



Polyphenol compounds of freeze-dried *Moringa oleifera* Lam pods and their anti-inflammatory effects on RAW 264.7 macrophages stimulated with lipopolysaccharide

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ABSTRACT

Background: *Moringa oleifera* pods are the main constituent in typical diets throughout Thailand. Due to their nutrients and phytochemicals, moringa pods have potential as an antioxidant and anti-inflammatory agent. Freeze drying is a widely embraced method known for extending shelf life, resulting in preserved nutrients and high-quality products.

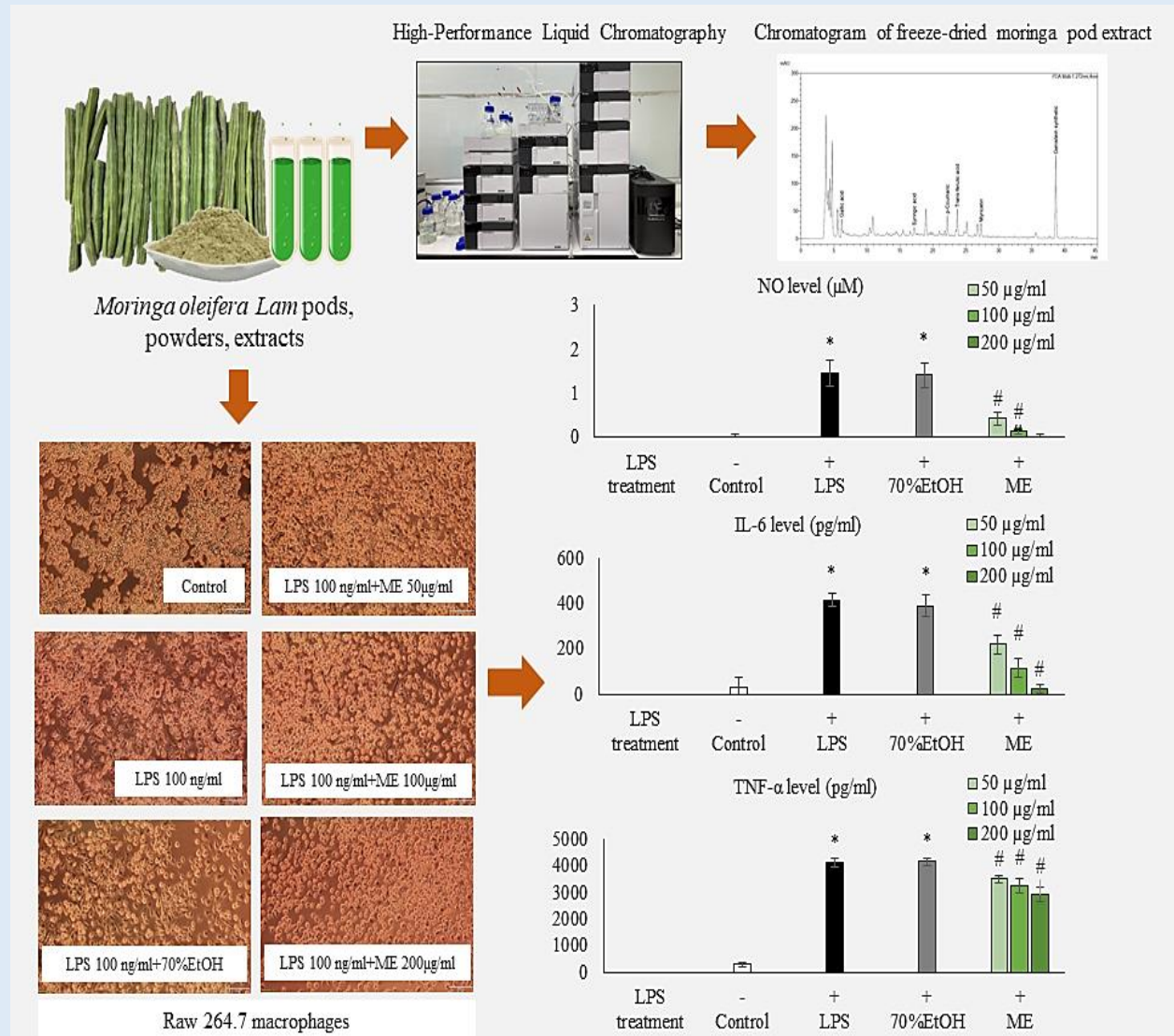
Objective: This study aimed to investigate the physical properties, nutrients, and bioactive compounds of freeze-dried moringa pods as well as their anti-inflammatory effects on lipopolysaccharide-induced Raw 264.7 macrophages.

