells in plates were washed gently 5 times with distilled water and dried at room temperature. The epithelial cells and cancer cells were stained with 50 µl of 0.4% w/v sulphorhodamine B (SRB) dissolved in 1% acetic acid solution for 30 min. Then 1% acetic acid was used to wash unbound the SRB solution 3-5 times and the plates left to air-dry. The culture plates were shaken on a plate shaker for 5 mins at room temperature. The absorbance of SRB dye in cells that was solubilized by 10 mM un-buffered Tris-based solution (pH 10.5) 200 µl /well was measured at 540 nm wavelength by Microplate Reader.

The anti-proliferative activity was calculated as a percentage of cell proliferation using the following formula: Percentage of cell proliferation = (Absorbance of treatment/ Absorbance of control) × 100, the absorbance of non-treatment control and flavonol treatment coming from triplicate wells in each experiment.

Immunoblotting: The cells were incubated with various reagents for 24h and washed by cold phosphate buffer saline (PBS) containing 138 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.46 mM KH_2PO_4 before being lysed with cold extract buffer (50 mM Tris base; 10 mM EDTA; 1% (V/V) Triton X-100; 0.57 mM PMSF; 1.5 µM pepstatin A; 2 µM leupeptin). Protein concentrations in each sample were determined by Bio-Rad protein assay kit (Life Science, Thailand). Equal amounts of protein were loaded into each lane of 12% separating SDS/PAGE gel and electrophorised for 2h at 120 constant voltages. The separated protein bands were transferred onto nitrocellulose membrane and blocked by 5% low fat milk in Tris buffer saline (20 mm Tris (pH 7.6) and 250 mm NaCl). The nitrocellulose membranes were incubated with primary monoclonal antibody for 90 min (Anti-total ERK [dilution 1:500], Anti-beta-actin [dilution 1:5,000]) (Cell Signaling, USA) followed by incubation with anti-rabbit IgG-conjugated horse radish peroxidase (dilution 1:2,000) (Cell Signaling, USA) for 60 mins. The p44/42 MAPK (ERK1/2) antibody was used to detect endogenous levels of total ERK1/2 protein. Immunoblots were developed with 3,3'-Diaminobenzidine; DAB substrate kit (Sigma, USA).

Statistical analysis: The percentage of cell proliferations compared with non-treatment cells are statistically shown as mean ± SD. All statistical analyses were performed using SPSS 21. The half maximal inhibitory concentration (IC_{50}) value was calculated by GraphPad Prism 6. The Image J program was used to determine the density of the protein band on blotting paper. The mean values of data among treatment groups were compared using one-way analysis of variance (ANOVA) and the t-test. The *P*-value < 0.05 was regarded as statistically significant.

Results

The cytotoxic effects of kaempferol, myricetin and quercetin on LLC-MK2 cells: After 24h incubation, various concentrations of kaempferol (1, 5, 10, 50, 100, 250, 500 and 1000 µM) had no effect on the viability of LLC-MK2 cells (Fig.1a). Thus, IC_{50} of kaempferol could not be calculated. Myricetin at 5 µM significantly inhibited normal epithelial cells by MTT assay (77.3 ± 5.6 %; IC_{50} = 81.55 µM). While the inhibitory concentration by SRB assay was 10 µM (IC_{50} = 707.10 µM) (Fig.1b). By both calorimetric assays, the inhibitory concentration of quercetin was 50 µM (86.2 ± 0.8%; IC_{50} = 2,322 µM and 60.2 ± 5.4%; IC_{50}=797.20 µM, respectively) (Fig.1c).

