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Protective Effect of *Syzygium jambos* (L.) Leaf Extract and Its Constituents Against LPS-induced Oxidative Stress

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Abstract: Oxidative stress is one of the leading causes that contribute to the pathogenesis of chronic diseases based on the imbalance between free radicals and the antioxidant defense system. Here, *Syzygium jambos* (L.) Aston, a traditional herb used to treat various diseases, was evaluated using RAW264.7 macrophages with lipopolysaccharide (LPS)-stimulation to demonstrate its effects and find a new therapy for oxidative stress. This study proved that *Syzygium jambos* leaf extract exerted its ability to protect cells against oxidative stress via activating the nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) pathway to suppress reactive oxygen species (ROS) production. Moreover, the extract also attenuated the nitric oxide (NO) production and the nitric oxide synthase (iNOS) expression, suggesting an anti-inflammatory response. In addition, four active compounds, including gallic acid, quercetin, myricetin, and caffeic acid, were isolated from ethyl acetate fraction using column chromatography. The structure of compounds was analyzed based on one-dimensional nuclear magnetic resonance (1D-NMR) and compared to literature data. Their biological activity was confirmed by promoting the induction of HO-1. Hence, this study provides the initial evidence of *Syzygium jambos* leaves protective effects and highlights its potential as an antioxidant agent.

Keywords: Macrophage cell; nuclear factor erythroid 2-related factor 2; oxidative stress; *Syzygium jambos* (L.) Aston.

1. Introduction

Many natural biological processes in the human body require oxygen to meet their metabolic demands; however, the metabolism energy process also generates free radicals that may harm cells [1]. While normal physiological levels of oxidative stress are protected by the body's self-defense mechanism, its overwhelming due to the imbalance of the redox state; either dysfunction of antioxidant systems can cause oxidative stress [2]. Increasingly, oxidative stress is known as a major reason in the normal aging process and the pathogenesis of various chronic inflammatory d diseases, including cardiovascular diseases, diabetes, cancer, and other diseases related to immune dysfunction [3]. Indeed, since the balance is disrupted, excessive reactive oxygen species (ROS) lead to

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environmental damage in the organism. Therefore, oxidative stress has been extensive research as a therapeutic target to attenuate cell damage and treat numerous diseases.

In the effort to find oxidative stress therapies, nuclear factor erythroid 2-related factor 2 (Nrf2) is a crucial factor that regulates the expression of enzymes for detoxification and antioxidant that can be modulated by dietary energy regulation [4]. Besides, the mechanism signaling pathway due to the expression of this gene not only protect cell under excessive ROS occur but also enhance the immune system to suppress inflammation [5]. Notably, macrophage activation in lipopolysaccharide (LPS)-stimulated is known as a model for developing functional diets and potential medicine to treat the diseases caused by oxidative stress and inflammation [6].

Syzygium jambos (L.) Aston, a traditional herb, has been used to treat numerous health disorders and infectious diseases. Moreover, plant extract and its compounds were reported to be antioxidant, antifungal, antidiabetic, and anticancer. Significantly, the chalcones as phloretin 4′-*O*-methyl ether, myrigalones B and G exerted strong antioxidant activity using DPPH radical [7]. However, to our knowledge, no detailed research has been conducted examining the molecular mechanism of antioxidative stress from *Syzygium jambos* leaves. Therefore, it is important to explore this research gap. This study examined the protective effects of *Syzygium jambos* leaf (SL) extract and its constituents during LPS-stimulated in RAW264.7 macrophage cells. Our findings demonstrated that the active compounds could enhance the expression of the antioxidant enzyme gene, confirming the effect of SL via promoting Nrf2/HO-1 signaling pathway. Hence, *S. jambos* might be used as a functional supplement diet or as a potential antioxidant agent to treat related diseases.

2. Materials and Methods

2.1. Plant Material

Syzygium jambos (L.) leaves (SL) were collected in a period of March 2020 in Kien Giang province, Vietnam. The plant was identified by Dr. Nguyen Thi Kim Hue and deposited under code number (SJ.2019-AG001) in the Laboratory of Biochemistry, Can Tho University, Vietnam. The plant was authenticated and compared with an herbarium specimen (voucher number: VNM00042985) at Institute of Tropical Biology, Vietnam.

