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Anti-inflammatory Effect of Mangosteen (*Garcinia mangostana* L.) Peel Extract and its Compounds in LPS-induced RAW264.7 Cells

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Abstract – Inflammation plays an important role in host defense against external stimuli such as infection by pathogen, endotoxin or chemical exposure by the production of the inflammatory mediators that produced by macrophage. Anti-inflammatory factor is important to treat the dangers of chronic inflammation associated with chronic disease. This research aims to analyze the anti-inflammatory effects of *Garcinia mangostana* L. peel extract (GMPE), α -mangostin, and γ -mangostin in LPS-induced murine macrophage cell line (RAW 264.7) by inhibiting the production of inflammatory mediators. The cytotoxic assay of *G. mangostana* L. extract, α -mangostin, and γ -mangostin were performed by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) to determine the safe and non-toxic concentration in RAW 264.7 for the further assay. The concentration of inflammatory mediators (COX-2, IL-6, and IL-1 β) were measured by the ELISA-based assay and NO by the nitrate/nitrite colorimetric assay in treated LPS-induced RAW 264.7 cells. The inhibitory activity was determined by the reducing concentration of inflammatory mediators in treated LPS-induced RAW 264.7 over the untreated cells. This research revealed that GMPE, α -mangostin, and γ -mangostin possess the anti-inflammatory effect by reducing COX-2, IL-6, IL-1 β , and NO production in LPS-induced RAW 264.7 cells.

Keywords – Anti-inflammatory, GMPE, α -Mangostin, γ -Mangostin, Macrophages, Inflammatory mediator

Introduction

Natural products have over the years contributed to the development of new therapeutic drugs for a variety of human diseases.¹ Many tropical plants have interesting biological activities with their therapeutic potential, including *Garcinia mangostana* L. (mangosteen). Mangosteen has been used for hundreds of years around the world, mostly in Southeast Asia, as a medicine for a great variety of diseases.² Many studies have shown that the various parts extract contain varieties of secondary metabolites such as prenylated and oxygenated xanthenes. Xanthenes as the major bioactive secondary metabolites were reported to have many pharmacological activities including antioxidant, antifungal, anti-bacteria, cytotoxic, anti-inflammatory, antihistamine, anti-HIV, antimalarial and other activities.³⁻⁵ The previous study confirmed that the *G. mangostana* peel extract (GMPE) contained α -mangostin (105 ppm), γ -mangostin (7.20 ppm), garcinone

C (3.50 ppm), and garcinone D (9.92 ppm) based on high performance liquid chromatography (HPLC) analysis.⁶ In this study, the anti-inflammatory potential of *G. mangostana* L. peel extract (GMPE) and its compound were observed.

Inflammation plays an important role in host defense that encompasses multiple processes against external stimuli such as infection by pathogen, exposure to bacterial endotoxin or chemical exposure.⁷ The inflammation process involves changes in blood flow, increased vascular permeability, destruction of tissue via the activation and migration of leukocytes with the synthesis of reactive oxygen derivatives (oxidative burst) and synthesis of local inflammatory mediators.⁸ The secretion of the inflammatory mediators both of proinflammatory mediators such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)- α , and Nitric Oxide (NO) and anti-inflammatory such as IL-10 are the primary response to inflammation in addition to leukocyte recruitment.⁹ Inflammation relates to various diseases such as rheumatoid arthritis, inflammatory bowel disease, arteriosclerosis, Alzheimer's, and has a role in various kinds of cancer.¹⁰ Anti-inflammatory is important to treat the danger of chronic inflammation associated

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with chronic disease.¹¹

Several mechanisms of action have been proposed to describe the phytochemical potential for anti-inflammatory, such as: 1) antioxidant and radical scavenging activity; 2) modulation of cellular activities of inflammation-related cells (mast cells, macrophages, lymphocytes, and neutrophils); 3) modulation of proinflammatory enzyme activities; 4) modulation of the production of other proinflammatory molecules; and 5) modulation of proinflammatory gene expression.¹² This study focused on the potential of GMPE, α -mangostin, and γ -mangostin (Fig. 1) in modulation of the proinflammatory molecules production by inhibiting the proinflammatory cytokines production including COX-2, IL-6, IL-1 β , and NO in LPS-induced murine macrophage cell line model (RAW 264.7). The RAW 264.7 murine macrophage cell line is widely used as an inflammatory model *in vitro*.¹³

Experimental

General Experimental Procedure – *Garcinia mangostana* L. was collected from Cisalak-Subang, West Java, Indonesia plantation and identified by a staff of herbarium of the Department of Biology, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The murine macrophage cell line RAW 264.7 (ATCC[®] TIB-71[™]) was obtained from Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia.

