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Anti-Metalloproteinase-9 Activities of Selected Indonesian Zingiberaceae Rhizome Extracts in Lipopolysaccharide-Induced Human Vascular Endothelial Cells *In Vitro*

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Abstract: Problem statement: Atherosclerosis is associated with chronic inflammation triggered by bacterial infection that activates the breakdown of extracellular matrix protein by matrix Metalloproteinases (MMPs). Zingiberaceae, a group of tropical food crops grown in Indonesia and other Southeast Asia regions, has been traditionally used for food coloring, seasoning, culinary and medicinal purposes. However, its efficacy as natural vascular protection has not been explored. **Approach:** The research was aimed to investigate the effects of 10 Indonesian Zingiberaceae rhizome extracts on inhibition of MMP-9 expression in human vascular endothelial cells treated with Lipopolysaccharide (LPS) *in vitro* by conducting gelatin zymogram, Western blotting and RT-PCR assays. **Results:** LPS (2 μg mL^{-1}) significantly elevated the expression of MMP-9 secretion, protein and mRNA in the vascular endothelial cells. Selected Zingiberaceae exctracts (5 μg mL^{-1}), i.e., *Curcuma xanthorrhiza*, *C. aeruginosa, C. mangga, C. longa*, *Kaempferia galanga*, *Alpinia galanga* and *Zingiberaceae officinale*, effectively attenuated the expression of MMP-9 secretion, protein and mRNA in LPS-induced vascular endothelial cells. Furthermore, MMP-9 expression was specifically blocked by MAPK inhibitors, i.e., PD98059 (ERK1/2 inhibitor), SB203580 (p38 inhibitor), SP600125 (JNK inhibitor) and PI3K inhibitor (LY294002), indicating that MAPK and PI3K signaling pathways are involved in regulation of MMP-9 gene expression in LPS-induced vascular endothelial cells. **Conclusion:** These results suggest that selected Indonesian Zingiberaceae rhizomes with potent MMP-9 inhibitory activity may scientifically offer the promising therapeutic target in vascular diseases, particularly atherosclerosis.

Key words: Zingiberaceae rhizomes, anti-metalloproteinase-9 activity, Lipopolysaccharide (LPS), vascular endothelial cells, atherosclerotic plaque, pathological processes, extracellular matrix protein, zingiberaceae plant, cell viability

INTRODUCTION

Matrix Metalloproteinases (MMPs) are a group of zinc- and calcium-dependent endopeptidases that play a key role in degradation of Extracellular Matrix (ECM) proteins. These enzymes have been associated with several major cellular physiological and pathological processes, such as inflammation, cancer and vascular-related diseases. In atherosclerosis, MMPs are involved in each stage of atherosclerotic plaque formation, from initial stages to the disintegration of plaque. Plaque formation occurs through the cellular migration and proliferation due to the accumulation of ECM. Degradation of ECM by MMP-2 and MMP-9 causes the migration of smooth muscle cells and lead to plaque formation. At the later stage, thrombotic complications occur by atherosclerotic plaque disruption that caused by the erosion of endothelial cells due to the excessive ECM solution which is mediated by MMP-2 and MMP-9 (Galis and Khatri, 2002).

The use of synthetic inhibitors of MMPs and antibiotics were effectively found to reduce the vascular expression of MMPs, in particular MMP-2 and MMP-9 and delay the progression of atherosclerotic plaque (Rodriguez *et al*., 2007). However, it is noticed that long-term tetracycline use may cause side effects that limit their clinical use. Thus, screening of natural products from tropical plants has recently been a focus f investigation for their possible roles in management of atherosclerosis.

Zingiberaceae, known as ginger family, is a perennial herb plant, terrestrial and rich in aromatic compounds. Zingiberaceae is mostly distributed in Indonesia and other Asian regions and widely used for food coloring, seasoning, culinary and medicinal purposes (Delin and Larsen, 2000). It has been noted

that there are 50 genera and 1300 species of Zingiberaceae plants and most of them are *Curcuma* sp., *Kaempferia* sp., *Alpinia* sp. and *Zingiber* sp. Research evidences demonstrated that Zingiberaceae rhizomes possessed various pharmacological effects such as antioxidant, anti-inflammatory, antimicrobial and anticaries properties (Ross, 2005; Rao *et al*., 2010; Shimoda *et al*., 2010). The rhizome extract of *Zingiber officinale* (ginger) has been reported to have an anti-fungal potential against *Candida albicans* (Atai *et al*., 2009). Among Zingiberaceae rhizomes, *Curcuma mangga* showed the significant antibacterial activities against most Gram-positive and Gram-negative bacteria (Philip *et al*., 2009). Meanwhile, our previous study also demonstrated that *Kaempferia pandurata* Roxb. or finger root strongly exerted MMP-2 and MMP-9 inhibitory effects in *Porphyromonas gingivalis*-treated human gingival and oral epithelial cells *in vitro*, suggesting its potential therapeutic for natural periodontal therapy (Yanti *et al*., 2009; Yanti and Hwang, 2010).