2.2. Antioxidant activity and Total Polyphenolic, Flavonoid contents

The radical scavenging effect of SL extract using 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, USA) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS; Sigma-Aldrich) radicals were determined according to the previous description method with minor modifications [5,8]. Briefly, an equal volume of SL extract at various concentrations (0 – 200 µg/mL) and DPPH was added to 96 well-plate and incubated without slight for 60 min at room temperature. Finally, the absorbance was recorded at 517 nm using a Epoch Spectrophotometer (BioTek, USA).

The ABTS stock solution was prepared by a mixture between 7 mM ABTS solution and 2.4 mM potassium persulfate solution, adjusted pH at 7.4, and allowed to generate the radicals under room temperature without slight for 16 h. Then, the solution was diluted with methanol to obtain suitable absorbance, reaching 0.7 at 734 nm. The antioxidant activity was determined using a mixed reaction of 990 µL ABTS solution with 10 µL of various SL concentrations after incubating at 6 min. Finally, 200 µL of each sample was added to 96 well-plate, and the absorbance was recorded at 734 nm.

Total antioxidant activity (TAC) was determined according to a previous report with a slight modification [9]. The reduction of Mo (VI) to Mo (V) was caused by the effect of SL extract to generate a green-colored phosphate/Mo (V) complex at acidic pH (4.0). Briefly, 0.1 mL of various concentrations of SL extract was added to 1 mL of a mixture solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Then, the reaction was incubated at 95 ^{o}C for 90 min, and the absorbance was measured at 695 nm after cooling. The TAC was expressed as mg/g ascorbic acid equivalent (AE) of SL dried extract. The experiment was performed in triplicates, and

the standard curve of ascorbic acid (Merck, Germany) was prepared as y = 0.0416 – 0.0098, R^2 = 0.9879.

The total polyphenolic content (TPC) of SL extract was evaluated using the Folin–Ciocalteu assay with modification slightly [10]. An equal volume of SL in methanol (500 µg/mL) and 10% Folin–Ciocalteu reagent was mixed, then added 2.5 mL of 10% Na_2CO_3 (*w/v*). The mixture was mixed and incubated at 40°C for 30 min using intermittent shaking for color development. Then, the reaction was measured at 765 nm using a UV-Vis Spectrophotometer (V-730 UV-Vis; Jasco, Tokyo, Japan). The TPC was expressed as mg/g gallic acid equivalent (GAE) of extract following the calibration curve of Gallic acid (Merck, Germany) as a control (y = 0.0246x + 0.0387, R^2 = 0.9957).

The total flavonoid content of SL extract was investigated according to a previous method with minor modifications [5]. Briefly, 0.2 mL of SL extract was mixed with an equal volume of $AlCl_3$ (10%; *w/v)* and potassium acetate (1 M), then 0.2 mL distilled water was added. The absorbance was analyzed at 415 nm after incubation at room temperature for 30 min. The standard curve (y = 0.0052x + 0.0039, R^2 = 0.9932) was prepared using the standard quercetin compound. Total flavonoid content was calculated by mg/g quercetin equivalent (QE) of SL dried extract.

2.3. Extraction and Isolation of Antioxidative Stress Compounds

The dried powder was extracted with 100 mL of ethanol (Merck, Germany) at 25 °C, centrifuged, and filtered through a filter paper to obtain a supernatant. The residue was extracted thrice, and the resulting supernatants were combined. Then, the crude extract was concentrated using a rotary evaporator (Rotavapor R-300; BUCHI, Flawil, Switzerland), freeze-dried, and then stored at 4 °C for further experiments.

10 g ethanol extract of SL was suspended in water under ultrasonic irradiation and fractionated using hexane and ethyl acetate, respectively. Then, each fraction was evaporated using a rotary evaporator to obtain hexane extract (1.76 g) and ethyl acetate extract (2.94 g). The remaining water layer was condensed to afford aqueous extract (1.74 g).

