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Research Article

In vitro study of *Myristica fragrans* seed (Nutmeg) ethanolic extract and quercetin compound as anti-inflammatory agent

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ABSTRACT

Background: Inflammation is one of an important biological response toward injury. Cytokine and mediator are produced by macrophage during the inflammatory process. Anti-inflammatory is important to treat the dangerous of chronic inflammation associated with chronic disease. Various plants and their derived compounds have been used in the treatment of inflammation including *Myristica fragrans*. The present study was designed to determine anti-inflammatory potential of *M. fragrans* seed (Nutmeg) ethanolic extract and pure quercetin extract from *M. fragrans* on LPS stimulated-murine macrophage cell line (RAW 264.7).
Methods: Cell viability assay to evaluate the non toxic concentration in cell line was performed by MTS assay. The anti-inflammatory potential was assayed through the inhibitory activity of *M. fragrans* seed extract and quercetin on NO, TNF-α, IL-6, and IL-1β production.
Results: The lowest cytotoxic activity and safe substance on RAW 264.7 cell were 50 and 10 µg/mL concentration of the *M. fragrans* seed ethanolic extract and quercetin compound. *M. fragrans* dose-dependently inhibited NO, TNF-α, IL-6 and IL-1β production on LPS stimulated-RAW 264.7. The 50 µg/mL of *M. fragrans* seed ethanolic extract showed the highest TNF-α, IL-6, IL-1β and nitrite-associated with NO inhibitory activity.
Conclusions: This research suggested that *M. fragrans* seed extract and quercetin compound possess the anti-inflammatory potential showed through the inhibition of TNF-α, IL-6, IL-1β and NO secretion.

Keywords: Anti-inflammatory, *Myristica fragrans*, Quercetin, Cytokine, RAW 264.7

INTRODUCTION

Inflammation is the complex biological response to overcome the harmful stimuli which aimed as host defense and usually activated in most disease conditions including pathogen exposure, cell injury and irritation.[1,2] Inflammation relates to various diseases such as rheumatoid arthritis, inflammatory bowel disease, artherosclerosis, Alzeimer's, and has a role in various kinds of cancer.[3] Macrophage plays an important role in Reactive Oxygene Species (ROS), Reactive Nitrogene Species (RNS), cytokines [Interleukine (IL)-1β , IL-6, Tumor Necrosis Factor (TNF)-α] production and Nitric Oxide (NO) mediated inflammation and prostalglandin. Chronic inflammation is a disregulated response to persistent noxious stimuli and seems to be related to tissue malfunction. Prolonged inflammation is associated with some chronic human disorders including cancer, allergy, arthritis, artherosclerosis and autoimmune dissease, so that anti-inflammatory agent become important.[4,5]

Various substances from plants are playing beneficial role in the prevention and even treatment of different disseases.[6,7] Plants extract are rich of bioactive chemicals and most of them free from adverse effects.[8] Some

herbals shows anti-inflammatory potential.[9] Lipopolysaccharide (LPS)-stimulated-murine macrophage cell line (RAW 264.7) is an appropriate model for evaluating and screening of antiinflammatory agents from plants extract.[10] Macrophages in mammalian immune system play a significant role in immediate defense against foreign agents.[11] LPS is a component of the cell walls of gram negative bacteria that the most powerful activators of macrophages and involves the production of pro-inflammatory cytokines.[12]

M. fragrans is one of medicinal plant that have variety of active phytochemical including vitamins, alkaloids, flavonoids, lignans and phenolic, etc. These compounds render their effects via different mechanism such as metal chelation, inhibition of lipid peroxidation and quenching of singlet oxygent to act as antioxidants.[13] The utility of *Myristica fragrans* has been known used as spice in Indonesia and introduced to Europe. *M. fragrans* has aromatic, stimulant, narcotic, carminative, astringent, aphrodisiac, hypolipidaemic, antithrombotic, anti-platelet aggregation, antifungal, antidysenteric, and antiinflammatory activities.[14] Quercetin is one of the major classes of phytochemicals in *M. fragrans* seeds. Quercetin, the most commonly occuring flavonoid is an excellent antioxidant that is also suggested to possess other beneficial activities.[15,16] The aim of this research is to determine anti-inflammatory potential of *M. fragrans* seed (Nutmeg) ethanolic extract and pure quercetin extract from *M. fragrans* on LPS stimulated-murine macrophage cell line (RAW 264.7).