In this study, on searching for potential candidate of several Zingiberaceae plant extracts (Kaempferia sp., Zingiber sp. and Curcuma sp.) with MMP-9 inhibitory effect, we conducted *in vitro* culture cell experiment using human vascular endothelial cells induced by bacterial virulence (lipopolysaccharide/LPS). *In vitro* study was designed to help determine the initial appropriate dose of potential agents.

MATERIALS AND METHODS

Plant materials and sample preparation: Ten Zingiberaceae rhizome plants, i.e. such as *Kaempferia pandurata*, *K. galanga, Alpinia galanga, Zingiber officinale, Z. officinale* Roxb. Var Rubra, *Curcuma xanthorrhiza*, *C. longa, C. zedoria, C. mangga* and *C. aeruginosa* were collected from traditional markets in Bogor. All samples were identified by Herbarium Bogoriense, Bogor Botanical Garden, Bogor. Samples were dried and grinded, followed by extraction two times with 70% ethanol at room temperature for 3 days each and the combined extracts were concentrated in vacuo and freeze dried (yield: ~10% w/w).

Cell culture and cell viability: Human umbilical vascular endothelial cells (HUVEC; ATCC CRL-1730; American Type Culture Collection, Manassas, VA, USA) were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum, 100 units mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin. Cells were incubated in the presence of 5% CO_2 at 37°C. The cells (passage 14-20) were seeded at a concentration of 2×10^5 cells mL^{-1} per 75-cm^2 flask and cultured for 24 h. Cells were then activated with *Eschericia coli* O157:H7 lipopolysaccharide (LPS; Sigma-Aldrich, St. Louis, MO, USA) to enhance the production of MMP-9.

The effects of LPS and Zingiberaceae rhizome extracts on cell viability were evaluated with the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Sigma-Aldrich) colorimetric assay. Zingiberaceae rhizome extracts were dissolved in 100% DMSO and the stock solution of the extract at a concentration of 1.000 μg mL^{-1} was prepared in 10% DMSO. The final concentrations of the extracts ranged from 1-50 μg mL^{-1} in the culture media and all cells were treated with DMSO at a final concentration of 0.1%.

Sample treatment: Cells were seeded at a concentration of 2×10^5 cells mL^{-1} in 6-well plates and cultured for 24 h in DMEM-FBS. After washing with Dulbecco's phosphate-buffered saline (DPBS), the cells were incubated in serum free-DMEM without LPS (negative control group), with 2 μg mL^{-1} LPS (positive control group), or with 2 μg mL^{-1} LPS plus treatment for 24 h. The treatment groups included Zingiberaceae rhizome extract (5 μg mL^{-1}), MMP inhibitors (doxycycline (Sigma-Aldrich) at 5 μg mL^{-1} and GM6001 (Calbiochem, San Diego, CA, USA) at 10 μM) and signaling inhibitors (PD98059, SB203580, SP600125 and LY294002 at total concentrations of 10 μM). All signaling inhibitors were purchased from Calbiochem. Conditioned medium and cellular lysates were collected for further experiments.

Gelatin zymogram: Secretion of MMP-2 and MMP-9 in the conditioned medium was measured by gelatin zymography. Briefly, the conditioned media from the negative control, positive control and treatment group (Zingiberaceae rhizome extracts, MMP inhibitors and signaling inhibitors) were collected and subjected to electrophoresis with 10% SDS polyacrylamide gels containing 0.1% gelatin. Electrophoresis was run at 90 V for 1.5 h in an electrophoretic apparatus (Bio-Rad Mini Protean 3 Cell, Bio-Rad Laboratories, Hercules, CA, USA). After electrophoresis, gels were washed twice with 25 mL of 2.5% Triton X-100 on a gyratory shaker for 1 h at room temperature to remove SDS. Gel was then incubated in 25 mL reaction buffer (50 mM Tris-HCl, pH 7.5, 10 mM $CaCl_2$, 0.15 M NaCl) at 37ºC for 24 h, stained with Coomassie brilliant blue R-250 and destained with methanol-acetic acid in water.