α -mangostin (95-99% purity), and γ -mangostin (95-99% purity) were bought from Biopurify Phytochemical Ltd. (Chengdu, China). The medium and its component for cell culture such as Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), penicillin, streptomycin, and Trypsin-EDTA were purchased from Biowest. 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) used for cell viability assay was purchased from Promega (Madison, WI, USA). COX-2 ELISA kit and protocol (E-EL-M0959) was purchased from Elabscience. IL-6 and IL-1 β ELISA kit and protocol (431301, and 432601) were purchased from BioLegend. Nitrate/Nitrite colorimetric assay kit (KA1342) was purchased from Abnova. MultiSkan Go (Thermo Scientific), a microplate reader, was used for viability assay, measuring COX-2, IL-6, IL-1 β , and NO concentration.

Plant Extract preparation – The peels of *G mangostana* L. were collected, chopped, and kept in drier tunnel service. Extraction was performed based on the maceration method using ethanol 70% as the solvent for

collecting *G mangostana* L. peel extract (GMPE).^{14,15}

RAW 264.7 Cells Culture and Viability Assay – The murine macrophage cells were grown in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin. The cells then maintained at the 37 °C humidified atmosphere incubator with 5% CO₂ until the cells were confluence. The cells were washed and harvested using Trypsin-EDTA and centrifuged at 2500 rpm for 4 minutes.¹⁶⁻¹⁹

Viability assay – The viability assay was performed to determine the safe and nontoxic concentration for the next assay, evaluated by MTS assay. Briefly, 100 μ L cells (5×10^3 cells per well) in medium (DMEM supplemented with 10% FBS and 1% penicillin-streptomycin) were plated in 96-well plate and incubated for 24 hours at 37 °C in a humidified atmosphere incubator with 5% CO₂. The medium then washed and added with 99 μ L new medium and 1 μ L of GMPE, α -mangostin, and γ -mangostin in different concentration and DMSO in triplicate then the plate were incubated for 24 hours. Untreated cells were served as the control. Briefly, 20 μ L MTS was added to each well. The plate was incubated in 5% CO₂ at 37 °C incubator for 4 hours. The absorbance was measured at 490 nm on a microplate reader.¹⁸⁻²¹

Proinflammatory activation of cells and treatment – The pro-inflammatory activation of cells was performed based on Yoon, *et al.* (2009) and modified method.^{17-19, 22} The cells were seeded in 6-well plate in density of 5×10^5 cells per well and incubated for 24 hours at 37 °C in a humidified atmosphere and 5% CO₂.^{18,19-23} The medium (DMEM supplemented with 10% FBS and 1% penicillin-streptomycin) then washed and supplemented with 1,600 μ L growth medium and 200 μ L GMPE (20, 10, 5 μ g/mL), α -mangostin, and γ -mangostin (75, 50, 25 μ M). Around 1-2 hours later, the medium was added with 200 μ L LPS (1 μ g/mL) and incubated for 24 hours at 37 °C in a humidified atmosphere and 5% CO₂.^{18,19} After incubation of RAW 264.7 cells with LPS for 24 hours, the quantity of COX-2, IL-6, IL-1 β , and NO was accumulated in the cell-free supernatant. The cell-free supernatant then was taken for the next assay by centrifugation at 2,000 g for 10 minutes. The supernatant was stored at -79 °C for the COX-2, IL-6, IL-1 β , and NO concentration.