METHODS

Plant extract preparation

This study was performed to continue the previous study of the antioxidant properties of spices extracts including *M. fragrans* seed extract.[17] The dried seeds of *M. fragrans* were crushed then extracted using ethanol 70%. Extraction was performed using maceration technique with ethanol 70% as a solvent. Every 24 hours the ethanol filtrate was filtered and collected until the filtrate become colorless. Evaporation was performed using 40°C evaporator until the pasta form product was obtained. The extracts were store at 4°C.[18,19] The *M. fragrans* extract and quercetin compound from *M. fragrans* extract was used as the treatment.

Cell culture

The murine macrophage cell line RAW 264.7 (ATCC®TIB-71™) was given by Biomolecular and Biomedical Research Center, Aretha Medika Utama. The RAW 264.7 cells were grown an maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 20% FBS, 100 U/mL penicillin (Invitrogen), 100 µg/mL streptomycin (Invitrogen). The culture was incubated at 37°C in humidified atmosphere and 5% CO_2 until the cells were confluent. The cells then washed and resuspended in DMEM. The cells then seeded on plates and treated using *M. fragrans* extract in different concentration (100 mg/mL, 50 mg/mL. 25 mg/mL, 0 mg/mL) and different concentration of quercetin (100 µM, 50 µM, and 25 µM).[20]

Viability assay

MTS (3- (4,5 – dimethylthiazol-2-yl) – 5 - (3-carboxymethoxyphenyl) -2- (4-sulfophenyl) - 2H-tetrazolium) assay (Promega, Madison, WI, USA) is a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays.[21] The cells were seeded in 96-well plate (5 x 10^3 cells per well) in 100 µL medium (DMEM supplemented with 10% FBS and 100 U/mL penicillin and streptomycin) and incubated for 24 hours at 37°C in a humidified atmosphere and 5% CO_2. The medium then washed and supplemented with 99 µL new medium and 1 µL of *M. fragrans* extract in various concentration (100 mg/mL, 50 mg/mL. 25 mg/mL, 0 mg/mL), quercetin in various concentrations (100 µM, 50 µM, and 25 µM) and DMSO in different plate then incubated for 24 hours. Untreated cells served as the control. 20 µL MTS was added to each well. The plate was incubated in 5% CO_2 at 37°C incubator for 4 hours. The absorbance was measured at 490 nm on microplate reader. The data are presented as the percentage of viable cells (%).[18,19] The viability assay was performed to determine the suitable concentration for the next assay.

Pro-inflammatory activation of cells

Inflammation cell triggered using modified method from Yoon et al.[10] and Khan et al.[22] The cells were seeded in 6-well plate (5 x 10^3 cells per well) and incubated for 24 hours at 37°C in a humidified aatmosphere and 5% CO_2. The medium (DMEM supplemented with 10% FBS and 100 U/mL penicillin and streptomycin) then washed and supplemented with 1600 µL growth medium and 200 µL extract solution or quercetin in different concentration then incubated for 1-2 hours. 200 µL LPS (1 µg/mL) was added into the medium and incubated for 24 hours at 37°C in a humidified atmosphere and 5% CO_2. The growth medium was taken for assay and centrifuged at 2000 g for 10 minutes. Supernatan was stored at -79°C.

Quantification of nitrite concentration and inhibitory activity asssay

Abnova kit (No cat. KA 1342) was used to determine the concentration of nitrite associated with NO production. After pre-incubation of RAW 264.7 cells with LPS and *M. fragrans* extract/quercetin for 24 h, the quantity of nitrite accumulated in the culture medium (DMEM supplemented with 10% FBS and 100 U/mL penicillin and streptomycin) was measured as an indicator of NO production. 200 µL assay buffer was added into the blank well and 100 µL of standard solution with 100 µL assay buffer was added into standard. Briefly, 100 µL of cell

medium was mixed with 100 µL assay buffer. The mixture was incubated at room temperature for 10 minutes and the absorbance at 540 nm was measured in a microplate reader. The quantity of nitrite was determined from sodium nitrite standard curve. The LPS-stimulated cells without extract or quercetin was used as positive control. The normal cell was used as negative control.