MMP-2 and MMP-9 were detected at 72 and 92 kDa as clear zones against the dark background. Relative band densities were analyzed by LAS 3000 Bio Imaging Analysis System (Lab Science, Fuji Film, Tokyo, Japan) and calculated by Multi Gauge software (Lab Science, Fuji Film, Tokyo, Japan).

Western blotting: To determine MMP-9 protein expression, the conditioned media from the negative control, positive control and treatment group (Zingiberaceae rhizome extracts) were concentrated with Fast-Con Protein Concentration kit (Corebio, Belmont, CA, USA) and subjected to Western blotting. Proteins (30 μg) were resolved by 10% SDS-PAGE and transferred to nitrocellulose transfer membranes. The membranes were blocked with 5% skim milk for 1 h at room temperature and then probed with the primary anti-rabbit polyclonal MMP-9 (Cell Signaling Technology, Beverly, MA, USA) at a 1:1000 dilution overnight at 4°C. After three washes, the blots were subsequently incubated with the secondary antibody peroxidase-conjugated anti-rabbit IgG (Cell Signaling Technology) at a 1:4000 dilution for 2 h at room temperature. The blots were stained with SuperSignal West Femto Maximum sensitivity substrate (Thermo Scientific, Rockford, IL, USA) and visualized using a LAS 3000 Bio Imaging Analysis System (Lab Science) and calculated by Multi Gauge software (Lab Science).

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay: MMP-9 and TIMP-1 mRNAs in the cellular lysates were determined by RT-PCR. Total RNAs from cellular lysates of negative control, positive control and treatment group (Zingiberaceae rhizome extracts) were extracted as previously described. The human oligonucleotide primers for MMP-9, TIMP-1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were designed according to a PCR primer selection program at the website of the Virtual Genomic Center from the GenBank database. MMP-9 primers were set up as 5' AATGCTGATGGGAAACCCTGCCAG3' for forward and 5'ACTTCTTGTCGCTGTCAAAGTTCG3' for reverse. TIMP-1 primers were designed as 5'CCTTCTGCAATTCCGACCTC3' for forward and 5'CGGGCAGGATTCAGGCTAT3' for reserve. GAPDH primers were 5' ATTGTTGCCATCAATGACCC3' for forward and 5'AGTAGAGGCAGGGATGAT3' for reverse. The GAPDH housekeeping gene was used as an internal control to standardize the relative expression levels for MMP-9 and TIMP-1. PCR products were separated electrophoretically in a 2% agarose DNA gel and stained with ethidium bromide. Relative band densities were analyzed by LAS 3000 Bio Imaging Analysis System (Lab Science) and calculated by Multi Gauge software (Lab Science).

Statistical analysis: Triplicate experiments were performed throughout this study. All data are presented as the mean ± Standard Deviation (SD). The significance of differences between control and treated groups were statistically analyzed by the paired Student's t-test (*$p<0.05$).

RESULTS

Effect of lipopolysaccharide on induction of matrix metalloproteinase-9 in human vascular endothelial cells: As shown in Fig. 1, LPS at 2 and 5 μg mL^{-1} significantly enhanced MMP-9 secretion in the vascular endothelial cells. Furthermore, we measured the viability of vascular endothelial cells treated with LPS and Zingiberaceae rhizome extracts. MTT colorimetric assay demonstrated that LPS (2 μg mL^{-1}) and Zingiberaceae rhizome extracts (1 and 5 μg mL^{-1}) did not show any cytotoxic effects in the vascular endothelial cells *in vitro* (Fig. 2). Thus, we employed these concentrations for the further study. In general, LPS at 2 μg mL^{-1} was found to significantly increase the level of MMP-9 secretion, protein and mRNA in the vascular endothelial cells as compared with the untreated control (Fig. 3).

Effect of Zingiberaceae rhizome extracts on inhibition of matrix metalloproteinase-9 secretion, protein and mRNA in lipopolysaccharide-induced human vascular endothelial cells: We further determined if Zingiberaceae rhizome extracts possess anti-atherosclerotic potential on the expression of MMP-9 secretion, protein and mRNA in LPS-induced vascular endothelial cells. As shown in Fig. 3, the rhizome extracts of *C. xanthorrhiza*, C. *aeruginosa*, *C. mangga*, *C. longa*, *K. galanga*, *A. galanga* and *Z. officinale* at 5 μg mL^{-1} were found to significantly inhibit the expression of MMP-9 secretion, protein and mRNA as compared to the LPS treatment alone. Interestingly, all rhizome extracts had no effect on TIMP-1 mRNA expression in vascular endothelial cells treated with LPS (Fig. 3d).