Methods: Moringa pods were freeze-dried and ground. The powders were extracted with 70% ethanol at room temperature for 24 hr. and then centrifuged at 5000 rpm and 25°C. The supernatant-filtered extracts were collected to determine bioactive compound contents and antioxidant as well as anti-inflammatory properties.

Results: The results illustrated that freeze-dried moringa pod extract had bioactive compounds including genistein, ferulic acid, p-Coumaric, myricetin, gallic acid, and syringic acid, which demonstrated antioxidant activity. In terms of anti-inflammatory effects, 50-200 µg/ml of extract inhibited pro-inflammatory mediators including NO production and IL-6 and TNF-α levels on LPS-induced Raw 264.7 macrophages.

Results: These results indicate that the antioxidant and anti-inflammatory properties of freeze-dried moringa pods make them a promising new raw material for functional product development.

Keywords: Anti-inflammation, *Moringa oleifera*, genistein synthetic, trans-ferulic acid, p-Coumaric, myricetin, gallic acid, syringic acid.



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INTRODUCTION

Inflammation is a body response that occurs to protect the body from various stimuli such as infection, injury, and irritation. Inflammation causes tissue damage and

leads to chronic inflammatory diseases such as cardiovascular disease, diabetes, auto-immune disease, cancer, and Alzheimer's disease. Chronic inflammatory diseases are indeed acknowledged as a major contributor

to global mortality, accounting for over 50% of deaths related to inflammation diseases [1]. Therefore, reducing or suppressing the inflammatory response could be an alternative approach to preventing and treating chronic inflammatory-associated diseases.

Moringa oleifera Lam. or drumstick tree belongs to the Moringaceae family. Moringa is a multi-purpose plant used for food and medicinal purposes. With its nutrients and phytochemicals, various parts of this tree have been widely used in tropical countries. For example, the leaves are rich in fiber, minerals, vitamins, and phytochemicals, which show antioxidant, antidiabetic, and anti-inflammation properties as well as reducing blood pressure and cholesterol. Meanwhile, the moringa seed serves as a source of fat that has been reported to possess both anti-cancer and anti-inflammatory effects. Moringa flowers are used for ulcer treatment and have displayed anti-inflammatory activity. Moringa pods have been reported to exhibit antioxidant and anti-inflammatory activities [2-6]. As a result, moringa are traditionally used for health prevention of chronic diseases, as well as managing associated symptoms, thus making them attributable to functional food species [7-9].

It is well known that freeze-drying is a process used to remove water in the form of ice under low pressure and low temperature in materials. Currently, freeze-drying is the preeminent drying method due to its ability to produce high-quality, easy-to-handle, and non-perishable food products [10]. Thus, the freeze-drying process in fruits and vegetables is commonly used to preserve their nutrients and bioactive compounds, which have shown antioxidant, anti-inflammatory, antidiabetic, and anticancer benefits in previous studies. Furthermore,

to prevent diseases, it is recommended to consume a variety of fruits and vegetables totaling to 400 grams per day [11].

While there's been significant exploration into the antioxidant and anti-inflammatory properties of *Moringa oleifera* Lam., there's a gap in the understanding of the bioactive compounds and biological functions of freeze-dried moringa pods that requires further investigation. Therefore, the objective of this study was to investigate the phenolic and flavonoid compounds of freeze-dried moringa pod extract and their anti-inflammatory effects on the LPS-induced Raw264.7 macrophage cell line.

MATERIALS AND METHODS

Materials and chemicals: Fully mature *Moringa oleifera* Lam.) pods were acquired from a local market in Ayutthaya, Thailand, while various chemicals including 2,2 diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and a selection of polyphenol standards like gallic acid, methyl gallate, caffeic acid, syringic acid, rutin hydrate, p-Coumaric acid, trans-ferulic acid, myricetin, daidzein, quercetin, cinnamic acid, synthetic genistein, and kaempferol were obtained from Sigma-Aldrich (St Louis, MO, USA). The murine RAW 264.7 cell line was sourced from the American Type Culture Collection (ATCC, Rockville, MD, USA), and components for cell culture medium (Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS)) were procured from GIBCO (Grand Island, NY, USA). All chemicals and reagents employed were of either analytical or biological grade.