Quantification of TNF-α concentration and inhibitory activity assay

BioLegend kit (No. cat 421701) was used to determine the TNF-α concentration in the culture medium.[23] The plate was washed four times using 300 µL wash buffer then 50 µL matrix C was added into the standard well. 50 µL assay buffer was added into each of the sample well. 50 µL sample was added into each of the sample well and 50 µL standard solution was used as standard. The LPS-stimulated cells without *M. fragrans* extract or quercetin was used as positive control. The normal cell was used as negative control. The plate then wrapped and incubated for 2 hours in 200 rpm orbital shaker. All of the solution was washed by 200 µL wash buffer, after two hours of incubation. 100 µL Rat-TNF-α detection antibody was added to each well and incubated in 100 rpm orbital shaker. The plate then washed again using 200 µL wash buffer, after one hour of incubation. 100 µL avidin HRP was added in each well and incubated for 30 minutes in 200 rpm orbital shaker. The plate then washed five times using 200 µL wash buffer. 100 µL substrate solution F was added into each well and incubated in the dark condition for 10 minutes. Stop solution was added into each well and the results then readed using MultiSkan Go Thermoscientific ELISA reader in the 450 nm wavelength.

Quantification of IL-6 concentration and inhibitory activity assay

The ammount of IL-6 in the culture supernatant was measured with a commercial kit (mouse max standard set, BioLegend). Plate was washed using 300 µL wash buffer four times, 50 µL matrixes C was added in to standard well and 50 µL assay buffer was added into sample well. 50 µL samples were introduced in sample well and 50 µL standard solutions were added in standard well then incubated in orbital shaker 200 ppm for 2 hour at room temperature. The LPS-stimulated cells without *M. fragrans* extract or quercetin was used as positive control. The normal cell was used as negative control. 100 µL of Rat IL-6 detection antibody was added then incubated for 1 hours in room temperature 200 rpm orbital shaker. The solution then discharged and plate was washed using 200 µL of wash buffer four times. 100 µL avidin HRP solution was added and kept at room temperature for 30 minutes in orbital shaker 200 ppm. Plate was washed again for 5 times and 100 µl substrate solution F was added then kept for 10 min in dark room. After 10 minutes, 100 µL stop solution was added and absorbance was read by using Miniskan ELISA Reader at 450 nm.[24]

Quantification of IL-1β concentration and inhibitory activity assay

The IL-1β concentration was determined based on BioLegend (No.Cat 432601) kit protocol. 300 µL wash buffer was used to wash the plate for times before the addition of 50 µL matrix C into standard well and 50 µL of assay buffer into sample well. 50 µL standard solution was added into standard well and 50 µL sample was added into the sample well. The LPS-stimulated cells without *M. fragrans* extract or quercetin was used as positive control. The normal cell was used as negative control. Plate then incubated in room temperature 100 rpm orbital shaker. After two hours of incubation the solution was discharged and washed four times using 200 µL wash buffer. 100 µL of substrat solution F then added to the each well then incubated in dark condition. Stop solution the andded into each well to terminite the reaction after 10 minutes of incubation. The absorbance was readed using this MultiSkan Go Thermoscientific ELISA reader in 450 nm of wavelength.[25]

Statistical analysis

All data were derived from three independent experiments. Statistical analysis was conducted using SPSS software (version 17.0). Value were presented as mean ± SD. Significant differences between the groups were determined using the analysis of variat (ANOVA) and Tukey Post Hoc test. Statistical significance was set at $P<0.05$.

RESULTS

Effects of M. fragrans on RAW 264.7 cells viability

RAW 264.7 cell viability assay was the preliminary study to test the effect of *M. fragrans* seed extract and quercetin compound toward RAW 264.7 cell viability. The aims of this assay is to determine the safe and non toxic concentration for the next assay. Viability was measured by MTS assay based on the conversion of yellow tetrazolium salt to form a purple formazan product.[21] The percent of cells viability was determined by comparing the cells viability value of treatments to the control. This viability assays (Table 1) shows that *M. fragrans* seed extract and quercetin in given concentration still available for the normal RAW 264.7 cells except for the concentration of 100 µg/mL. In the highest concentration the RAW 264.7 cells viability was low, indicate that concentration is little bit toxic to the cells so that concentration will not used in the next step of assay.