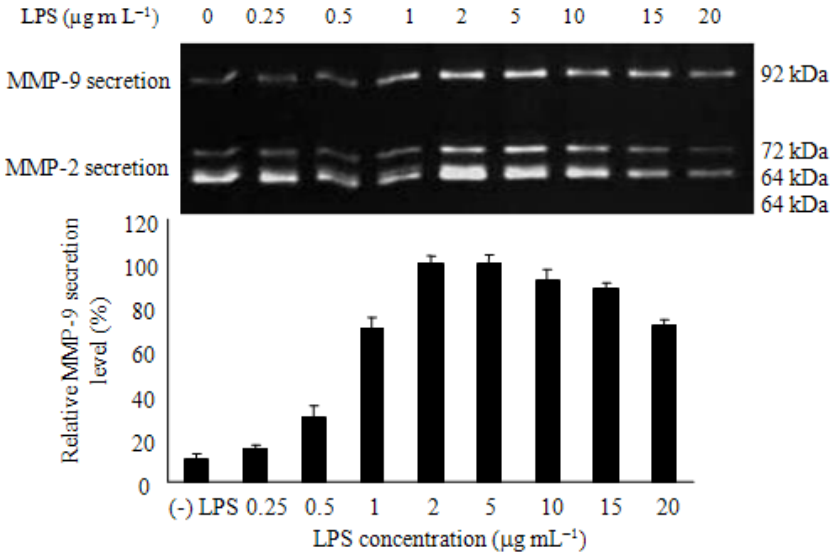


Fig. 1: Dose effect of LPS on regulation of MMP-9 expression in vascular endothelial cells assayed by gelatin zymography. Values represent the mean ± SD of triplicate experiments. * indicates $p<0.05$ against untreated cells

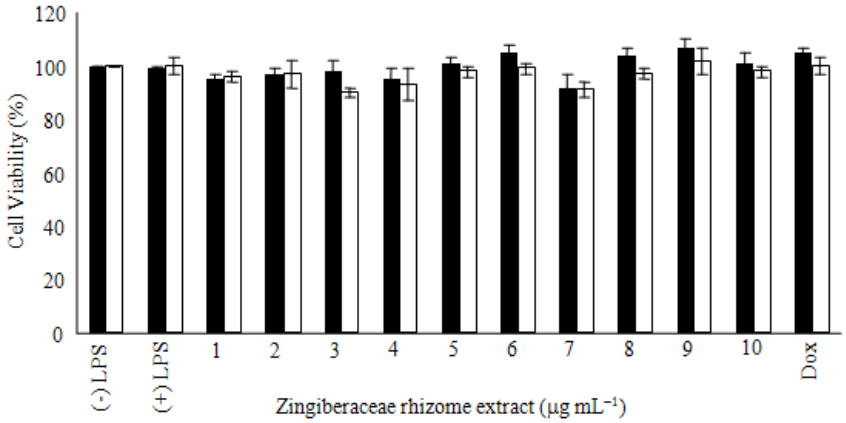
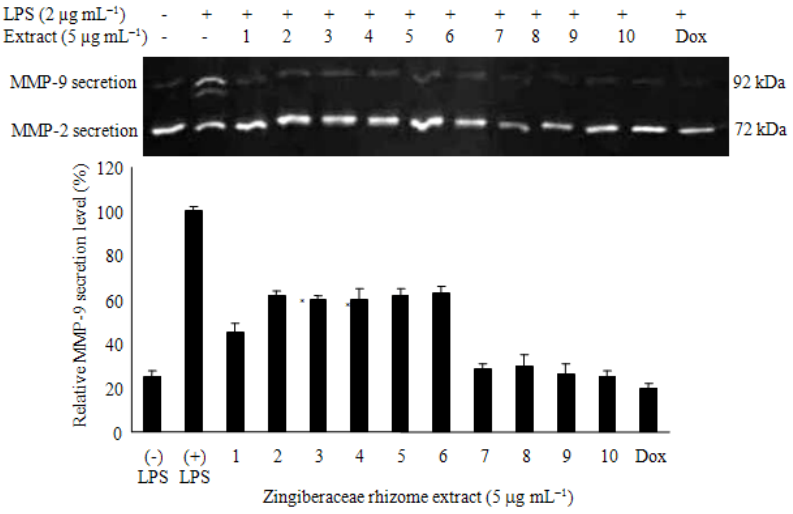
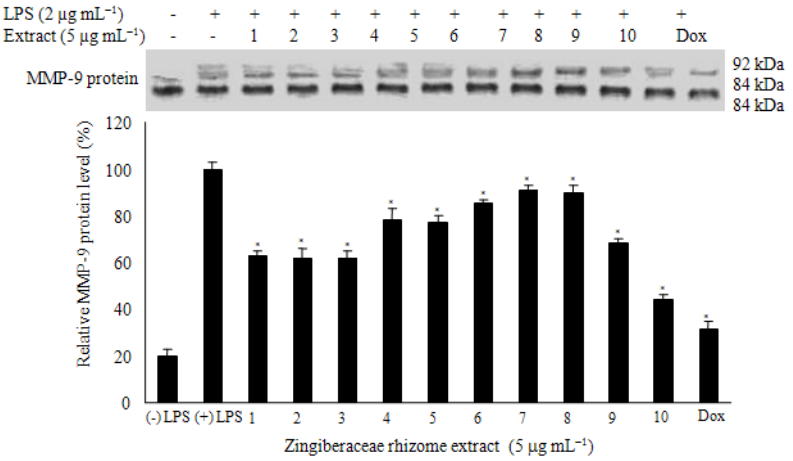