Sample preparation: Moringa pods were washed, chopped, and frozen. After that, the frozen moringa pods

were freeze-dried and subsequently ground to powder. All samples were stored in a humidity chamber at room temperature before testing. For the extract, freeze-dried moringa pod powder was extracted with 70% ethanol for 24 hours at room temperature and then stored in a freezer.

Colorimetric measurements: The color of freeze-dried moringa pod powder was measured and represented in terms of L^* (lightness), $+a^*$ (redness), $-a^*$ (greenness), $+b^*$ (yellowness), $-b^*$ (blueness), and c^* (intensity) using a colorimeter and calculation (Ultrascan pro, Hunterlab, Reston, VA, USA). The color intensity and the hue angle (h_{ab}) were calculated from $C^* = (a^{*2} + b^{*2})^{0.5}$ and $h_{ab} = \tan^{-1}(b^*/a^*)$, where $h_{ab} = 0^\circ$ and 90° were for a red and yellow hue, respectively [12].

Determination of Chlorophyll contents: The chlorophyll content of freeze-dried moringa pod powder was analyzed using a microplate reader. Chlorophyll was extracted from 0.2 g of the powder by adding 5 ml of absolute methanol and shaking for 2 hours at 60 rpm using a rotator (Grant Bio PTR 360° Vertical Multi-Function Tube Rotators, Latvia). The extract underwent centrifugation at 2400 rpm for 10 minutes, after which the supernatant was carefully transferred to a second centrifuge tube. Subsequently, the absorbance of 200 μ l of the extract in the microplate was adjusted to account for a 1-cm path length [13].

$$A_{652,1\text{cm}} = (A_{652, \text{microplate-blank}}) / \text{path length}$$

$$A_{665,1\text{cm}} = (A_{665, \text{microplate-blank}}) / \text{path length}$$

Chlorophyll concentration was calculated from a 1-cm corrected path length using the formula below:

$$\text{Chlorophyll a } (\mu\text{g/ml}) = -8.0962 A_{652, 1\text{cm}} + 16.5169$$

$$A_{665, 1\text{cm}}$$

$$\text{Chlorophyll b } (\mu\text{g/ml}) = 27.4405 A_{652, 1\text{cm}} - 12.1688$$

$$A_{665, 1\text{cm}}$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Composition analysis: Moisture, solid, and fat of freeze-dried moringa pod powder were performed by a rapid NMR fat analyzer (CEM, Oracle, North Carolina, USA). Protein content was determined using combustion (LECO, FP828 series, Michigan, USA) and calculated, with a nutrition factor of 6.25.

Determination of bioactive compounds (phenolic acid and flavonoid) and antioxidant activity:

Polyphenol content was assessed using High-Performance Liquid Chromatography (HPLC) employing a Shimadzu Model prominence LC20 series instrument equipped with a UV visible detector set at a wavelength of 272 nm. The methodology was adapted from a prior study focusing on quantitative HPLC analysis of phenolic acid and flavonoids [14]. A 10 μ l injection volume was utilized. Analysis of phenolic acid and flavonoid compounds was conducted on an Ascentis Express Column (C18, 5.0 μ m, 15 cm \times 4.6mm) using 1% acetic acid in water and 100% acetonitrile as mobile phases A and B, respectively. The flow rate was maintained at 0.4 ml/min, with the column temperature set at 28°C. A gradient elution was performed from 10% to 40% B over 28 minutes, followed by 40% to 60% B over 39 minutes, and then 60% to 90% B over 45 minutes, with 90% B held for 50 minutes. Each compound was identified based on its retention time compared to standards under identical conditions. Linearity was confirmed by preparing a stock solution of the standard (1 mg/ml) and diluting it to six different concentrations (2.5, 5, 10, 20, 30, 40 μ g/ml) with a square

of the correlation coefficient ($R^2 > 0.99$). Analysis of the freeze-dried moringa pod extract was conducted in

triplicate, and results were reported with convergence limits.