Quantification of COX-2 concentration and inhibitory activity assay after treatment – The quantitative determination of COX-2 concentration in the cell-free supernatant was performed using COX-2 ELISA Kit Elabscience (E-EL-M0959). Briefly, 100 μ L of standard, blank, and sample solution was added into each well then sealed and incubated for 90 minutes at 37 °C. The cell-free supernatant after treated with GMPE, α -mangostin, and γ -

mangostin were served as the sample. The LPS-induced cells free supernatant without extract and compounds were used as positive control. The normal cell or untreated cell was used as negative control. Subsequently, the liquid of each well removed and 100 μ L of Biotinylated Detection AB working solution was added into each well, incubated for 1 hour at 37 °C. The plate then washed prior to the addition of 100 μ L HRP conjugate, incubated for 30 minutes at 37 °C. The plate was washed again and 90 μ L of substrate solution was added into each well, incubated for about 15 minutes at 37 °C. Following that, 50 μ L of stop solution was added and the absorbance measured using microplate reader at 450 nm.²⁴ The concentration of COX-2 was determined from COX-2 standard curve, the regression equation obtained was $y = 0.09543x - 0.01039$ with $r^2 = 0.99$. The percentage of inhibitory activity was calculated using following equation:

$$\text{COX2 inhibitory activity (\%)} = \frac{(\text{COX2}_p - \text{COX2}_s)}{\text{COX2}_p} \times 100$$

where COX2_p : COX-2 concentration of positive control (ng/mL); COX2_s : COX-2 concentration of samples (ng/mL).

Quantification of IL-6 and IL-1 β concentration and inhibitory activity assay after treatment – The quantitative determination of IL-6 and IL-1 β concentration in the cell-free supernatant was performed using Mouse IL-6 ELISA MAX Standard Sets (BioLegend. 431301) and Mouse IL-1 β ELISA MAX Standard Sets (BioLegend 432601), respectively. Approximately 100 μ L of diluted capture antibody solution was added to each well, incubated overnight at 4 °C. Following that, the plate was washed 4 times and 200 μ L of assay diluent added into each well, incubated at room temperature for 1 hour with shaking. The plate then washed again 4 times, prior to addition of the diluted standards and samples. The cell-free supernatant after treated with GMPE, α -mangostin, and γ -mangostin were served as the sample. The LPS-induced cells free supernatant without extract and compounds were used as positive control. The normal cell was used as negative control. The plate was incubated for 2 hours at room temperature with shaking, then washed for 4 times. Subsequently, 100 μ L of diluted detection antibody solution was added into each well and the plate was incubated for 1 hour with shaking at room temperature. After the plate was washed again for 4 times, 100 μ L of diluted Avidin-HRP solution was added, incubated for 30 minutes with shaking at room temperature. The plate was washed 5 times, then 100 μ L of

TMB substrate solution was added to each well and incubated for 15 minutes in the dark. Finally, 100 μ L of stop solution was added and the absorbance was read at 450 nm using microplate reader.^{17-19,25} The quantity of IL-6 and IL-1 β were determined from the standard curve. The regression equation obtained from IL-6 standard curve was $y = 0.0007x - 0.0412$ with $r^2 = 0.98$, and from IL-1 β standard curve was $y = 0.0013x - 0.0157$ with $r^2 = 0.99$. The percentage of inhibitory activity was calculated using following equation:

$$\text{Inhibitory activity (\%)} = \frac{(C_p - C_s)}{C_p} \times 100$$

where C_p : IL-6 or IL-1 β concentration of positive control (pg/mL); C_s : IL-6 or IL-1 β concentration of samples (pg/mL).

Quantification of nitrite associated with NO concentration and inhibitory activity assay after treatment –

The determination of nitrite associated with NO production was performed using Nitrate/Nitrite colorimetric assay kit (Abnova KA1342).²⁴ The cell-free supernatant after treated with GMPE, α -mangostin, and γ -mangostin were served as the sample. The LPS-induced cells without GMPE or compound free supernatant was used as positive control. The normal cell-free supernatant was used as negative control. The samples were read in 540 nm of wavelength in a microplate reader. The quantity of nitrite was determined from the sodium nitrite standard curve, the regression equation obtained was $y = 0.0253x - 0.0550$ with $r^2 = 0.98$. The percentage of inhibitory activity was calculated using following equation:

$$\text{NO inhibitory activity (\%)} = \frac{(\text{NO}_p - \text{NO}_s)}{\text{NO}_p} \times 100$$

where NO_p : NO concentration of positive control (μ M); NO_s : NO concentration of samples (μ M).