Fig. 2: Effect of LPS (2 μg mL^{-1}) and Zingiberaceae rhizome extracts (1 and 5 μg mL^{-1}) on vascular endothelial cell viability. 1, *Kaempferia pandurata*; 2. *Curcuma xanthorriza*; 3.*C. aeruginosa*; 4. *C. mangga*; 5. *C. zedoaria*; 6. *C. longa*; 7. *K. galanga*; 8. *Alpinia galanga*; 9. *Zingiber officinale*; 10. *Z. officinale Roxb*. Var Rubra.; Dx. Doxycycline (standar MMP inhibitor). Values represent the mean ± SD of triplicate experiments. * indicates $p<0.05$ against LPS-treated cells

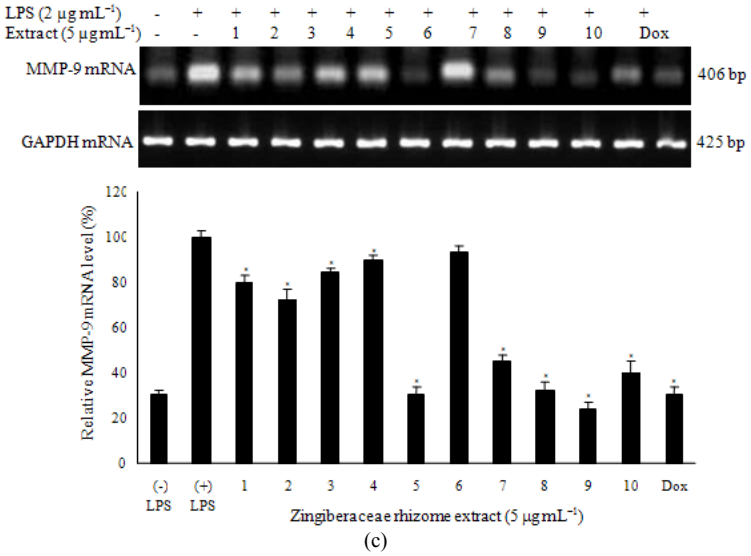
Signaling pathways involved in matrix metalloproteinase-9 expression in lipopolysaccharide-induced human vascular endothelial cells: To determine the signaling pathways mediated the expression of MMP-9 in LPS-induced vascular endothelial cells, we examined the effect of various mitogen-activated protein kinase (MAPK) inhibitors (PD98059, SB203580 and SP600125) and phosphatidylinositol-3 kinase (PI3K) inhibitor (LY294002) in the cell system by performing gelatin zymogram. Our results exhibited that signaling inhibitors of MAPK family and PI3K strongly ameliorated the level of MMP-9 secretion (Fig. 4). Furthermore, the broad spectrum MMP inhibitor, GM6001, was also tested to verify its potential on the expression of MMP-9 in LPS-induced vascular endothelial cells. The results showed that GM6001 strongly blocked MMP-9 secretion (Fig. 4).