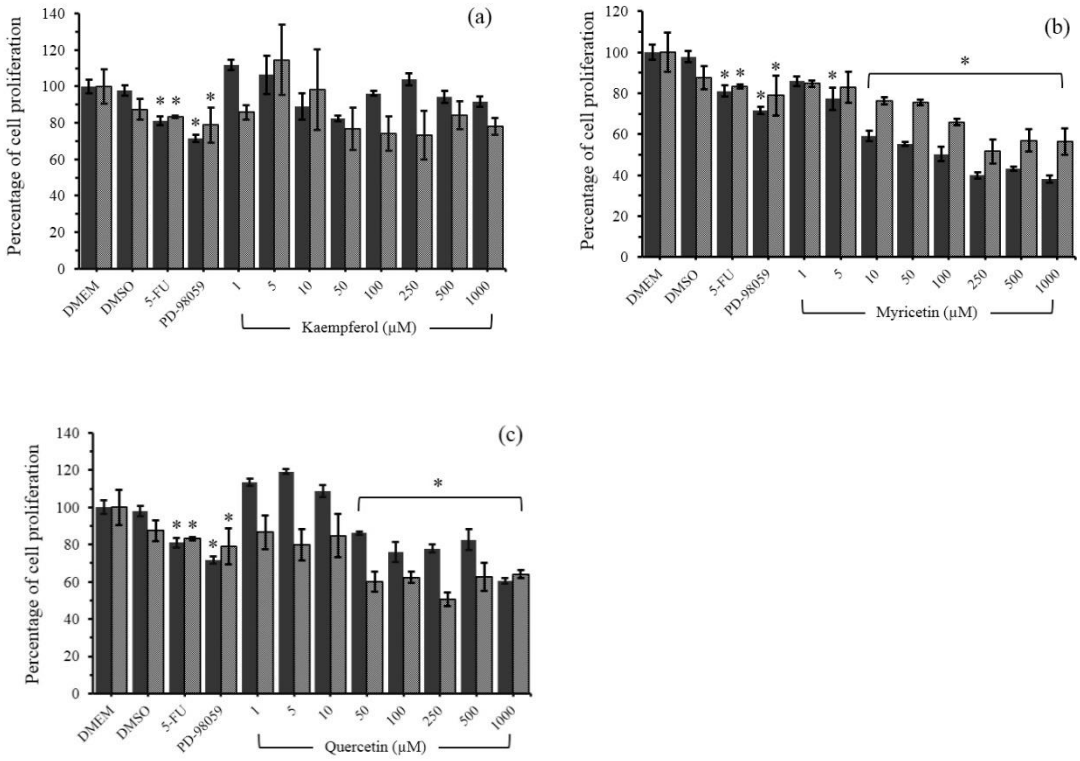

Figure 1 Cytotoxicity of kaempferol (a), myricetin (b) and quercetin (c) on rhesus monkey kidney cells (LLC-MK2) by MTT (Black color bar) and SRB (Gray color bar) assays. Percentage of cell proliferation compared with non-treatment group (control) is represented as mean ± SD (n = 6). * $P < 0.001$ when compared with the control.

Note: Figs.1-4, DMEM: free serum Dulbecco's modified Eagle's medium, DMSO: 0.001 % dimethyl sulfoxide, 5-FU: 20 µM 5-fluorouracil, PD-98059: 20 µM PD-98059.

Anti-proliferative effects of kaempferol, myricetin and quercetin on HeLa cells: The 0.001 % DMSO that was used as a solvent in flavonol solution did not have any cytotoxic effect on either both LLC-MK2 on HeLa cells. The positive control, 5-FU (20 µM) and PD-98059 (20 µM) had inhibitory effects on the proliferations of normal and cancer cells. Fig.2 represents the effects of three flavonols on cervical cancer cell proliferations compared with non-treatment control. After 24h treatment, kaempferol initially had a significant effect on HeLa cell proliferations at 5 µM by MTT method (69.5 ± 3.1%; IC_{50} > 1000 µM). Myricetin significant concentration was 1 µM by both colorimetric assays (MTT-IC_{50}= 9.06 µM, SRB-IC_{50} = 3.27 µM). The results of quercetin were not similar for the two methods. By MTT assay, quercetin could significantly inhibit HeLa cells proliferation at 50 µM (47.3 ± 1.9%; IC_{50} = 43.57 µM). By SRB assay, the inhibitory concentration was 10 µM (61.7 ± 7.5%; IC_{50} = 27.97 µM).