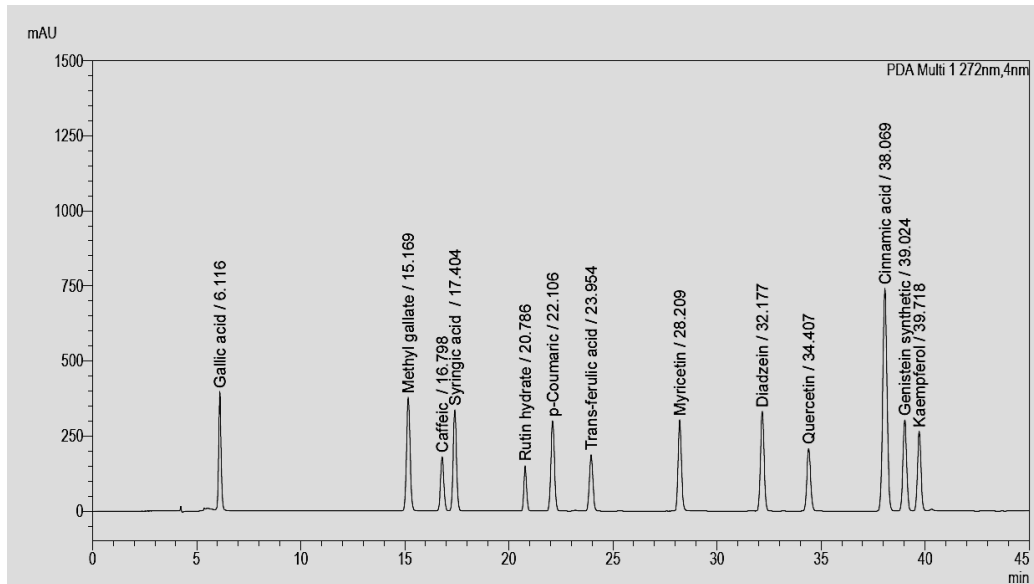


Figure 1. Chromatogram of mixed 13 phenolic acid and flavonoid standards by HPLC

The antioxidant activity of freeze-dried moringa pod extract was determined by a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [15]. The extract was mixed and incubated with a DPPH solution. Absorbance was measured at 525 nm after 30 minutes. The antioxidant activity was declared as mg Trolox equivalents per mL sample.

Effects of freeze-dried moringa pod extract on Raw

264.7 macrophages: Raw 264.7 macrophage cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C with 5% CO₂. The cells were utilized once they reached 80% confluence. Cell viability was assessed using the MTS assay with a cell density of 1×10⁴ cells per well. The cells were exposed to either 70% ethanol or freeze-dried moringa pod extract at concentrations ranging from 25 to 1000 µg/mL. After 24 hours of treatment, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS reagent) was added, and absorbance was measured at 490 nm using a

microplate reader (Omega, BMG Labtech, Ortenberg, Germany) [16]. Nitric oxide (NO) level was determined at cells density 5×10⁴ cells per well by the Griess method. Cells were co-treated with lipopolysaccharide (LPS) and freeze-dried moringa pod extract at concentrations of 50, 100, and 200 µg/mL for 24 hours. The supernatants from the culture media were combined with an equal volume of Griess reagent, comprising 50 µL of 1% sulphanilamide in 5% phosphoric acid and 50 µL of 0.1% naphthylethylenediamine dihydrochloride. Absorbance was measured at 540 nm and calculated to nitrite concentration of sample-treated cells using the sodium nitrite (NaNO₂) standard curve [17]. The other medium supernatants were stored at 4°C before cytokine testing.

Anti-inflammatory (IL-6 and TNF-α) cytokine levels were determined in medium supernatants using an enzyme-linked immunosorbent assay of mouse ELISA kit from Invitrogen (Frederick, MD, USA). The concentrations of cytokine levels were calculated from the standard curve.

Statistical analysis: The experiment followed a completely randomized design (CRD), with each treatment conducted in triplicate. Treatment comparisons were conducted using one-way ANOVA followed by Tukey's multiple comparisons test. A p-value of 0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism software version 10.2.2 (California, USA).

RESULTS AND DISCUSSION

Physical and chemical compositions: Freeze-dried moringa pods were ground to powder, as shown in Figure 2B. The lightness (L^*), green color ($-a^*$), and yellow color (b^*) values of the powder were 78.51 ± 1.10 , -4.35 ± 0.12 , and 22.67 ± 0.72 , respectively. Meanwhile, this sample

had a low chroma level (23.08 ± 0.72) but high hue angle (79.14 ± 0.38), as shown in Table 2. This value implied that freeze-dried moringa pod powder had a pale yellow-green tone color.

The primary pigment found in moringa pods is chlorophyll, ranking among the most notable bioactive compounds and demonstrating numerous health benefits, which include antioxidant, anti-inflammatory, and anti-cancer properties [18]. In this study, the contents of total chlorophyll (chlorophyll a and b) were determined. Freeze-dried moringa pod powder had 32.05 mg/ml of chlorophyll a, 13.28 mg/100 g of chlorophyll b, and 45.33 mg/100 g of total chlorophyll, as shown in Table 1.