Next, the ethyl acetate extract exerted the highest activity against LPS-induced via ROS experiments and was selected to separate 5 fractions using silicagel-column chromatography (Silica gel 60; Merck, 230-400 mesh ASTM). These fractions were eluted with the ratio of hexane and ethyl acetate (Hex:EtOAc, 100 : 0 to 0 : 100) and repeatedly purified by the mixture between chloroform and methanol ($CHCl_3$:MeOH, 9:1) to obtain 4 compounds. The yield of each compound collected was 5 mg (compound 1), 4 mg (compound 2), 16 mg (compound 3), and 7 mg (compound 4). The purification of each fraction was verified by thin-layer chromatography (TLC) plates coated with fluorescent indicator F-254 (Merck, Germany). The spots of each compound were visualized using 10 % (*v/v*) phosphomolybdic acid in ethanol and heating in a hot plate to develop the color.

2.4. Cell Viability

RAW264.7 cells, a murine leukemia macrophage cell line (ATCC TIB-71™, Manasas, VA, USA), was maintained in Dulbecco's Modified Eagle's Medium (DMEM; FUJIFILM-Wako, Japan) containing 10% fetal bovine serum (FBS; Merck, Germany). The culture media was supplemented with 1 U/mL penicillin-streptomycin and 1% L-glutamine (FUJIFILM-Wako, Japan). Cells were plated in 25 cm^2 plates under a humidified incubator with 5% CO_2 at 37 °C. Then, each sample was dissolved in dimethyl sulfoxide (DMSO) and diluted to an appropriate concentration with DMEM in 96-well plates for 24 h incubated [5]. The concentration of DMSO was maintained at 0.1% (v/v).

The cell viability was evaluated via the intracellular dehydrogenase activity using Cell Counting Kit-8 assay (CCK-8; Dojindo Molecular Technologies Inc., Rockville, USA). Briefly, the cells were treated with a serial concentration of SL extract in 96-well plates (a density of 2×10^5 cells/well) for 24 h, followed by incubation with or without 1 µg/mL LPS (Merck, Germany) for an additional 16 h. Finally, the absorption was read at 450 nm using an SH-1200 microplate reader (CORONA Electric, Japan).

2.5. Reactive oxygen species (ROS) and Nitric oxide (NO) Production

Cells were pretreated with the appropriate concentration of SL extract in DMEM for 24 h, and the old medium was removed and washed with phosphate-buffered saline (PBS). Next, cells were stimulated with 1 µg/mL LPS in DMEM at 37 °C after removing old media and washing with PBS. The fluorescence, corresponding to the intracellular ROS level, was analyzed using the Cellular ROS assay Kit (Abcam, Tokyo, Japan). Briefly, cells were incubated with 10 µM 2′,7′-dichlorodihydrofluorescein diacetate ($DCFH_2$–DA) for 45 min. Intracellular ROS level was determined using the Wallac 1420 spectrofluorometer (Perkin-Elmer, Finland) at 488/535 nm wavelength [5].

NO production was measured by Griess reagent (FUJIFILM-Wako, Japan), following the previous report [5]. 100 µL of the conditioned medium was mixed with an equal volume of Griess reagent. After 10 min incubated at room temperature, the absorbance was recorded at 540 nm by SH-1200 microplate reader. The standard curve was generated with serial concentrations of sodium nitrite ranging from 0 to 100 µM ($y = 0.00627x + 0.0759$, $R^2 = 0.9956$). N-Nitro-**L**-arginine methyl ester (L-NAME, 100 µM; FUJIFILM-Wako, Japan) was used as a positive control.

2.6. Reverse Transcription Quantitative PCR (RT-qPCR)

RAW264.7 cells were cultured for 24 h in 6-well plates, then incubated with the indicated concentration of SL extract or compounds. Then, cells were washed with PBS and stimulated with or without LPS for 16 h. The total RNA was extracted using Qiazol reagent, which was subsequently purified using a Qiagen RNeasy Kit (Qiagen, Germany) following the manufacturer's instructions. Total RNA was stored at -80^{o}C for further experiments. Finally, the effect of SL extract on mRNA expression was determined by *q*RT-PCR, as described in our previous study [11]. *β*-actin was used as a normalizing control, and the following primers were described in Supporting Information.