***M. fragrans* seed extract exhibited the higher nitric oxide (NO) inhibitory activity**

The positive control of this test shows the highest concentration of nitrite concentration compared to the negative control and extract or quercetin treated cells (Table 2). That indicate the LPS succesfully induce the inflammation on the RAW 264.7 cells.[26] The percent of inhibitory activity was determine by the value of positive control nitrite concentration minus the nitrite concentration of treatment devided to the nitrite concentration of positive control. 50 µg/mL *M. fragrans* seed ethanolic extracts shows the highest nitrite inhibitory activity (Table 2). The negative control shows the highest value of nitrite inhibitory too because the normal cell was used. The normal cell did not release much nitrite like LPS-stimulated cells so the inhibitory activity become high.

Table 1: Mean and Tukey HSD post hoc test of RAW 264.7 cell's viability of various concentration extract and quercetin measured in triplicate.

Samples	Viability		
	100 µg/mL	50 µg/mL	10 µg/mL
M. fragrans seed extract	85.27 ± 1.34[a]	90.84 ± 2.08[b]	97.75 ± 1.34[c]
Quercetin	83.21 ± 6.4[a]	92.34 ± 5.78[b]	99.52 ± 9.47[c]

Data were presented as mean ± standard deviation. Different superscript letters ([a,b,c]) in the same row (concentrations) are significant at P<0.05 (Tukey HSD post hoc test)

Table 2: Mean and Tukey HSD post hoc test of nitrite content and nitrite inhibitory activity over the positive control of various concentration extract and quercetin measured in triplicate.

Samples	Nitrite oxide	
	Nitrite concentration (µM)	Nitrite inhibitory activity (%)
Positive control	35.68 ± 0.23[f]	0.0067 ± 0.65[a]
Negative control	4.92 ± 0.49[a]	86.2250 ± 1.37[f]
M. fragrans seed extract 50 µg/mL	21.27 ± 0.03[b]	40.3883 ± 0.091[e]
M. fragrans seed extract 10 µg/mL	25.38 ± 0.10[c]	28.8733 ± 0.29[d]
Quercetin 50 µg/mL	25.81 ± 0.10[d]	27.6650 ± 0.27[c]
Quercetin 10 µg/mL	30.84 ± 0.14[e]	13.5733 ± 0.38[b]

Data were presented as mean ± standard deviation. Different superscript letters ([a,b,c,d,e,f]) in the same row (nitrite concentration and nitrite inhibitory activity) are significant at P<0.05 (Tukey HSD post hoc test)

M. fragrans seed extract posses the higher TNF-α inhibitory activity

Tumor necrosis factor-α (TNF-α) plays an important role as the main cytokine of inflammation processes. The highest TNF-α concentration was shown by the positive control. The *M. fragrans* ethanolic seed extract or quercetin-treated cells shows the lower TNF-α concentration compared to the positive (Table 3). The percent of inhibition was determine by using the value of positive control TNF-α concentration minus the TNF-α concentration of treated cells compare to the TNF-α concentration of positive control. The *M. fragrans* seed extract in 50 µg/mL concentration have the highest inhibitory activity (Table 3). The negative control-LPS-nonstimulated cells show the high value of TNF-α inhibitory activity because it did not release much TNF-α like the LPS-stimulated cells so the inhibition value become high.

Table 3: Mean and Tukey HSD post hoc test of TNF-α concentration and TNF-α inhibitory activity over the positive control of various concentration extract and quercetin measured in triplicate.

Samples	TNF-α	
	TNF-α concentration (pg/mL)	TNF-α inhibitory activity (%)
Positive control	500.17 ± 7.74[f]	0.00 ± 1.55[a]
Negative control	251.20 ± 14.06[a]	49.78 ± 2.81[f]
M. fragrans seed extract 50 µg/mL	280.90 ± 6.20[b]	43.84 ± 1.24[e]
M. fragrans seed extract 10 µg/mL	348.20 ±10.36[c]	30.38 ± 2.07[d]
Quercetin 50 µg/mL	368.30 ± 15.92[d]	26.37 ± 3.18[c]
Quercetin 10 µg/mL	438.73 ± 13.83[e]	12.28 ± 2.77[b]

Data were presented as mean ± standard deviation. Different letters (^{a,b,c,d,e,f}) in the same row (TNF-α concentration and inhibitory activity) are significant at P<0.05 (Tukey HSD post hoc test)

Both of M. fragrans seed extract and quercetin posses IL-6 inhibitory activity

M. fragrans seed extract and quercetin posses the IL-6 inhibitory activity in concentration dependent manner. The highest inhibitory activity was showed by *M. fragrans* seed extract in the concentration of 50 µg/mL (Table 4). IL-6 is one of cytokine that takes a part in acute inflammation including hematopoiesis, 'response immune' regulation, and inflammation.[24,27] The negative control-LPS-nonstimulated cells show the high value of IL-6 inhibitory activity because it did not release much IL-6 like the LPS-stimulated cells so the inhibition value become high when it compared to the positive control.