Statistical analysis – All data were derived from three independent experiments. Statistical analysis was performed using SPSS software (version 20.0). The data were presented as mean \pm standard deviation. Significant differences between the groups were determined using the Analysis of Variance (ANOVA) followed by Duncan Post Hoc Test $P < 0.05$ were considered as statistical significance.^{18,19}

Result and Discussion

Natural plant compounds are now gaining more pharmacological attention as many plant products are still unexplored and show a wide range of activities.²⁶ Major compounds of several commonly used botanicals, including

Table 1. Effect of GMPE and mangostins in various concentrations toward RAW 264.7 cell viability

Samples	Viability (%)
Control	100.00±0.00 ^{def}
GMPE 100 µg/mL	7.36±2.67 ^a
GMPE 75 µg/mL	8.13±1.69 ^a
GMPE 50 µg/mL	48.86±12.81 ^b
GMPE 25 µg/mL	102.49±8.14 ^{cfg}
GMPE 20 µg/mL	100.80±4.45 ^{def}
GMPE 10 µg/mL	115.23±16.03 ^{figh}
GMPE 5 µg/mL	123.10±12.93 ^h
α-mangostin 100 µM	85.81±4.75 ^{cd}
α-mangostin 75 µM	98.50±6.36 ^{de}
α-mangostin 50 µM	112.76±8.86 ^{cfigh}
α-mangostin 25 µM	116.95±9.43 ^{gh}
γ-mangostin 100 µM	83.30±11.55 ^c
γ-mangostin 75 µM	97.23±3.70 ^{cde}
γ-mangostin 50 µM	103.38±6.54 ^{cfig}
γ-mangostin 25 µM	105.34±5.43 ^{cfig}

The data are presented as mean ± standard deviation. Different superscript letters (^a,^b,^c,^d,^e,^f,^g,^h,ⁱ,^j,^k,^l,^m,ⁿ,^o,^p,^q,^r,^s,^t,^u,^v,^w,^x,^y,^z) in the same column (the viability among concentrations of the samples) are significant at $p < 0.05$ based on Duncan's *post-hoc* comparisons ($p < 0.05$). The experiment was conducted in triplicate

mangostin have been reported to have anti-inflammatory actions. The xanthenes, such as α-mangostin and γ-mangostin are major bioactive compounds found in the fruit peel of mangosteen.^{6,27} In this study, we evaluated the biological effects of GMPE, α-mangostin, and γ-mangostin on inflammatory mediators production in LPS-induced RAW 264.7 cell line as the model. The cytotoxic assay was performed to determine the safe and nontoxic concentration of GMPE and compounds for the next assay. Nontoxicity of the substrate was indicated by over 90% of cells viability by MTS assay. Viability test is an important aspect of pharmacology which deals with the adverse effect of bioactive substance on living organism prior to the use of substances as drug or chemical in clinical use.²⁸⁻³⁰ The α-mangostin and γ mangostin in the concentration of 100 µM were toxic toward RAW 264.7 cells, therefore the respective concentration was not used for the treatments and the concentration of 75, 50, and 25 was chosen instead (Table 1). In other hand, GMPE treatment showed low viability at 100, 75, 50, and 25 µg/mL. Hence, the lower concentration of GMPE were tested. Finally, the cells in the concentration of 20, 10, and 5 µg/mL of GMPE treatment showed high viability and nontoxic to the cells (Table 1).

A stimuli of LPS can activate the macrophages that

involved in the pathological processes in several acute and chronic disorders by secreting several inflammatory mediators.^{7,18,19,31} The overproduction of the inflammatory mediators contributes to the pathogenesis of several diseases such as sepsis, rheumatoid arthritis, atherosclerosis, pulmonary fibrosis, and chronic hepatitis.³² Several nonsteroidal anti-inflammatory drugs were the currently available drugs to reduce the inflammation, but it possess a side effect and major problem.³³ Therefore, the development of new anti-inflammatory agents from natural sources that more active and have fewer side effects become important.