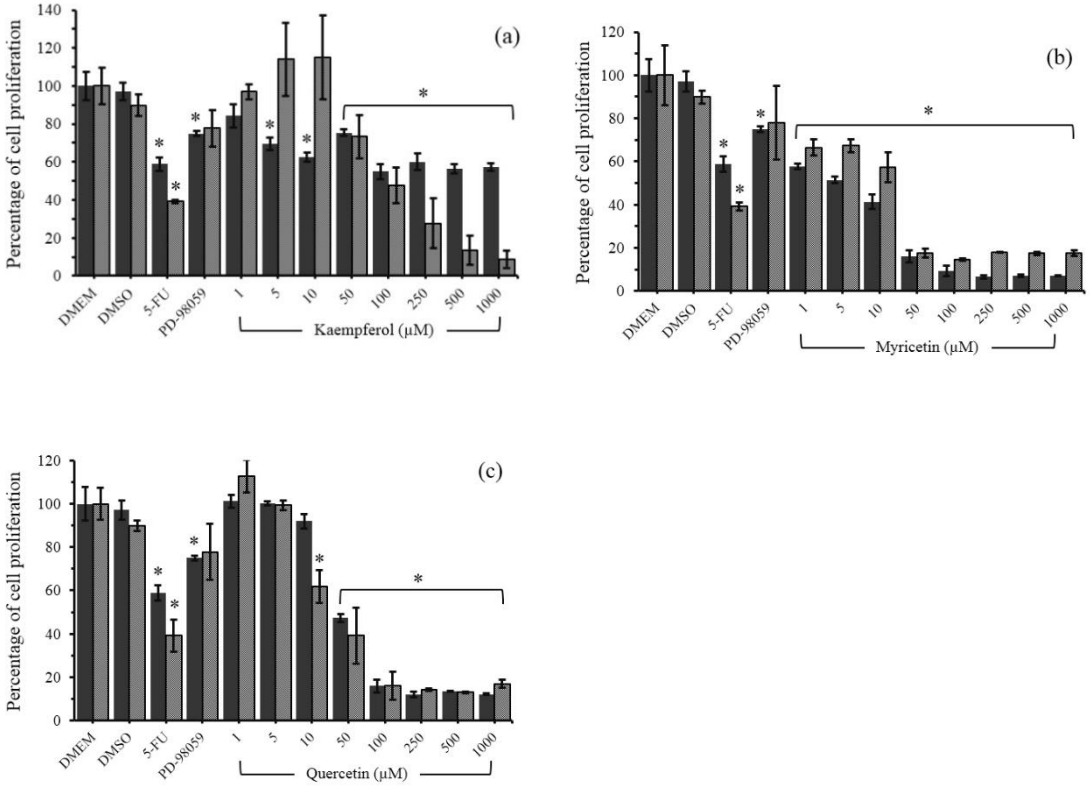

Figure 2 Effects of kaempferol (a), myricetin (b) and quercetin (c) on Human cervical adenocarcinoma (HeLa) cell proliferations by MTT (Black color bar) and SRB (Gray color bar) assays. Percentage of cell proliferation compared with non-treatment group (control) was represented as mean ± SD (n = 6). * $P < 0.001$ when compared with the control.

The effects of three flavonoids on Total ERK1/2 protein expression in LLC-MK2 and HeLa cells: LLC-MK2 and HeLa cells were sub-cultured into 6-well plates with free-bovine serum DMEM before treatment with various reagents for 24h. According to Western blot analysis of total ERK1/2 protein expression, kaempferol, myricetin and quercetin (1, 5, 10 and 50 µM) did not effect normal epithelial cells after incubation (Fig.3). On HeLa cells, myricetin could not inhibit total ERK1/2 protein expression. However, two other flavonols: kaempferol and quercetin had obvious inhibitory effects on cancer cells (Fig.4). According to protein density measurement, all concentrations of kaempferol (1, 5, 10 and 50 µM) decreased the density of protein expression more then 0.57, 0.60, 0.70 and 0.66 times, respectively when compared with non-treatment control. After 24h treatment, 1, 5, 10 and 50 µM quercetin could supress total ERK1/2 less effectively than kaempferol (0.36, 0.46, 0.40 and 0.32 times, respectively). The phosphorylated ERK protein expression at 24h was also evaluated but the protein band did not express at this incubation time (data not shown).

Discussion

The assays of cytotoxic effects revealed the high safety of kaempferol on normal epithelial cells. Compared with the epithelial cells from the human umbilical vein, the IC_{50} of kaempferol, myricetin and quercetin were 20, 100 and 50 µM, respectively (Kim et al., 2006). Myricetin and quercetin exhibited more cytotoxicity than kaempferol on enterocytes of guinea pigs (Canada et al., 1989).

Starting from 5 µM, kaempferol significantly affected HeLa cell proliferations via reduction of total ERK1/2 protein at 24h. Compared with the kaempferol

extracted from *Ginkgo Biloba*, this natural active ingredient inhibited pancreatic cancer cells at a higher concentration (70 μM) and for longer periods (4 days) (Zhang et al., 2008). In osteosarcoma and oral cancer, the anti-cancer activities of kaempferol related to the inhibition of phosphorylated ERK (Lin et al, 2013) but did not effect on total ERK1/2 protein. Differently from lung cancer (Ikari et al., 2012) and cholangiocarcinoma (Qin et al., 2016), kaempferol exhibited anti-proliferation activities without any effects on the ERK protein. Hence, the mechanism of kaempferol may not usually inhibit cancer cell proliferations by reducing the MEK/ERK pathway (Sonoki et al., 2017). Kaempferol had many alternative pathways including suppression of claudin-2 protein (Ikari et al., 2012) or angiogenesis-derived VEGF expression (Luo et al., 2012) after MEK and ERK activations.