Table 4: Mean and Tukey HSD post hoc test of IL-6 concentration and IL-6 inhibitory activity over the positive control of various concentration extract and quercetin measured in triplicate.

Samples	IL-6	
	IL-6 concentration (pg/mL)	IL-6 inhibitory activity (%)
Positive control	912.14 ± 201.31^{b}	0.00 ± 0.00^{a}
Negative control	110.81 ± 12.93^{ab}	87.34 ± 3.55^{c}
M. fragrans seed extract 50 µg/mL	44.76 ± 10.04^{a}	94.83 ± 2.03^{c}
M. fragrans seed extract 10 µg/mL	693.47 ± 176.32^{ab}	24.32 ± 2.57^{ab}
Quercetin 50 µg/mL	307.10 ± 17.13^{ab}	65.32 ± 7.10^{bc}
Quercetin 10 µg/mL	890.90 ± 409.59^{ab}	9.73 ± 51.65^{ab}

Data were presented as mean ± standard deviation. Different letters (^{a,b,ab,bc}) in the same row (IL-6 concentration and inhibitory activity) are significant at P<0.05 (Tukey HSD post hoc test)

Both of M. fragrans seed extract and quercetin exhibited IL-1β inhibitory activity

IL-1β expression as pro-inflammatory cytokine is enhanced following crush injury, infection, invasion and become an important sign of inflammation.[28] *M. fragrans* seed extract posses the highest activity in inhibiting IL-1β. The negative control-LPS-nonstimulated cells show the high value of IL-1β inhibity activity because it did not release IL-1β much like the LPS-stimulated cells so the inhibition value become high (Table 5).

Table 5: Mean and Tukey HSD post hoc test of IL-1β concentration and IL-1β inhibitory activity over the positive control of various concentration extract and quercetin measured in triplicate.

Samples	IL-1β	
	IL-1β concentration (pg/mL)	IL-1β inhibitory activity (%)
Positive control	51.93 ± 8.60^{a}	0.00 ± 0.00^{a}
Negative control	80.77 ± 23.83^{a}	54.38 ± 30.88^{ab}
M. fragrans seed extract 50 µg/mL	59.97 ± 16.78^{a}	100.10 ± 98.25^{b}
M. fragrans seed extract 10 µg/mL	112.36 ± 43.60^{a}	14.56 ± 18.67^{ab}
Quercetin 50 µg/mL	74.97 ± 18.31^{a}	43.66 ± 20.01^{ab}
Quercetin 10 µg/mL	72.84 ± 28.63^{a}	10.00 ± 5.26^{ab}

Data were presented as mean ± standard deviation. Different letters (^{a,b,ab}) in the same row (IL-1β concentration and inhibitory activity) are significant at P<0.05 (Tukey HSD post hoc test)

DISCUSSION

Inflammation is one of an important biological response toward injury. Cytokine and mediator are produced by macrophage during the inflammatory process.[29] Anti-inflammatory is important to treat the dangerous of chronic inflammation associated with chronic disease. Natural phytochemicals plays a significant role in drug discovery. Various plants and their derived compounds have been used in the treatment of inflammation.[9] Anti-inflammatory drugs should have effect on prime causative factors, inhibitory effect or blocking effect on initial reaction set in a biological model by the prime cause and thereby inhibit the established inflammation, and effect on end results of established inflammation.[30] In this *in vitro* study, we evaluated the anti-inflammatory potential of *Myristica fragrans* seed (nutmeg) extract and the quercetin compound from *M. fragrans* through NO,

TNF-α, IL-6 and IL-1β inhibitory activity assays in LPS stimulated-murine macrophages cell line (RAW 264.7). Lipopolysaccharide (LPS) is a pro-inflammatory glycolipid component of the gram negative bacterial cell wall that stimulated inflammation process.[31] The activation of macrophage by LPS leads to secretion of inflammatory molecules such as the inflammatory cytokine including TNF-α, IL-6, IL-1β and the free radical NO, which play an important role in inflammation.[32,33]

The *M. fragrans* seed extract and quercetin compound in 10 and 50 µg/mL of concentration displayed positive result and no toxicity to RAW 264.7. Non toxicity of that substrate was indicated by high percentage of viable cells in viability test. Viability test is an important aspect of pharmacology that deals with the adverse effect of bioactive substance on living organism prior to the use as drug or chemical in clinical use.[34-36] The *M. fragrans* seed extract and quercetin compound from *M. fragrans* dose-dependently inhibited NO, TNF-α, IL-6 and IL-1β production on LPS stimulated-RAW 264.7.