2.7. Western Blotting

Cells were seeded on a 6-well plate, then incubated with selected compound concentrations for 24 h prior to LPS stimulation (1 µg/mL) for 16 h. The nuclear proteins were extracted using a Nuclear Extraction Kit (Abcam, Japan). Then, the protein concentration was determined by BCA protein assay (Thermo Fisher Scientific, USA). Next, each sample was separated into SDS-PAGE (10%, *v/v*) and transferred to PVDF membranes. The membrane was blocked using a specific solution (5% skim milk) for 1 h. After that, the sample was incubated with the primary antibody (Nrf2; Proteintech, Japan) overnight at 4^{o}C until use. The membrane was washed with Tris-buffered saline (TBST; 0.5% Tween-20) and incubated with horseradish-peroxidase-conjugated secondary antibody in TBST, which supplemented 3% bovine serum albumin (BSA; Roche, Germany) for 1 h at room temperature. Finally, the membrane was visualized using ECL Western blotting Substrate. The protein bands were observed and normalized to lamin-B1 [11].

2.8. Nuclear magnetic Resonance (NMR) Spectrometry Analysis

The isolated compounds were analyzed using NMR (Avance III-600 MHz, Bruker, Rheinstetten, Germany) to elucidate the chemical structures with CD_3OD and tetramethylsilane (TMS) as the solvent and internal standard, respectively.

2.9. Statistical Analysis

Experiments were repeated at least three times. All data are expressed by the mean ± standard deviation (S.D.). Statistical significance was evaluated using one-way ANOVA, where $p < 0.05$ was considered significant.

3. Results and Discussion

3.1. Antioxidant activity of SL extract

Free radicals are essential for life activities; however, the excessive production of free radicals induces cell damage and apoptosis, contributing to many diseases [12]. Therefore, the body creates several enzymatic mechanisms to minimize the damage and protect the body against it [13]. Antioxidants play an essential role in these defense mechanisms. The antioxidant activity of SL extract was first indirectly evaluated via radical scavenging ability by DPPH and ABTS assay. As shown in Figure 1, the free radical-scavenging activity enhanced linearly with increasing of SL extract concentration, whereas the concentration required to scavenge 50 % of the free radicals corresponded to 46.33 ± 0.329 µg/mL (equivalent to 8.8 µg/mL vitamin C) and 14.66 ± 0.27 µg/mL (equivalent to 1.8 µg/mL Gallic acid), respectively. Besides, the total antioxidant capacity was found as 686.97 ± 2.28 mg AE/g of dried extract (data not shown). Notably, the DPPH radical result was higher than a previous study in India at 114.56 µg/mL, while another study reported this value at around 14.10 µg/mL [14,15]. Similarly, the scavenging activity of SL extract using ABTS radical and the total antioxidant capacity were also stronger than another study in Bangladesh at 57.8 µg/mL and 628.5 mg AE/g of extract, respectively [16].

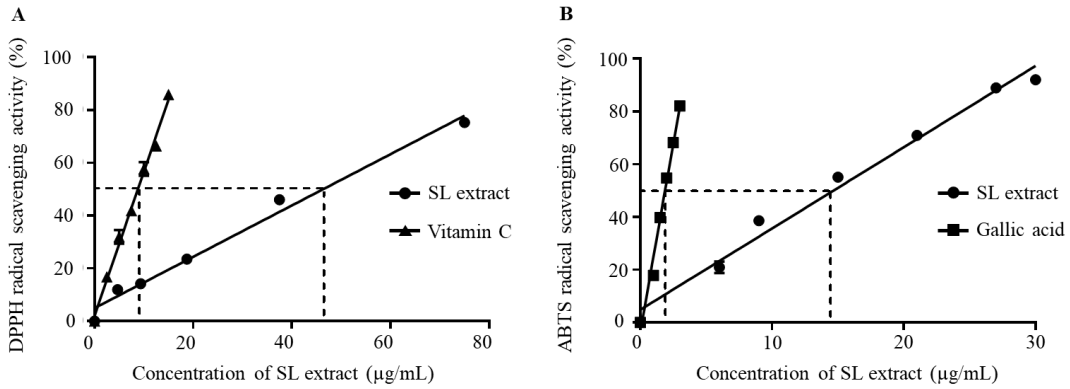

Figure 1. Radical scavenging activity of SL extract. The scavenging activity of various concentrations of SL extract was obtained using DPPH (A) and ABTS (B). Vitamin C or Gallic acid were used as positive controls, respectively. The dotted line indicated the SL concentration or control required to scavenge 50 % radical (SC_{50}). Values are expressed by the means ± S.D (n=3).