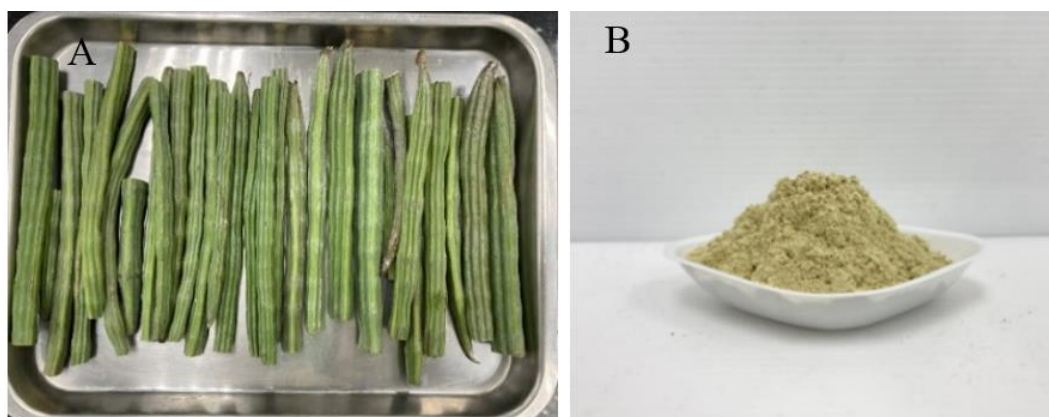


Figure 2. Freeze-dried moringa pods (A) and its powder (B)

Table 1. Color parameter and chlorophyll content of freeze-dried moringa pod powder

Freeze-dried moringa pod powder		Average \pm SD
Color parameter		
	L^*	78.51 ± 1.10
	a^*	-4.35 ± 0.12
	b^*	22.67 ± 0.72
	C^*	23.08 ± 0.72
	H_{ab}	79.14 ± 0.38
Chlorophyll (mg/100g)		
	Chlorophyll a	32.05 ± 1.18
	Chlorophyll b	13.28 ± 0.49
	Total chlorophyll	45.33 ± 1.30

The composition of freeze-dried moringa pod powder was determined (Table 2). Freeze-dried moringa pod powder has low water activity and moisture content (0.2 and 3.28, respectively), which inhibits the microbial cells growth, germination of spores, toxin production, and enzyme activities [19]. In this study, moringa pods contained whole seeds and coats, which had 0.84 g of fat and 15.50 g of protein per 100 g sample, similar to other investigations that also showed low amounts of fat in

moringa pods (0.4-1.0%) [4, 20]. However, protein amount comparison with other studies reported various ranges from 10.1-17.2%, which could have been influenced by the growing region, cultivation area, and level of nitrogen available [4, 20]. Although moringa pods had low protein content, essential amino acids including isoleucine, leucine, arginine, glycine, glutamine, and tyrosine were reported in sufficient amounts [20].

Table 2. Composition of freeze-dried moringa pod powder

Freeze-dried moringa pod powder	Average \pm SD
Water activity (A_w)	0.20 \pm 0.00
Moisture (%)	3.28 \pm 0.25
Solid (%)	96.72 \pm 0.25
Fat (g/100g)	0.84 \pm 0.06
Protein (g/100g)	15.50 \pm 0.49

Bioactive compounds and antioxidant activities: A mixture chromatogram with high-value $R^2 > 0.99$ recorded at 272 nm is presented in Figure 1. The assessment of the bioactive compounds in freeze-dried moringa pod extract in this study consisted of phenolics and flavonoids. A chromatogram of the phenolic and flavonoid compounds in freeze-dried moringa pod extract is shown in Figure 3. The results illustrated that freeze-dried moringa pod extract had genistein synthetic, trans-ferulic acid, p-Coumaric, myricetin, gallic acid, and syringic acid (39.70, 19.50, 9.41, 8.57, 7.16 and 4.89 mg/100g, respectively), as shown in Table 3. The detected peaks did not match any compounds identified in our laboratory. However, it has been reported that other compounds observed may primarily be derivatives, such as glucosides, specifically quercetin and kaempferol [21]. Additionally, the various

bioactive compounds of Moringa are influenced by the growing region, the specific part of the Moringa plant, and the extraction method [22].