LPS (2 μg mL⁻¹) - + + + + + + + + + + + +
Extract (5 μg mL⁻¹) - - 1 2 3 4 5 6 7 8 9 10 Dox
MMP-9 secretion
MMP-2 secretion
92 kDa
72 kDa
Relative MMP-9 secretion level (%)
120
100
80
60
40
20
0
(-) LPS (+) LPS 1 2 3 4 5 6 7 8 9 10 Dox
Zingiberaceae rhizome extract (5 μg mL⁻¹)

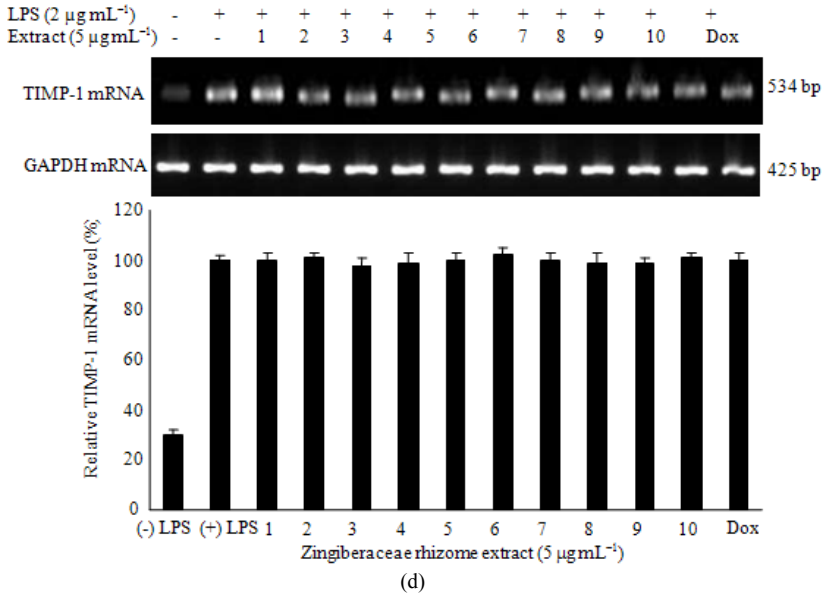
(a)


LPS (2 μg mL⁻¹) - + + + + + + + + + + + +
Extract (5 μg mL⁻¹) - - 1 2 3 4 5 6 7 8 9 10 Dox
MMP-9 protein
92 kDa
84 kDa
84 kDa
Relative MMP-9 protein level (%)
120
100
80
60
40
20
0
(-) LPS (+) LPS 1 2 3 4 5 6 7 8 9 10 Dox
Zingiberaceae rhizome extract (5 μg mL⁻¹)

(b)


LPS (2 μg mL⁻¹) - + + + + + + + + + + + +
Extract (5 μg mL⁻¹) - - 1 2 3 4 5 6 7 8 9 10 Dox
MMP-9 mRNA
GAPDH mRNA
406 bp
425 bp
Relative MMP-9 mRNA level (%)
120
100
80
60
40
20
0
(-) LPS (+) LPS 1 2 3 4 5 6 7 8 9 10 Dox
Zingiberaceae rhizome extract (5 μg mL⁻¹)

(c)



(d)

Fig. 3: Effect of Zingiberaceae rhizome extracts on the expression of MMP-9 secretion (a), MMP-9 protein (b), MMP-9 mRNA (c) and TIMP-1 mRNA (d) in LPS-induced vascular endothelial cells assayed by gelatin zymography, Western blot and RT-PCR. GAPDH was used as the internal control. 1, *Kaempferia pandurata*; 2. *Curcuma xanthorriza*; 3.*C. aeruginosa*; 4. *C. mangga*; 5. *C. zedoaria*; 6. *C. longa*; 7. *K. galanga*; 8. *Alpinia galanga*; 9. *Zingiber officinale*; 10. *Z. officinale Roxb*. Var Rubra.; Dx. Doxycycline (standar MMP inhibitor). Values represent the mean ± SD of triplicate experiments. * indicates $P < 0.05$ against LPS-treated cells