Moreover, the total polyphenolic and flavonoid content were recorded to be 857.24 ± 9.06 mg GAE/g of dried extract and 559.81 ± 6,93 mg QE/g of dried extract, respectively (data not shown). Phenolic compounds, one of diverse plant secondary metabolites, ranging from phenols to flavonoids, … are well-known as antioxidants and important bioactive agents necessary for human health and for preventing many diseases [17]. The results were higher than the previous studies, with a maximum total phenolic content of approximately 135 mg GAE/g or 466 mg GAE/g, indicating the significant difference in chemical constituents depending on geography and nutrition [18-20]. Therefore, the result demonstrated that SL extract exerted its strong antioxidant activity dependent on the high content of phenolic compounds.

3.2 Effect of SL extract on Cell viability, ROS, and NO Production

The cytotoxicity of SL extract was performed to find the optimal concentration for further experiments that effective with the minimum toxicity. The SL concentration of 400 µg/mL reduced cell survival by about 10%, causing cell toxicity. However, no significant effect was observed on cell viability upon SL treatment with a concentration of up to 200 µg/mL (Figure 2A). To this end, the SL

extract did not exert cytotoxicity with a safe concentration of about 200 µg/mL. Moreover, three fractions, including hexane, ethyl acetate, and aqueous extract, were assayed for cytotoxicity effect. Notably, only ethyl acetate extract (SL-EtOAc) was not significantly different in cell survival with the concentration investigated (data not shown).

To investigate the antioxidative stress activity of *Syzygium jambos* L. leaf extract, RAW264.7 cells were pretreated with a serial concentration of SL extract or SL-EtOAc for 24 h, followed by stimulating with LPS for 16 h. As mentioned in the previous report, LPS can produce excessive ROS, leading to oxidative stress and triggering cell damage [21]. As expected, cell survival was reduced significantly upon LPS-stimulated than the non-treated group. This fact could be indicated that pre-treatment with SL extract or SL-EtOAc suppressed the cell toxicity and protected cells from LPS-induced cell damage (Figure 2B,C).

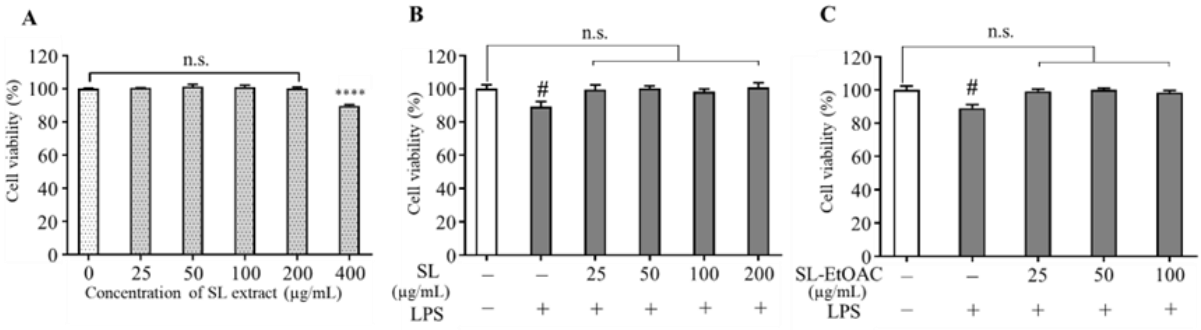

Figure 2. The cytotoxicity of *Syzygium jambos* leaf extract. (A). Cell viability was treated with SL extract alone; (B). Cell viability was pretreated with a serial concentration of SL extract for 24 h, followed LPS stimulation for an additional 16 h. (C). Cell viability was pretreated with a serial concentration of SL-EtOAc for 24 h, followed LPS stimulation for an additional 16 h. Data are expressed by the mean ± S.D; (n=6); n.s, not significant; #, $p<0.05$ vs. cell treated with media only; ****, $p<0.0001$ is expressed in comparison with LPS treatment only.