Tumor Necrosis Factor (TNF-α) is one of the proinflammatory cytokines that plays an important role in inflammation.[37] TNF-α is involved in inflammatory response by activating Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB), cytokine, and adhesion molecule inducer.[5,38,39] Increased concentration of TNF-α are believed to cause the cardinal signs of inflammation to occur. TNF-α will stimulate white cell phagocytosis and the production of inflammatory lipid prostaglandin E2 (PGE2).[40] The TNF-α inhibitory activity measurement is important in anti-inflammatory potential agent screening since this cytokine is an important mediator of inflammation.[31] Blocking one cytokine can be sufficient as anti-inflammatory because cytokine exist in cascades.[37] In particular, TNF-α may stimulate additional inflammatory pathways result in additional inflammatory pathways resulting Nitric Oxide (NO).[5] IL-6 act as both pro- and anti-inflammatory cytokines.[41,42] IL-6 along with TNF-α and IL-1β serve as endogenous pyrogens that causes fever during inflammation by up regulating the inflammatory responses and stimulating the production of acute phase reactans.[43]

M. fragrans seed ethanolic extract and quercetin compound from *M. fragrans* showed the inhibitory activity potential on TNF-α, IL-6 and IL-1β production. Our study is in the line with study showing that macelignan as an active compound of *M. fragrans* can targeted NF-κB and COX-2 gene. NF-κB induced inflammation by activating several cytokine, chemokine and the gene including TNF-α, IL-1β, IL-6, IL-8, COX-2, 5-LOX nd iNOS.[44-46] Inhibition on NF-κB could inhibit the production of TNF-α, IL-6, and IL-1β because there is positive feedback between NF-κB and that cytokine.[47] Recent research also confirmed that quercetin as one of active compound from *M. fragrans* can inhibit various cytokines including TNF-α, IL-6, and IL-1β.[15,16,31,48,49]

NO plays a significant role in host immune defense, vascular regulation, neurotransmission, and other system in normal condition. Overproduction of inducible NO Synthase (iNOS) is especially related to various human diseases including inflammation.[50] LPS can induce iNOS transcription and transduction with subsequent NO production in murine macrophage RAW 264.7 cells. Among the oversecreted inflammatory mediator, NO has been strongly implicated the pathogenesis of several disease.[51] NO inhibitions activity is important for anti-inflammatory agent screening. Our result showed that *M. fragrans* seed ethanolic extract and quercetin compound from *M. fragrans* can inhibit the nitrite production-an indicator of nitric oxide (NO) synthesis. Cao et al. (2013) identified six compounds (licarin B, 3'methoxylicarin B, myrisfrageal A, isodihydrocainatidin, dehydrodiisoeugenol, and myrisfrageal B) of *M. fragrans* that showed inhibition of nitric oxide production in LPS-stimulated RAW 264.7.[52] Our result also in line with the research that showed quercetin can inhibit NO production and iNOS protein and mRNA expression.[53] *M. fragrans* seed contains several active phytochemicals including isoeugenol, methyl-eugenol, eugenol, dihydroguaiaretic acid, γ-terpinene, terpinen-4-ol, myristic acid, oleanolic acid, palmitic acid, camphene, lauric acid, myrecne, kaempferol, and also quercetin as the most common compound found in the *M. fragrans* seed.[54]

CONCLUSION

This research suggested that *M. fragrans* seed extract and quercetin compound possess the anti-inflammatory potential showed by the inhibition of TNF-α, IL-6, IL-1β and NO production. It is well known that NO and PGE_2 are main macrophage-derived inflammatory mediators.[50,55,56] Furthermore, it has been reported that TNF-α, IL-6 and IL-1β is an important inflammatory cytokine. Therefore, it has been thought suppression on TNF-α, IL-6, IL-1β and NO is a good strategy to reduce inflammation.[50] Validation of active samples in animal models should eventually follow for further study.

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