DISCUSSION

The HUVECs were used as the *in vitro* culture cell model for vascular diseases particularly atherosclerosis since they represent the similarity with in vivo experiments. Human atherosclerotic plaques are heterogenous tissues containing various cell types, including macrophages, endothelial and smooth muscle cells within an accumulation of lipid and extracellular matrix proteins (Ekmekci and Ekmekci, 2006). Vascular endothelial cells were found to primarily produce MMP-2 and LPS treatment at various doses induced the production of MMP-9 secretion in the cells. In our study, LPS strongly increased MMP-9 secretion in the cells (Fig. 1), suggesting that bacterial infection also involved in inflammation-related vascular diseases including atherosclerosis. Other evidences also reported that both LPS and thrombin exerted the potential stimulating effect on MMP-9 secretion in vascular endothelial cells (Bedoui *et al*., 2005; Snoek-Van Beurden and Von den Hoff, 2005). LPS has also been recognized as the major inducer for the production of pro-inflammatory cytokines, including Tumor Necrosis Factor (TNF)-α and interleukin (IL)-6, which in turn stimulates inducible Nitric Oxide Synthase (iNOS) induction during the inflammatory process in various inflammatory cells (Chang *et al*., 2009).

The rhizomes of Zingiberaceae have been frequently used as spices and traditional drugs in medicine. However, their potentials as candidates for cardiovascular protection have not been explored yet. In this study, we found that selected Zingiberaceae rhizomes, i.e., *C. xanthorrhiza, C. aeruginosa, C. mangga, C. longa, K. galanga, A. galanga* and *Z. officinale*, exerted the potential inhibitory effects on MMP-9 expression at secretion, protein, and gene levels in LPS-induced vascular endothelial cells *in vitro* (Fig. 3). In addition, our results also showed that all rhizome extracts did not have inhibitory effect on TIMP-1 mRNA expression in vascular endothelial cells treated with LPS (Fig. 3d), suggesting that they might not be involved in regulation of the endogenous TIMPs in the cell system. In contrast, *A. pricei* extract, the Taiwanese Zingiberaceae rhizome, possessed a dual potential activities for decreasing MMP-9 expression and increasing MMP-9 endogenous inhibitor (TIMP-1) expression in squamous carcinoma cells *in vitro* (Hseu *et al*., 2009).

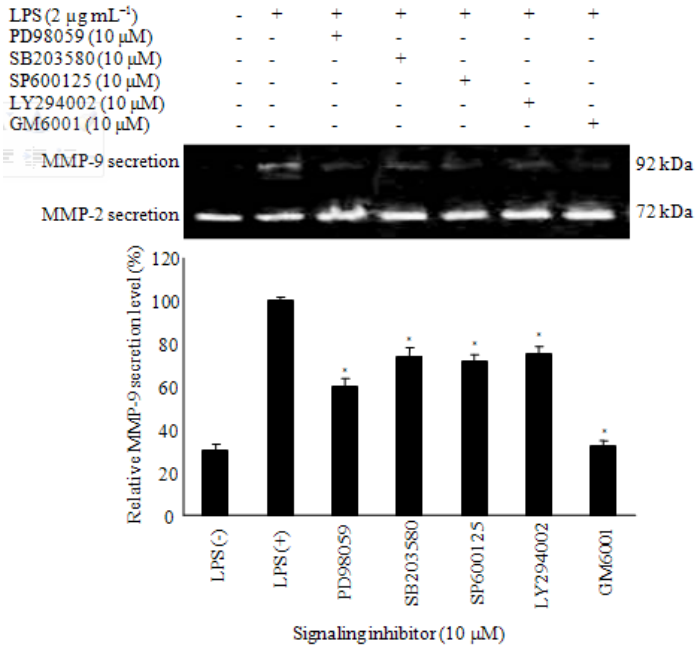


Fig. 4: Effect of various signaling inhibitors on the expression of MMP-9 secretion in LPS-induced vascular endothelial cells assayed by gelatin zymography. PD98059, ERK1/2 inhibitor; SB203580, p38 inhibitor; SP600125, JNK inhibitor;LY294002, PI3K inhibitor; GM6001, MMP inhibitor. Values represent the mean ± SD of triplicate experiments. * indicates $P < 0.05$ against LPS-treated cells