Genistein, the most abundant phytochemical in moringa pod extract, is renowned for its diverse biological activities. These include anti-cancer, antioxidant, and anti-inflammatory effects, as well as protection against osteoporosis and reduction in the risk of cardiovascular disease [23]. Meanwhile, trans-ferulic acid is a potent antioxidant with anti-aging, anti-inflammation, and anti-cancer benefits. Furthermore, genistein aids in reducing cholesterol levels and inhibits the oxidation of low-density lipoprotein (LDL) [24, 25]. Other phenolic acids such as p-coumaric acid and gallic acid, are secondary phenolic metabolites which are

widely distributed in the plant. It exhibits numerous bioactivities encompassing antioxidant, anti-inflammatory, anti-cancer, and anti-diabetic properties [26, 27]. Moreover, it has been reported that myricetin and syringic acid have anticancer, antidiabetic, and anti-obesity benefits, as well as providing cardiovascular protection, osteoporosis protection, and anti-inflammatory effects [28, 29].

The antioxidant activity of freeze-dried moringa pod extract was determined using a DPPH assay. The results of this study showed that the freeze-dried moringa pod extract had 510.13 mg Trolox/ 100g. It is well known that antioxidants play a role in scavenging and neutralizing

free radicals, which have anti-inflammatory properties that prevent inflammatory responses in the body. Antioxidants help by donating electrons to stabilize these free radicals, thereby reducing the inflammatory process [30]. It has been reported that the antioxidant activities of moringa extract varied in a range of 40-675 mg /100g of dry weight, which could be influenced by the amount of raw moringa, growing region, cultivation area, extraction solvent, the extraction method [15, 31].

From the polyphenol compounds and antioxidant activity of freeze-dried moringa pod extract, the authors hypothesized that the extract could show anti-inflammatory effects.

Table 3. Bioactive compound and antioxidant activity of Moringa pod extract

Bioactive compound and antioxidant activity	Moringa pod extract (mg/100 g)
Phenolic acid	
Gallic acid	7.16±0.36
Methyl gallate	ND
Caffeic	ND
Syringic acid	4.89±0.41
p-Coumaric	9.41±0.75
Trans-ferulic acid	19.50±1.56
Cinnamic acid	ND
Flavonoid	
Rutin hydrate	ND
Myricetin	8.57±0.57
Diadzein	ND
Quercetin	ND
Genistein synthetic	39.70±1.73
Kaempferol	ND
Antioxidant activity	
DPPH method (mg Trolox/100g)	510.13±3.75

Note: ND means not detected in this method; mean ± standard deviation from three different experiments performed in triplicate.

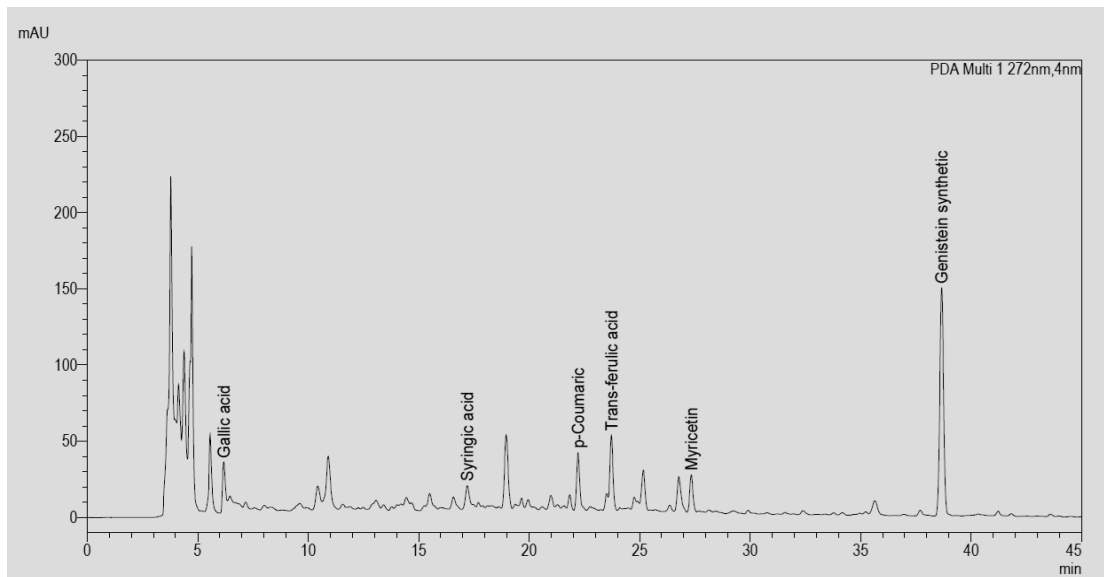


Figure 3. Chromatogram of freeze-dried moringa pod extract.