Reactive oxygen species (ROS) or reactive nitrogen species (RNS) are generated from oxidative stress as the result of the imbalance between pro-oxidant and antioxidant homeostasis [22]. Furthermore, NO and ROS, as pro-inflammatory mediators, play an essential role in the inflammation process [11]. In addition, LPS triggers ROS production and NO production leading to the expression of inflammation-associated genes [23]. Thus, the effect of *Syzygium jambos* leaf extract on oxidative stress production was evaluated. LPS stimulation remarkably enhanced the generation of ROS level and NO production, as shown in Figure 3. However, pre-treatment with various concentrations of SL extract or SL-EtOAc suppressed ROS levels. As expected, NO production was downregulated significantly upon pre-treatment with SL extract compared with LPS treated (Figure 3C). Collectively, these results demonstrate that *Syzygium jambos* leaf extract not only suppresses oxidative stress via the inhibition of intracellular ROS but also alleviates NO production related to anti-inflammatory. For further experiments, SL-EtOAc was chosen to evaluate the mechanism underlying its effects and isolate the active compounds.

Noteworthy, the bacterial invasion could change the redox state leading to the initiation of various signaling cascades and regulating the transcriptional and post-transcriptional to control the pro-inflammatory mediators [23]. Cyclooxygenase-2 (COX-2) is related to oxidative stress, ROS production, and initiated inflammation development [24]. Besides, the overexpression of inducible nitric oxide synthase (iNOS) is a major leading to excessive NO production. Hence, to improve the hypothesis, the transcriptional levels of *iNOS (NOS2)*, and *COX-2* were analyzed by *q*RT-PCR. As shown in Figure 4, the mRNA expression of *COX-2* and *iNOS* was enhanced by LPS stimulation. On the contrary, pre-treatment of SL extract and SL-EtOAc at the 25 µg/mL concentration significantly suppressed the gene expression. Moreover, interleukin-10 (IL-10) as an anti-inflammatory cytokine is essential to autoimmune pathologies [2]. A recent study reported that IL-10 suppresses oxidative stress

and attenuates entire stages of autophagy-related marker mRNA [25]. Here, the messenger RNA (mRNA) level of IL-10 was remarkably increased at the SL-EtOAc concentration of 50 µg/mL, except for 25 µg/mL compared with the control and LPS groups. Similarly, a previous study reported the efficiency of *Syzygium jambos* bark extract, which significantly increased IL-10 in pancreatic tissues via oral supplementation for diabetic rats model [26]. Taken together, these findings confirm the ability of *Syzygium jambos* leaf extract can alleviate oxidative stress and inflammation in LPS-induced.

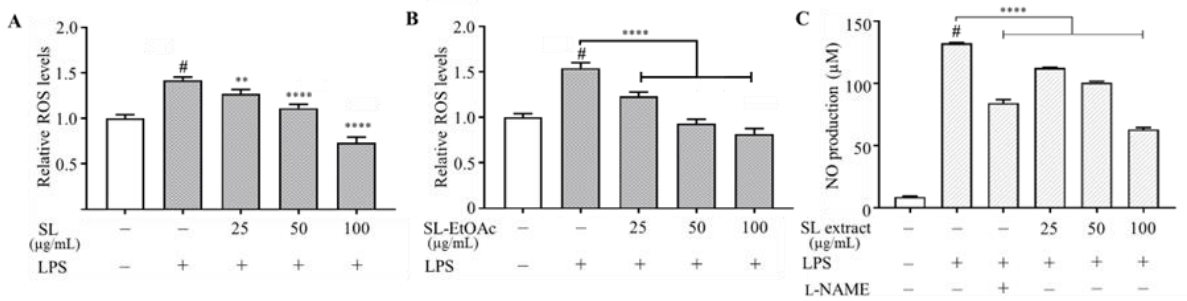

Figure 3. The effect of *Syzygium jambos* leaf extract on ROS and NO production. (A). ROS levels in LPS-stimulated were pretreated with SL extract; (B). ROS levels in LPS-stimulated were pretreated with SL-EtOAc; (C). NO production was pretreated with a serial concentration of SL-EtOAc. Data are expressed by the mean ± S.D; (n=6); n.s, not significant; *#, p<0.05* vs. cell treated with media only*; **, p<0.01; ****, p<0.0001* is expressed in comparison with LPS treatment only.