The inhibitory molecular mechanisms of selected Zingiberaceae rhizome extracts on MMP-9 expression in LPS-induced vascular endothelial cells are not clearly understood. The role of MMP-9 is complex because multiple pathways are involved, including the transcriptional and post-transcriptional levels, secretion, zymogen activation and proteolytic inhibition (Chakraborti *et al*., 2003). Evidences demonstrated that regular consumption of fruits and vegetables containing polyphenolic compounds, i.e. cranberry, grape, green tea, citrus fruit, pumpkin, cocoa and red wine, is associated with decreased risk for cardiovascular disease (Stoclet *et al*., 2004; Santaangelo *et al*., 2007). It has been assumed that the presence of polyphenols in plant-derived foods is linked to their antioxidant activity and their direct effect on vascular endothelial cell growth and the expression of genes involved in atherosclerosis and angiogenesis (Nicholson *et al*., 2008). Our findings indicate that selected Zingiberaceae rhizome extracts with potential anti-MMP-9 activities could represent beneficial diet in terms of cardiovascular protection. Further study is still needed to investigate whether polyphenols derived from Zingiberaceae rhizome extracts are responsible for anti-atherosclerotic activity through modulation of MMP-9 expression in LPS-induced vascular endothelial cells.

Inhibition of MMP-9 expression via signaling pathways in LPS-induced vascular endothelial cells was preliminary determined by conducting gelatin zymography. All MAPK inhibitors (PD98059, SB203580 and SP600125) and PI3K inhibitor (LY294002) significantly abolished the expression of MMP-9 secretion (Fig. 4), indicating that these signaling pathways involved in MMP-9 expression in LPS-induced vascular endothelial cells. Among MAPK family, p38 and JNK have been considered to play critical roles in the regulation of the expression of pro-inflammatory mediators. It has been reported that LPS stimulated phosphorylation of p38 and JNK in macrophages since these signaling pathways were mostly associated with various cellular stress responses, such as oxidative stress, inflammation and apoptosis (Lee and Kim, 2009). LPS was also known to activate the expression of MAPK family and promote the upregulation of intercellular adhesion molecule (ICAM)-1 protein and mRNA expression in the vascular endothelial cells *in vitro* (Yan *et al*., 2002). Other study exhibited that MMP-9 expression was strongly induced by ICAM-1 mediated cell adhesion to the endothelial cells (Sampson *et al*., 2004). Moreover, the induction of proinflammatory cytokine IL-1β also significantly downregulated MMP-9 expression in

fibroblasts *in vitro*, thus subsequently inhibited PI3K signaling pathway mediated the prevention of inflammation (Ruhul *et al*., 2003).

In addition, GM6001, the broad spectrum MMP inhibitor, was also employed to confirm the molecular mechanisms participated in the expression of MMP-9 in LPS-induced vascular endothelial cells. GM6001 significantly reduced the production of MMP-9 secretion, indicating its potent MMP-9 inhibitory activity and its exact signaling mechanisms involved in LPS-induced vascular endothelial cells *in vitro* (Fig. 4). MMP-9 plays a pivotel role in the pathogenesis of vascular diseases, such as atherosclerosis and angiogenesis. In the presence of inflammatory cells and stimulation by virulence factors, the expression of MMP-9 in atherosclerotic plaques is enhanced, that subsequently leads to weaken the arterial wall and thus contributes to destabilization and rupture of atherosclerotic plaques (Galis and Khatri, 2002). Meanwhile, recent report showed that cardiotrophin (CT)-1, a member of the IL-6 cytokines, has contributed to both atherosclerotic plaque development and plaque destabilization in patients with acute myocardial infarction (Jahromi *et al*., 2010). It has been recognized that the efficacy of MMP-9 inhibitors is correlated with the use of cell types and inducers. Induction of phorbol 12-myristate 13-acetate (PMA) increased MMP-9 expression in endothelial cells, i.e. ECV304 and HUVECs, *in vitro*. In contrast, stimulation with proinflammatory cytokine TNF-α only significanlty triggered MMP-9 expression in ECV304 cells, but not in HUVECs (Genersch *et al*., 2000).

CONCLUSION

In summary, our results demonstrated that selected Zingiberaceae rhizome exctracts, i.e., *C. xanthorrhiza*, C. *aeruginosa*, C. *mangga*, C. *longa*, *K. galanga, A. galanga* and *Z. officinale*, significantly inhibited the expression of MMP-9 secretion, protein and mRNA in LPS-induced vascular endothelial cells. Signaling pathways of MAPK and PI3K families were mainly involved in MMP-9 expression in LPS-induced vascular endothelial cells. Daily consumption of Zingiberaceae rhizomes may represent a beneficial diet in terms of cardiovascular protection. Further study on searching for the potent bioactive compounds derived from Zingiberaceae rhizome plants which significantly responsible for inhibition of MMP-9 expression in LPS-induced vascular endothelial cells is still needed.

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