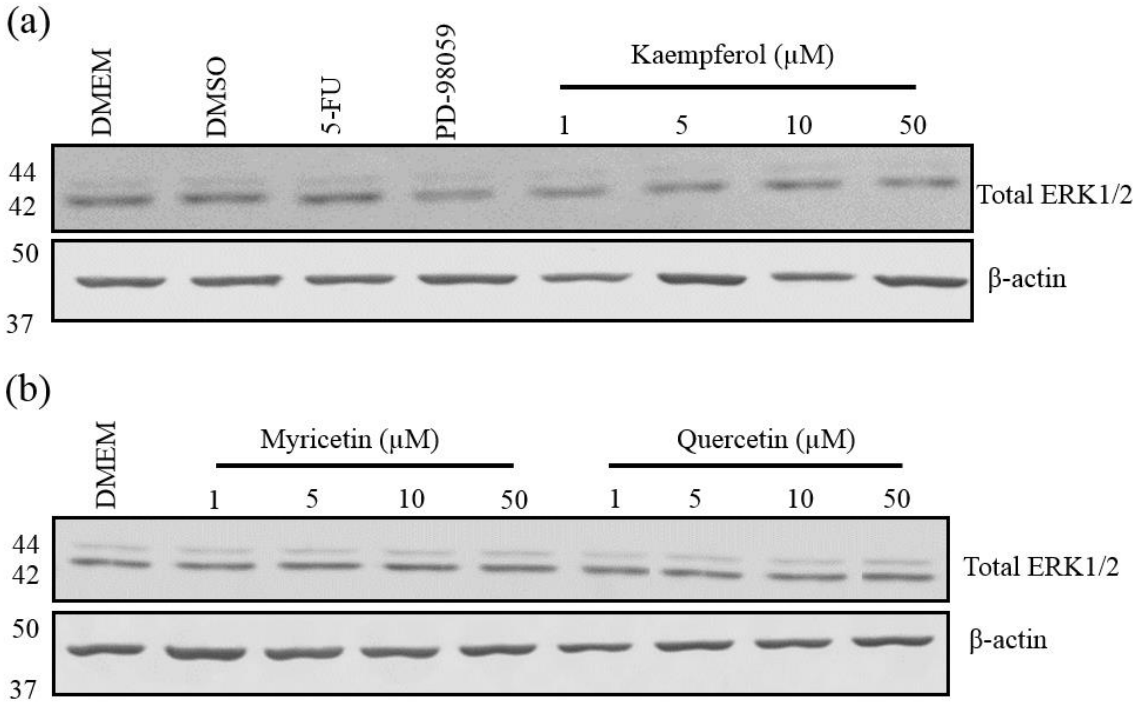

Figure 3 Effects of kaempferol (a), myricetin and quercetin (b) on total ERK protein expression in LLC-MK2 cells.

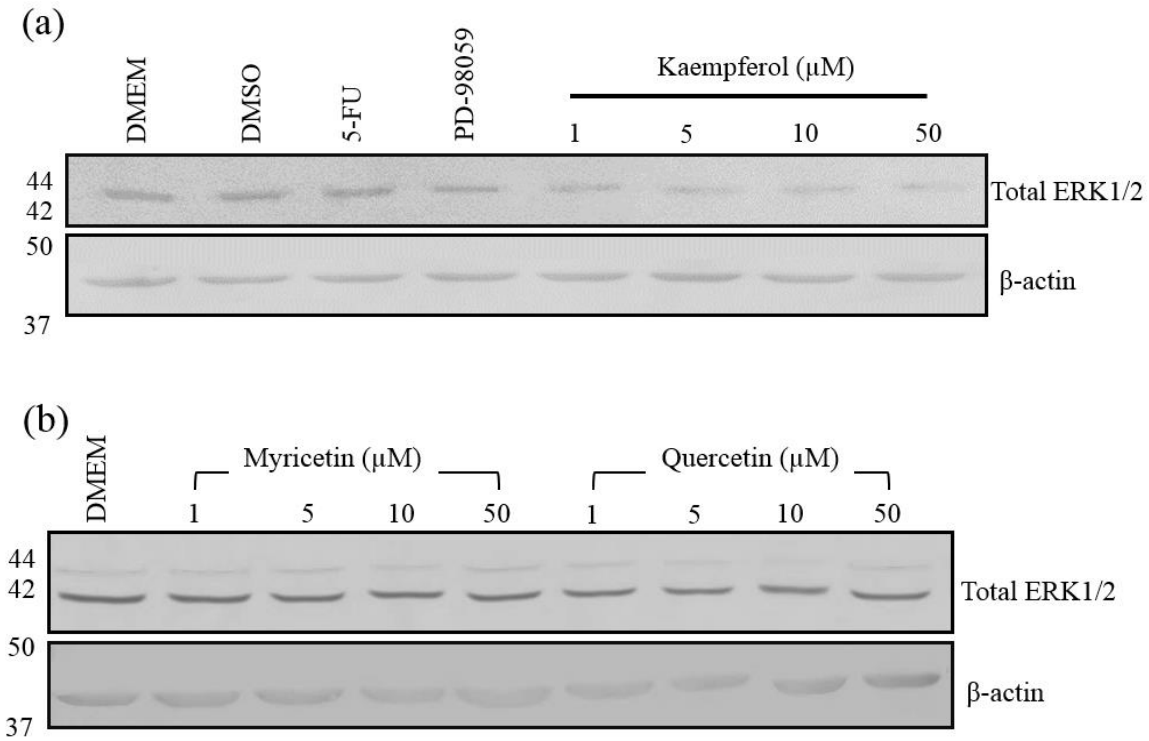

Figure 4 Effects of kaempferol (a), myricetin and quercetin (b) on total ERK protein expression in HeLa cells.