Based on cytotoxic result, three concentration of GMPE (20, 10, 5 µg/mL), α-mangostin (75, 50, 25 µg/mL), and β-mangostin (75, 50, 25 µg/mL) were applied for IL-6, IL-1β, NO, and COX-2 assay. All of the anti-inflammatory assays showed that GMPE possessed potent COX-2, IL-1β, IL-6, and NO inhibitory activity. The isolated compounds from GMPE including α-mangostin and γ-mangostin also possessed the same activity. Inhibiting the synthesis of mediators that plays a role in inflammation will be useful for autoimmune diseases and inflammation treatment. COX-2 is a key regulatory enzyme of the prostaglandin/eicosanoid pathway that highly induced by pro-inflammatory cytokines in an NF-κβ-dependent manner.³⁴ COX-2 induced several stimuli and is responsible for the pro-inflammatory cytokine at the inflammatory sites.³⁵ COX-2 is highly induced by pro-inflammatory cytokines (IL-1β and IL-6) that serve as endogenous pyrogens that causes fever during inflammation by up-regulating the inflammatory responses and stimulating the production of acute phase reactants.³⁶ In addition to COX-2 inhibitory activity, NO inhibitory activity may be as attractive as one of anti-inflammatory agent screening indicators. NO plays a significant role in host immune defense, vascular regulation, neurotransmission, and other system in normal condition. In human body, a family of nitric oxide synthase (NOS) enzyme is responsible for catalyzing the synthesis of NO.³⁷ The expression of inducible NOS (iNOS) in various inflammatory and tissue cells can be induced by LPS or proinflammatory cytokines such as interleukin (IL-1).³⁷ Overproduction of NO and iNOS are especially related to various human diseases including inflammation.^{16,18,19,38}

GMPE and α-mangostin in the highest concentration showed the highest inhibitory activity against COX-2 production (Table 2). COX-2 with COX-1 are the key players in the inflammatory response which catalyze the conversion of arachidonic acid into pro-inflammatory prostaglandins and triggers the production of other pro-inflammatory mediators.³⁴ IL-6 is a pleiotropic cytokine

Table 2. Effect various concentrations of GMPE and mangostins toward COX-2 concentration in RAW 264.7 cell

Samples	COX-2	
	COX-2 concentration (ng/mL)	COX-2 inhibitory activity (%)
Negative control	0.81±0.03 ^a	72.93±0.97 ^f
Positive control	2.98±0.170 ^g	0.11±5.72 ^a
GMPE 20 µg/mL	1.35±0.18 ^b	54.59±6.09 ^c
GMPE 10 µg/mL	2.02±0.12 ^{dc}	32.21±3.87 ^{cd}
GMPE 5 µg/mL	2.16±0.07 ^{ef}	27.40±2.28 ^{bc}
α-mangostin 75 µM	1.43±0.08 ^b	52.13±2.61 ^c
α-mangostin 50 µM	1.75±0.12 ^c	41.16±4.00 ^d
α-mangostin 25 µM	2.06±0.05 ^{dc}	30.87±1.78 ^{bc}
γ-mangostin 75 µM	1.89±0.03 ^{cd}	36.58±0.89 ^{cd}
γ-mangostin 50 µM	1.98±0.03 ^d	33.56±1.16 ^{cd}
γ-mangostin 25 µM	2.31±0.03 ^f	22.48±0.89 ^b

The data are presented as mean ± standard deviation. Different superscript letters (^{a,b,c,d,d,d,d,e,e,e}) in the same column (among various concentrations of GMPE, mangostins in COX-2 concentrations and inhibitory activity) are significant at $p < 0.05$ based on Duncan's *post-hoc* comparisons ($p < 0.05$). The experiment was conducted in triplicate

Table 3. Effect various concentrations of GMPE and mangostins toward IL-6 concentration in RAW 264.7 cell