Effects of freeze-dried moringa pod extract on Raw 264.7 macrophages: Lipopolysaccharide (LPS) is a complex composed of lipids and polysaccharides, serving as a potent bacterial toxin commonly employed to elicit inflammation in the Raw264.7 macrophage cell line. Its mechanism involves interaction with the membrane receptor CD14, triggering the production of pro-inflammatory cytokines such as TNF- α , IL-6, and nitric oxide (NO) [32]. In this study, Raw264.7 macrophages were co-incubated with LPS at a concentration of 100 ng/ml along with varying concentrations of freeze-dried moringa pod extract (25, 50, 100, 200, 500, and 1000

$\mu\text{g/ml}$) for a duration of 24 hours. The cell viability values of freeze-dried moringa pod extract, at a concentration of 25-200 $\mu\text{g/ml}$, were over 90%. However, the cell viabilities were decreased to less than 30% in 500 and 1000 $\mu\text{g/ml}$ freeze-dried moringa pod extract incubation, as shown in Figure 4. This indicated that a high concentration of freeze-dried moringa pod extract may influence cell viability. Therefore, 50-200 $\mu\text{g/mL}$ of freeze-dried moringa pod extracts were selected to perform the anti-inflammatory effects in term of NO production, IL-6 and TNF- α cytokine secretion.

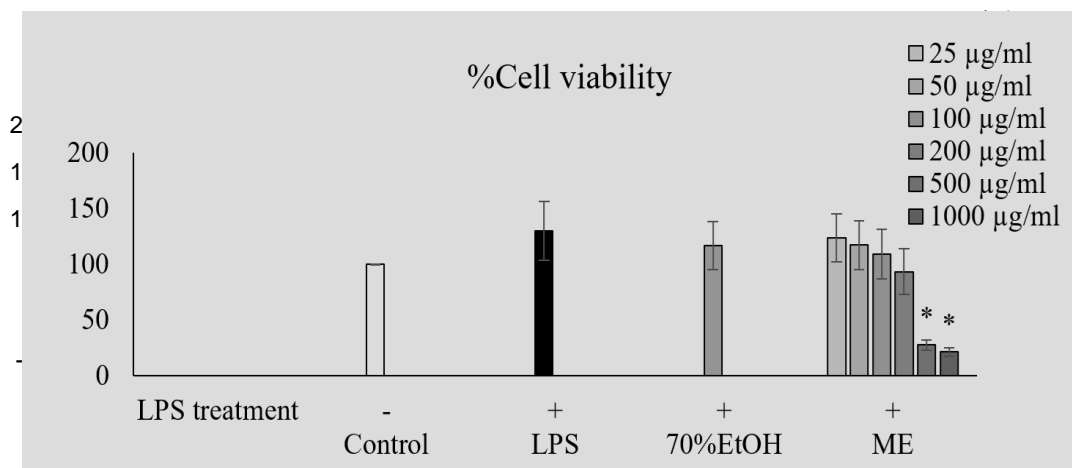


Figure 4. Cell viability of freeze-dried moringa pod extract (ME) at concentrations of 25, 50, 100, 200, 500, and 1000 $\mu\text{g/ml}$ on RAW 264.7 macrophages stimulated with 100 ng/ml of lipopolysaccharide (LPS) for 24 hr. Control = Untreated, LPS = LPS 100 ng/ml treated, 70%EtOH = 70% ethanol value as same as high-value solvent extraction in sample. Values are expressed as mean \pm SD (n=3).