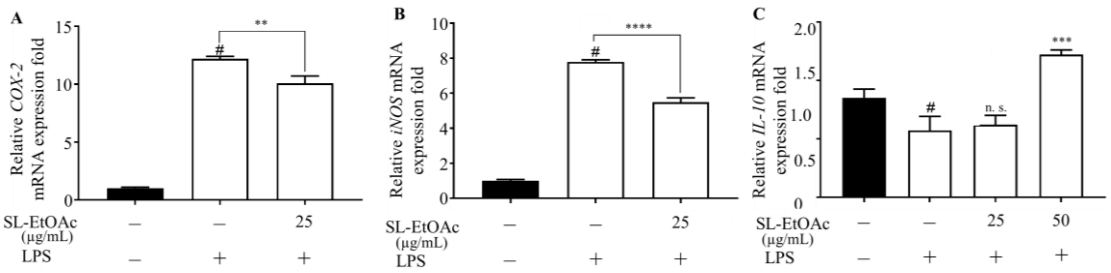

Figure 4. Effect of SL-EtOAc fraction on the expression of biomarkers in LPS-stimulated. The expression of *COX-2* (A), *iNOS* (B), and *IL-10* (C) was evaluated by *q*RT-PCR (n=3). Values represented by the mean ± S.D. n.s, not significant; *#, p<0.05* vs. cell treated with media only*; **, p<0.01; ***, p<0.001, ****, p<0.0001* is expressed in comparison with LPS treatment only.

3.3 Isolation and Evaluating the Effect of Antioxidative Stress Compounds

Four active compounds were isolated from SL-EtOAc using column chromatography. Then, the structure of these compounds, numbered as compounds **1**, **2**, **3**, and **4**, was performed using 1D-NMR compared to the available literature (Supporting Information). Based on the initial analysis and published data, the result proved that the structure of these compounds was elucidated as gallic acid, quercetin, myricetin, and caffeic acid, respectively (Figure 5) [27,28].

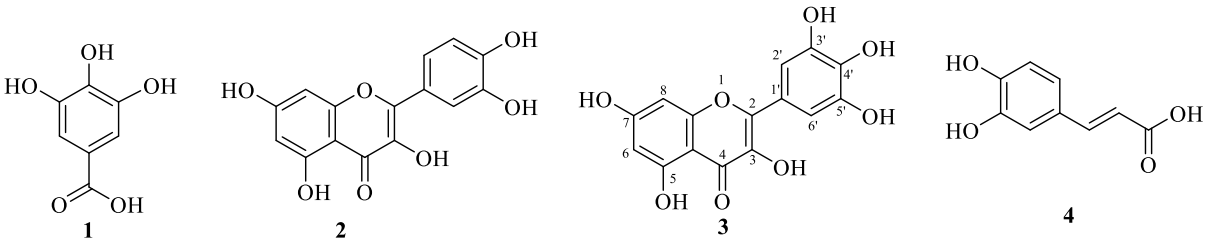

Figure 5. Structures of active compounds isolated from SL-EtOAc fraction

Heme oxygenase-1 (HO-1) is considered the initial defense factor against oxidative stress. The enhancement of HO-1 expression enabled cells against injury by ROS generated. Hence, the increase of HO-1 expression plays an essential role in antioxidant defense and exerts its cytoprotective effects [29]. Here, the effect of the isolated compounds on HO-1 activity was evaluated using *q*RT-PCR. As expected, pre-treatment with these active compounds enhanced the mRNA HO-1 expression, while LPS stimulation did not affect its activity (Figure 6). Especially, myricetin (compound 3) exerted its highest induction of HO-1 expression than other compounds. Besides, myricetin showed stronger activity levels than its structural analog quercetin (compound 2), suggesting that the addition of hydroxyl group (-OH) at position 5′ in ring B might significantly affect the HO-1 activity. Moreover, gallic acid (compound 1) and caffeic acid (compound 4), as a kind of phenolic compounds, were reported their activity to antioxidative stress and anti-inflammatory via inducible the expression of antioxidant enzyme (HO-1) [30,31]. This implies that the effects of chemical structures and the numbers of hydroxyl groups are critical structural factors for antioxidative stress.