Samples	IL-6	
	IL-6 concentration (pg/mL)	IL-6 inhibitory activity (%)
Negative control	176.57±5.14 ^a	73.87±0.76 ^g
Positive control	675.43±4.58 ^g	0.00±0.68 ^a
GMPE 20 µg/mL	304.33±55.85 ^b	54.95±8.27 ^f
GMPE 10 µg/mL	351.52±33.57 ^{bc}	47.96±4.97 ^{ef}
GMPE 5 µg/mL	602.48±7.83 ^{cfig}	10.80±1.16 ^{abc}
α-mangostin 75 µM	337.62±57.83 ^{bc}	50.02±8.56 ^{ef}
α-mangostin 50 µM	422.34±55.88 ^{cd}	37.48±8.28 ^{dc}
α-mangostin 25 µM	567.52±66.90 ^{ef}	15.98±9.91 ^{bc}
γ-mangostin 75 µM	500.24±53.87 ^{dc}	25.94±7.98 ^{cd}
γ-mangostin 50 µM	582.14±90.57 ^{efg}	13.81±13.41 ^{abc}
γ-mangostin 25 µM	645.14±92.87 ^{fg}	4.49±13.75 ^{ab}

The data are presented as mean ± standard deviation. Different superscript letters (^{a,ab,bc,abc,cd,ef,fg,efg,f,g}) in the same column (among various concentrations of GMPE, mangostins in IL-6 concentrations and inhibitory activity) are significant at $p < 0.05$ based on Duncan's *post-hoc* comparisons ($p < 0.05$). The experiment was conducted in triplicate

which modulates inflammatory responses.³⁹ GMPE in the concentration of 20 µg/mL showed the highest inhibitory activity (Table 3). Inhibiting the production of IL-1β was important in finding the anti-inflammatory agent. GMPE, α-mangostin, and γ-mangostin inhibited the production of

Table 4. Effect various concentrations of GMPE and mangostins toward IL-1β concentration in RAW 264.7 cell

Samples	IL-1β	
	IL-1β concentration (pg/mL)	IL-1β inhibitory activity (%)
Negative control	841.44±18.01 ^a	28.87±1.52 ^c
Positive control	1,183.03±35.09 ^c	0.00±2.97 ^a
GMPE 20 µg/mL	894.31±77.23 ^a	24.41±6.53 ^c
GMPE 10 µg/mL	950.70±115.33 ^{ab}	19.64±9.75 ^{bc}
GMPE 5 µg/mL	951.72±45.98 ^{ab}	19.55±3.89 ^{bc}
α-mangostin 75 µM	877.28±35.87 ^b	25.84±3.03 ^c
α-mangostin 50 µM	910.43±79.98 ^{ab}	23.04±6.76 ^{bc}
α-mangostin 25 µM	942.64±121.95 ^{ab}	20.32±10.31 ^{bc}
γ-mangostin 75 µM	817.69±3.80 ^a	30.88±0.32 ^c
γ-mangostin 50 µM	936.41±43.20 ^{ab}	20.85±3.65 ^{bc}
γ-mangostin 25 µM	1,041.41±110.80 ^b	11.97±9.37 ^b

The data are presented as mean ± standard deviation. Different superscript letters (^{a,ab,b,c,bc}) in the same column (among various concentrations of GMPE, mangostins in IL-1β concentrations and inhibitory activity) are significant at $p < 0.05$ based on Duncan's *post-hoc* comparisons ($p < 0.05$). The experiment was conducted in triplicate

IL-1β in a concentration-dependent manner. The highest concentration of GMPE, α-mangostin and γ-mangostin possessed the highest inhibitory activity with no significant differences observed among them (Table 4). IL-1β is a potent proinflammatory cytokine released by macrophages in systemic inflammatory responses that regulate inflammatory reaction and immune response.⁴⁰ In this study, the nitrite concentration was associated with NO production. Excessive levels of NO can mediate proinflammatory and have destructive effects, thus lowering the NO concentration can be used as an anti-inflammatory action. The GMPE showed the highest NO inhibitory activity, meanwhile the positive control showed the highest concentration of NO (Table 5). This research result indicated that the LPS successfully induced the inflammation of the RAW 264.7 cells⁴¹ and GMPE had anti-inflammatory properties by reducing the NO production. The inhibition of NO production might related to suppression of iNOS expression, as NO synthesis is catalyzed by iNOS.