Myricetin, the very common flavonols in berries, vegetables, teas and wines possess anti-proliferative activities against many types of cancer (Semwal et al., 2016). The molecular mechanism of myricetin on cancer cells is related to the restriction of Akt/PKB (protein kinase B), MEK, and Janus kinase–signal transducer and activator of transcription (JAK-STAT) oncoproteins (Devi et al., 2015). The synthetic myricetin derivatives had potency on telomerase inhibition and could down-regulate p65 and telomerase reverse transcriptase (TERT) expression in breast cancer (Xue et al., 2015). Moreover, the suppression of phosphorylated ERK by myricetin could depress cancer cell invasion and migration (Shih et al., 2009). Our data showed the anti-cancer potency of myricetin on human cervical cancer cells at 5 times lower than normal epithelial cells. However, these anti-proliferative effects did not exert via ERK1/2 protein reduction. Previous studies demonstrated that the anti-cancer mechanism of myricetin on human cervical cancer cells could occur by inducing caspase-3 activity (Yi et al., 2015) and a p53-dependent apoptotic pathway (Huang et al., 2015).

Quercetin had anti-proliferative effects on HeLa cells as well as other nine human cancer cells including colon, prostate, pheocromocytoma, breast, lymphoblastic leukemia, myeloma, lymphoid and ovarian cancer cells (Hashemzaei et al., 2017), with harmless effects on normal fibroblasts (Mier-Giraldo et al., 2017). Quercetin could exert anti-tumor effects via total ERK1/2 inhibition in HeLa cells and Human hepatocellular carcinoma (HepG2) (Boonmasawai et al., 2017). In addition, quercetin also down-regulated lipopolysaccharides (LPS)-induced JNK and ERK phosphorylation in macrophage (Park et al., 2016). In human neuroglioma cells, quercetin induced autophagy and apoptosis of cancer cells downstream after ERK activation (Lou et al., 2016). Autophagy had antitumor effects under certain conditions (Lei et al., 2017). The quercetin-induced autophagy could be reduced by ERK phosphorylation inhibitor (Zhao et al., 2017). Quercetin could inhibit cell proliferations and induce protective autophagy at low concentrations in HeLa cells (Wang et al., 2016).

In conclusion, kaempferol (5 μM) and quercetin (1 μM) could significantly inhibit HeLa cell proliferations through reduction of total ERK protein at 24h. The anti-proliferative effects of myricetin (1 μM) on these human cervical carcinoma cells did not exert via total ERK1/2 suppression. While the three flavonols also had significant antiproliferative effects on HepG2, kaempferol did not have the inhibitory effects via ERK1/2 protein reduction at 24h (Boonmasawai et al., 2017). Thus, these flavonols have a future in cancer cell therapeutic applications as chemotherapeutic agents and herbal supplements. Many edible plants naturally contain all three flavonols in single trees such as *Dryopteris erythrosora* (Cao et al., 2013), *Matricaria chamomilla* L. (Viapiana et al., 2016) and *Camellia sinensis* (Zhao et al., 2017). Kaempferol, myricetin and quercetin can totally produce the useful effects from some single herb consumption. Therefore, the synergistic effects of three flavonoids should be further investigated.

Conflict of interest: The authors have no conflict of interest to declare

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References

Boonmasawai S, Leesombun A, Chaichoun K, Taowan J, Sariya L and Thongjuy O. 2017. Effects of the three flavonoids; kaempferol, quercetin, and myricetin on Baby hamster kidney (BHK-21) cells and Human hepatocellular carcinoma cell (HepG2) cells proliferations and total Erk1/2 protein expression. J Appl Anim Sci. 10(2): 23-34.

Cagnol S and Chambard JC. 2010. ERK and cell death: mechanisms of ERK-induced cell death-apoptosis, autophagy and senescence. The FEBS journal. 277(1): 2-21.

Canada AT, Watkins WD and Nguyen TD. 1989. The toxicity of flavonoids to guinea pig enterocytes. Toxicol Appl Pharmacol. 99(2): 357-61.

Cao J, Xia X, Chen X, Xiao J and Wang Q. 2013. Characterization of flavonoids from *Dryopteris erythrosora* and evaluation of their antioxidant, anticancer and acetylcholinesterase inhibition activities. Food Chem Toxicol. 51: 242-50.

Carvalho D, Paulino M, Polticelli F, Arredondo F, Williams RJ and Abin-Carriquiry JA. 2017. Structural evidence of quercetin multi-target bioactivity: A reverse virtual screening strategy. Eur J Pharm Sci. 106: 393-403.

Chang HK, Kim DS, Chae JJ, Kim M, Myong JP, Lee KH, Lee MW and Park TC. 2017. Inhibition of ERK activity enhances the cytotoxic effect of peroxisome proliferator-activated receptor gamma (PPARgamma) agonists in HeLa cells. Biochem Biophys Res Commun. 482(4): 843-8.

Chen HJ, Lin CM, Lee CY, Shih NC, Peng SF, Tsuzuki M, Amagaya S, Huang WW and Yang JS. 2013. Kaempferol suppresses cell metastasis via inhibition of the ERK-p38-JNK and AP-1 signaling pathways in U-2 OS human osteosarcoma cells. Oncol Rep. 30(2): 925-32.

Devi KP, Rajavel T, Habtemariam S, Nabavi SF and Nabavi SM. 2015. Molecular mechanisms underlying anticancer effects of myricetin. Life Sci. 142: 19-25.