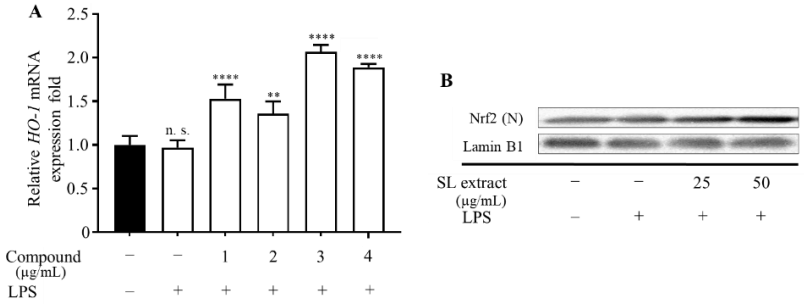

Figure 6. Active compounds isolated from SL-EtOAc promoted the Nrf2/HO-1 activation. (A). The mRNA level of *HO-1* following pre-treatment with 10 μM concentration for each compound was analyzed by *q*RT-PCR. (B). The expression of Nuclear (N) Nrf2 following pre-treatment with SL extract was analyzed by Western blot analysis (n = 3) (A). Lamin B1, as a nuclear loading control. Data represented by the mean ± S.D. n.s, not significant; **, *p<0.01;* ****, *p<0.0001* is expressed in comparison with LPS treatment only.

Regarding the expression of the phase II antioxidant enzyme gene (HO-1), the nuclear factor-erythroid-2-related factor (Nrf2) is an upstream mediator, exerts its interaction via dissociating from Kelch-like encoded in human (ECH)-associated protein (Keap1) and translocate to the nucleus in oxidative stress response [11]. Therefore, SL extract was analyzed to reconfirm its effect on Nrf2 underlying the antioxidative stress response induced by LPS stimulation. As expected, SL extract promoted the nuclear translocation of Nrf2 in LPS-induced macrophage activation (Figure 6B). These compounds also proved their effects on inflammation and oxidative stress by modulating the Nrf2 pathway [32–36]. To this end, these results demonstrated that *Syzygium jambos* leaf extract exerts strong antioxidative stress efficiency due to the presence of these compounds.

A previous study showed that 17 secondary metabolites were found in *Syzygium jambos* leaf extract using HPLC-PDA-MS/MS, which exerted substantial antioxidant and hepatoprotective activities *in vivo* via preventing the p-38 phosphorylation, targeting MAPKAPK-2 activation [37].

Moreover, the protective effect of *Syzygium jambos* water bark extract was mentioned on attenuating pancreatic oxidative stress and inflammation based on the activation of IRS-2/AKT/GLUT4 signaling pathway in diabetes [26]. Remarkably, Nrf2 is a major mechanism in cellular defense against oxidative stress through regulating an extensive panel of gene expression related to detoxicating and eliminating the reactive oxidants [38]. Furthermore, aberrant Nrf2 expression is ably led to the numerous pathologies associated with oxidative stress and inflammation, including neurodegenerative disease, psychiatric disorders, cardiovascular disease, and cancer [39]. The present study illustrates that SL extract could activate the Nrf2/HO-1 pathway to promote the downstream expression of antioxidant enzyme genes, thereby reducing ROS generation. In addition, SL extract also suppressed the NO secretion and the expression of iNOS, suggesting an effective anti-inflammatory response. Conclusively, our study provides the initial evidence of protective effects from *Syzygium jambos* leaf extract and its constituents against LPS-induced oxidative stress and highlights its potential as a phytotherapeutic agent. Further research is needed to isolate more active compounds and investigate the mechanism underlying their effects on inflammation.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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