GMPE possessed the highest inhibitory activity against COX-2, IL-6, and NO production, but all of the tested concentration both for GMPE and its compounds have no significant differences in inhibitory activity of IL-1β. This research was consistent with previous research that *G mangostana* fruit hull inhibited the inflammation-related diseases through NO and PGE2 releases.⁴² Likewise, α-mangostin and γ-mangostin inhibited NO and PGE2 production and COX-2 activity in LPS-induced RAW

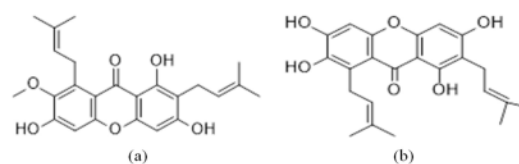
Table 5. Effect various concentrations of GMPE and mangostins toward NO concentration in RAW 264.7 cell

Samples	NO	
	NO concentration (μM)	NO inhibitory activity (%)
Negative control	6.06 \pm 0.17 ^a	82.74 \pm 0.50 ^j
Positive control	35.10 \pm 0.08 ⁱ	0.01 \pm 0.23 ^a
GMPE 20 $\mu\text{g}/\text{mL}$	23.29 \pm 0.07 ^b	33.66 \pm 0.19 ⁱ
GMPE 10 $\mu\text{g}/\text{mL}$	23.92 \pm 0.04 ^c	31.86 \pm 0.12 ^h
GMPE 5 $\mu\text{g}/\text{mL}$	27.07 \pm 0.07 ^f	22.89 \pm 0.19 ^e
α -mangostin 75 μM	24.55 \pm 0.03 ^d	30.07 \pm 0.09 ^g
α -mangostin 50 μM	25.86 \pm 0.02 ^e	26.33 \pm 0.07 ^f
α -mangostin 25 μM	28.88 \pm 0.12 ^g	17.73 \pm 0.34 ^d
γ -mangostin 75 μM	26.94 \pm 0.05 ^f	23.24 \pm 0.14 ^c
γ -mangostin 50 μM	29.37 \pm 0.08 ^h	16.32 \pm 0.23 ^c
γ -mangostin 25 μM	30.26 \pm 0.07 ⁱ	13.78 \pm 0.21 ^b

The data are presented as mean \pm standard deviation. Different superscript letters (^{a,ab,b,c,bc}) in the same column (among various concentrations of GMPE, mangostins in NO concentrations and inhibitory activity) are significant at $p < 0.05$ based on Duncan's *post-hoc* comparisons ($p < 0.05$). The experiment was conducted in triplicate

264.7 cells according to Chen, *et al.* (2007) study.²⁷ The inhibitory activity of α -mangostin and γ -mangostin from GMPE against IL-6 were also revealed by Bumrungpert, *et al.* (2010) study.⁴³ Furthermore, the α -mangostin and γ -mangostin revealed to attenuated LPS-induced inflammatory gene expression of TNF- α , IL-1 β , IL-6, IL-8, MCP-1, and Toll-like receptor-2.⁴⁴ The effect of *G. mangostana* and its compounds against inflammation in this study also in line with Chomnawang, *et al.* (2007) study which reported anti-inflammatory activity of *G. mangostana* on inflammation caused by *Propionibacterium acnes* through suppression of pro-inflammatory cytokines,⁴⁵ as well as Lee, *et al.* (2013) study which revealed that *G. mangostana* and its compounds has great potential in the treatment and prevention of rheumatoid arthritis, a chronic inflammatory disease, showed by inhibition of TNF- α and IL-6 production in LPS-stimulated mice, reduction of paw edema in the carrageenan-induced rats, and reduction of arthritis score in the CIA rats.⁴⁶

This research revealed that GMPE, α -mangostin, and γ -mangostin possess the anti-inflammatory potential by inhibiting COX-2, IL-6, IL-1 β , and NO. These extracts may have therapeutic potential for the modulation and regulation of macrophage activation, and may provide safe and effective treatment option for various inflammation-mediated diseases. However, the therapeutic potential of these plant extracts will be further clear after preclinical and clinical test were conducted.

**Fig. 1.** Chemical structure of (a) α -mangostin and (b) γ -mangostin.

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