George VC, Dellaire G and Rupasinghe HPV. 2017. Plant flavonoids in cancer chemoprevention: role in genome stability. J Nutr Biochem. 45: 1-14.

Guo H, Ren F, Zhang L, Zhang X, Yang R, Xie B, Li Z, Hu Z, Duan Z and Zhang J. 2016. Kaempferol induces apoptosis in HepG2 cells via activation of the endoplasmic reticulum stress pathway. Mol Med Rep. 13(3): 2791-800.

Hashemzaei M, Delarami Far A, Yari A, Heravi RE, Tabrizian K, Taghdisi SM, Sadegh SE, Tsarouhas K, Kouretas D, Tzanakakis G, Nikitovic D, Anisimov NY, Spandidos DA, Tsatsakis AM and

Rezaee R. 2017. Anticancer and apoptosis inducing effects of quercetin *in vitro* and *in vivo*. Oncol Rep. 38(2): 819-28.

Huang H, Chen AY, Ye X, Li B, Rojanasakul Y, Rankin GO and Chen YC. 2015. Myricetin inhibits proliferation of cisplatin-resistant cancer cells through a p53-dependent apoptotic pathway. Int J Oncol. 47(4): 1494-502.

Ikari A, Sato T, Watanabe R, Yamazaki Y and Sugatani J. 2012. Increase in claudin-2 expression by an EGFR/MEK/ERK/c-Fos pathway in lung adenocarcinoma A549 cells. Biochim Biophys Acta. 1823(6): 1110-8.

Jeong JC, Kim MS, Kim TH and Kim YK. 2009. Kaempferol induces cell death through ERK and Akt-dependent down-regulation of XIAP and survivin in human glioma cells. Neurochem Res. 34(5): 991-1001.

Jo S, Ha TK, Han SH, Kim ME, Jung I, Lee HW, Bae SK and Lee JS. 2017. Myricetin induces apoptosis of human anaplastic thyroid cancer cells via mitochondria dysfunction. Anticancer Res. 37(4): 1705-10.

Kim JD, Liu L, Guo W and Meydani M. 2006. Chemical structure of flavonols in relation to modulation of angiogenesis and immune-endothelial cell adhesion. J Nutr Biochem. 17(3): 165-76.

Kim ME, Ha TK, Yoon JH and Lee JS. 2014. Myricetin induces cell death of human colon cancer cells via BAX/BCL2-dependent pathway. Anticancer Res. 34(2): 701-6.

Kumar S and Pandey AK. 2013. Chemistry and biological activities of flavonoids: an overview. ScientificWorldJournal. 2013: 162750.

Lee GA, Choi KC and Hwang KA. 2017. Kaempferol, a phytoestrogen, suppressed triclosan-induced epithelial-mesenchymal transition and metastatic-related behaviors of MCF-7 breast cancer cells. Environ Toxicol Pharmacol. 49: 48-57.

Lee KW, Kang NJ, Heo YS, Rogozin EA, Pugliese A, Hwang MK, Bowden GT, Bode AM, Lee HJ and Dong Z. 2008. Raf and MEK protein kinases are direct molecular targets for the chemopreventive effect of quercetin, a major flavonol in red wine. Cancer Res. 68(3): 946-55.

Lee KW, Kang NJ, Rogozin EA, Kim HG, Cho YY, Bode AM, Lee HJ, Surh YJ, Bowden GT and Dong Z. 2007. Myricetin is a novel natural inhibitor of neoplastic cell transformation and MEK1. Carcinogenesis. 28(9): 1918-27.

Lei Y, Zhang D, Yu J, Dong H, Zhang J and Yang S. 2017. Targeting autophagy in cancer stem cells as an anticancer therapy. Cancer Lett. 393: 33-9.

Li H and Chen C. 2017. Quercetin has antimetastatic effects on gastric cancer cells via the interruption of uPA/uPAR function by modulating NF-kappab, PKC-delta, ERK1/2, and AMPKalpha. Integr Cancer Ther. 1534735417696702.

Lin CW, Chen PN, Chen MK, Yang WE, Tang CH, Yang SF and Hsieh YS. 2013. Kaempferol reduces matrix metalloproteinase-2 expression by down-regulating ERK1/2 and the activator protein-1 signaling pathways in oral cancer cells. PLoS One. 8(11): e80883.

Lou M, Zhang LN, Ji PG, Feng FQ, Liu JH, Yang C, Li BF and Wang L. 2016. Quercetin nanoparticles induced autophagy and apoptosis through AKT/ERK/Caspase-3 signaling pathway in human neuroglioma cells: *In vitro* and *in vivo*. Biomed Pharmacother. 84: 1-9.

Luo H, Rankin GO, Juliano N, Jiang BH and Chen YC. 2012. Kaempferol inhibits VEGF expression and *in vitro* angiogenesis through a novel ERK-NFkappaB-cMyc-p21 pathway. Food Chem. 130(2): 321-8.

Mendoza EE and Burd R. 2011. Quercetin as a systemic chemopreventative agent: structural and functional mechanisms. Mini Rev Med Chem. 11(14): 1216-21.

Miean KH and Mohamed S. 2001. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J Agric Food Chem. 49(6): 3106-12.

Mier-Giraldo H, Diaz-Barrera LE, Delgado-Murcia LG, Valero-Valdivieso MF and Caez-Ramirez G. 2017. Cytotoxic and immunomodulatory potential activity of *Physalis peruviana* fruit extracts on Cervical cancer (HeLa) and Fibroblast (L929) cells. J Evid Based Complementary Altern Med. 22(4): 777-787.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 65(1-2): 55-63.

Orellana EA and Kasinski AL. 2016. Sulforhodamine B (SRB) assay in cell culture to investigate cell proliferation. Bio Protoc. 5: 6(21).

Panche AN, Diwan AD and Chandra SR. 2016. Flavonoids: an overview. J Nutr Sci. 5: e47.

Park JY, Lim MS, Kim SI, Lee HJ, Kim SS, Kwon YS and Chun W. 2016. Quercetin-3-O-beta-D-glucuronide suppresses lipopolysaccharide-induced JNK and ERK phosphorylation in LPS-challenged RAW264.7 Cells. Biomol Ther (Seoul). 24(6): 610-5.

Patra S, Upadhyay M and Chhabra P. 2017. Awareness of cervical cancer and willingness to participate in screening program: Public health policy implications. J Cancer Res Ther. 13(2): 318-23.

Phillips P, Sangwan V, Borja-Cacho D, Dudeja V, Vickers S and Saluja A. 2011. Myricetin induces pancreatic cancer cell death via the induction of apoptosis and inhibition of the phosphatidylinositol 3-kinase (PI3K) signaling pathway. Cancer Lett. 308(2): 181-8.

Qin Y, Cui W, Yang X and Tong B. 2016. Kaempferol inhibits the growth and metastasis of cholangiocarcinoma *in vitro* and *in vivo*. Acta Biochim Biophys Sin (Shanghai). 48(3): 238-45.

Qiu W, Lin J, Zhu Y, Zhang J, Zeng L, Su M and Tian Y. 2017. Kaempferol modulates DNA methylation

and downregulates DNMT3B in bladder cancer. Cell Physiol Biochem. 41(4): 1325-35.

Semwal DK, Semwal RB, Combrinck S and Viljoen A. 2016. Myricetin: A dietary molecule with diverse biological activities. Nutrients. 8(2): 90.

Shih YW, Wu PF, Lee YC, Shi MD and Chiang TA. 2009. Myricetin suppresses invasion and migration of human lung adenocarcinoma A549 cells: possible mediation by blocking the ERK signaling pathway. J Agric Food Chem. 57(9): 3490-9.

Somerset SM and Johannot L. 2008. Dietary flavonoid sources in Australian adults. Nutr Cancer. 60(4): 442-9.

Sonoki H, Tanimae A, Endo S, Matsunaga T, Furuta T, Ichihara K and Ikari A. 2017. Kaempherol and luteolin decrease claudin-2 expression mediated by inhibition of STAT3 in lung adenocarcinoma A549 Cells. Nutrients. 9(6): E597.

Su Q, Peng M, Zhang Y, Xu W, Darko KO, Tao T, Huang Y, Tao X and Yang X. 2016. Quercetin induces bladder cancer cells apoptosis by activation of AMPK signaling pathway. Am J Cancer Res. 6(2): 498-508.

Tao T, He C, Deng J, Huang Y, Su Q, Peng M, Yi M, Darko KO, Zou H and Yang X. 2017. A novel synthetic derivative of quercetin, 8-trifluoromethyl- 3,5,7,3',4'-O-pentamethyl-quercetin, inhibits bladder cancer growth by targeting the AMPK/mTOR signaling pathway. Oncotarget. 8(42): 71657-71671.

van Tonder A, Joubert AM and Cromarty AD. 2015. Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC Res Notes. 20(8): 47.

Viapiana A, Struck-Lewicka W, Konieczynski P, Wesolowski M and Kaliszan R. 2016. An Approach based on HPLC-fingerprint and chemometrics to quality consistency evaluation of *Matricaria chamomilla* L. commercial samples. Front Plant Sci. 7: 1561.

Vichai V and Kirtikara K. 2006. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc. 1(3): 1112-6.

Wang Y, Zhang W, Lv Q, Zhang J and Zhu D. 2016. The critical role of quercetin in autophagy and apoptosis in HeLa cells. Tumour Biol. 37(1): 925-9.

Xie F, Su M, Qiu W, Zhang M, Guo Z, Su B, Liu J, Li X and Zhou L. 2013. Kaempferol promotes apoptosis in human bladder cancer cells by inducing the tumor suppressor, PTEN. Int J Mol Sci. 14(11): 21215-26.

Xue W, Song B-A, Zhao HJ, Qi XB, Huang YJ and Liu XH. 2015. Novel myricetin derivatives: Design, synthesis and anticancer activity. Eur J Med Chem. 97: 155-63.

Yao LH, Jiang YM, Shi J, Tomás-Barberán FA, Datta N, Singanusong R and Chen SS. 2004. Flavonoids in food and their health benefits. Plant Foods Hum Nutr. 59(3): 113-22.

Yi JL, Shi S, Shen YL, Wang L, Chen HY, Zhu J and Ding Y. 2015. Myricetin and methyl eugenol combination enhances the anticancer activity, cell cycle arrest and apoptosis induction of cis-platin against HeLa cervical cancer cell lines. Int J Clin Exp Pathol. 8(2): 1116-27.

Zeng Y, Cheng AS, Liu X and Chan CC. 2017. Cervical cancer survivors' perceived cognitive complaints and supportive care needs in mainland China: a qualitative study. BMJ Open. 7(6): e014078.

Zhang Y, Chen AY, Li M, Chen C and Yao Q. 2008. *Ginkgo biloba* extract kaempferol inhibits cell proliferation and induces apoptosis in pancreatic cancer cells. J Surg Res. 148(1): 17-23.

Zhao X, Wang P, Li M, Wang Y, Jiang X, Cui L, Qian Y, Zhuang J, Gao L and Xia T. 2017. Functional characterization of a new tea (*Camellia sinensis*) flavonoid glycosyltransferase. J Agric Food Chem. 65(10): 2074-83.

Zhao Y, Fan D, Zheng ZP, Li ET, Chen F, Cheng KW and Wang M. 2017. 8-C-(E-phenylethenyl) quercetin from onion/beef soup induces autophagic cell death in colon cancer cells through ERK activation. Mol Nutr Food Res. 61(2): 1600437.

บทคัดย่อ

การยับยั้งการเพิ่มจำนวนของเซลล์ และการแสดงออกของโปรตีน total ERK1/2 ของสารกลุ่มฟลาโวนอลจากพืช ที่มีต่อเซลล์มะเร็งปากมดลูก ชนิด Human cervical cancer (HeLa)

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kaempferol myricetin และ quercetin เป็นสารกลุ่มฟลาโวนอล (flavonols) ที่พบมากในผักและผลไม้ที่รับประทานได้ มีรายงานจำนวนมากก่อนหน้านี้ แสดงถึงประสิทธิภาพของฟลาโวนอลดังกล่าว ว่ามีคุณสมบัติอันเป็นประโยชน์ ในการรักษามะเร็งหลายชนิด การทดลองครั้งนี้ จึงได้ทำการทดสอบความเป็นพิษต่อเซลล์ (cytotoxic effects) ของ kaempferol myricetin และ quercetin ที่มีต่อเซลล์ชนิด rhesus monkey kidney epithelial cells (LLC-MK2) และการยับยั้งการเพิ่มจำนวนของเซลล์มะเร็ง (anti-proliferative effects) ผ่านการแสดงออกของโปรตีน total ERK ในเซลล์มะเร็งปากมดลูก (HeLa cells) ผลการทดลองที่ 24 ชั่วโมง แสดงว่าฟลาโวนอลทั้งสามชนิดมีความปลอดภัยสูงต่อเซลล์ชนิด LLC-MK2 ส่วน kaempferol ที่ความเข้มข้น 1-1000 μM ไม่มีผลต่อ viability ของ epithelial cells ความเข้มข้นของ myricetin ที่เป็นพิษต่อเซลล์ปกติ มีค่าเท่ากับ 5 μM สาร quercetin มีผลต่อเซลล์ปกติอย่างมีนัยสำคัญ ที่ความเข้มข้น 50 μM สารฟลาโวนอลทั้งหมด ไม่สามารถลดการแสดงออกของโปรตีน total ERK1/2 ในเซลล์ LLC-MK2 ได้ ส่วนการทดลองในเซลล์ HeLa พบว่า kaempferol (5 μM) และ quercetin (1 μM) ยับยั้งทั้งการเพิ่มจำนวนของเซลล์ HeLa และการแสดงออกของโปรตีน total ERK1/2 ส่วน myricetin ยับยั้งเซลล์มะเร็งอย่างมีนัยสำคัญทางสถิติ ที่ 1 μM แต่ไม่ยับยั้งการแสดงออกของโปรตีน total ERK1/2 สรุปได้ว่า kaempferol และ quercetin สามารถยับยั้งการเพิ่มจำนวนของเซลล์มะเร็งผ่านการแสดงออกของโปรตีน total ERK1/2 ที่ 24 ชั่วโมง แต่ผลการยับยั้งของ myricetin ไม่ได้กระตุ้นผ่านกลไกดังกล่าว การทดลองนี้ยืนยันผลของฟลาโวนอลทั้งสามชนิดว่า มีแนวโน้มที่จะเป็นได้ทั้งยารักษาโรคมะเร็งและอาหารเสริมสมุนไพรในอนาคต

คำสำคัญ: ฟลาโวนอล เซลล์มะเร็งปากมดลูก kaempferol myricetin quercetin

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