Nitric oxide (NO) serves as a crucial signaling molecule in cellular processes and contributes significantly to inflammation. Its production is catalyzed by nitric oxide synthases (NOS), which exist in three isoforms: neuronal NOS (nNOS), constitutive endothelial NOS (eNOS), and inducible NOS (iNOS) [33]. iNOS isoform is produced by macrophage cells and is induced by proinflammatory cytokine. The endotoxin NO can increase vascular permeability and induce vasodilation as well as inflammation [34]. Thus, NO inhibition is the key to decreasing inflammation in macrophage cells. In this study, the incubation of 100 ng/ml of LPS significantly produced NO compared to the control group, whereas

the 70% ethanol-treated group also significantly produced NO but had no significant difference to the LPS group on LPS-induced Raw 264.7 macrophage cells, as shown in Figure 5. Conversely, when incubated with freeze-dried moringa pod extract at concentrations of 50, 100, and 200 $\mu\text{g/ml}$ along with 100 ng/ml of LPS, a notable reduction in NO secretion was observed in a dose-dependent manner ($p < 0.05$). This finding aligns with a previous study that demonstrated pre-treatment with boiled moringa pod extract ranging from 31 to 250 $\mu\text{g/ml}$ effectively decreased NO production without inducing cytotoxic effects [35].

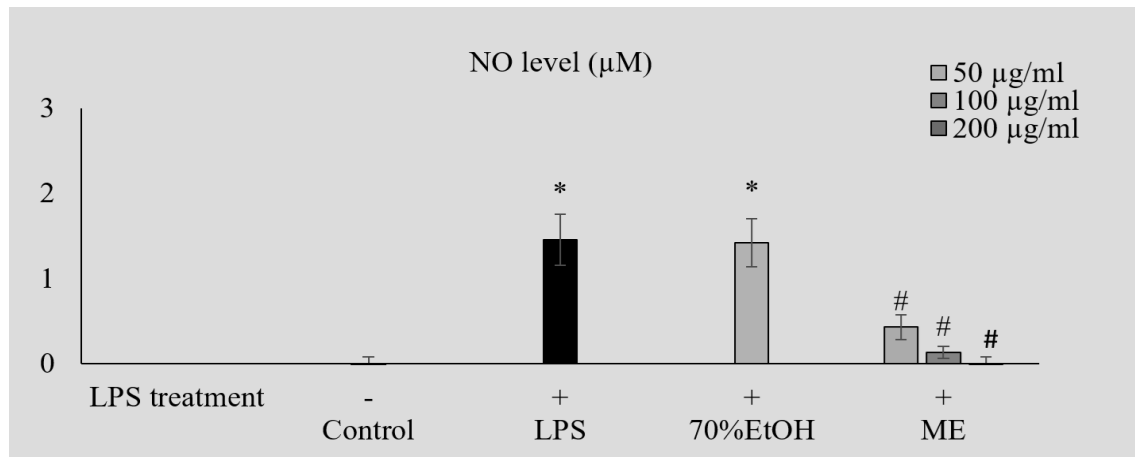


Figure 5. The nitric oxide (NO) levels were assessed in RAW 264.7 macrophages treated with lipopolysaccharide (LPS) for 24 hours, comparing various conditions: Control (Untreated), LPS (treated with LPS at 100 ng/ml), and 70%EtOH (treated with 70% ethanol, serving as the high-value solvent extraction control). Results are presented as mean \pm standard deviation ($n=3$); * indicates significance at $P < 0.05$ compared to the control, while # indicates significance at $P < 0.05$ compared to the LPS-treated group.

Figure 6 demonstrates the impact of ME on levels of other pro-inflammatory mediators, specifically IL-6 and TNF- α cytokines. The findings indicate a significant reduction in LPS-induced pro-inflammatory expression with ME treatment, exhibiting a dose-dependent effect ($p < 0.05$). Previous studies revealed that pre-treatment with boiled moringa pod extract significantly suppressed the pro-inflammatory cytokine on LPS-induced Raw264.7 macrophages [35]. Moreover, other parts of moringa

extract including the leaf, seed, flower, and root also showed anti-inflammatory effects on Raw 264.7 macrophages [2, 3].

Within the inflammation process, IL-6 and TNF- α cytokines emerge as primary pro-inflammatory mediators, predominantly generated by macrophages [16]. Polyphenols, such as phenolics and flavonoids have been reported to be able to suppress IL-6 and TNF- α cytokine levels and effectively inhibit LPS-induced NO

production. In this experiment, freeze-dried moringa pod extract at 50-200 $\mu\text{g/ml}$ had polyphenols including genistein (1.99-7.94 $\mu\text{g/ml}$), trans-ferulic acid (0.98-3.9 $\mu\text{g/ml}$), p-Coumaric (0.47-1.88 $\mu\text{g/ml}$), myricetin (0.43-1.71 $\mu\text{g/ml}$), gallic acid (0.36-1.43 $\mu\text{g/ml}$), and syringic acid (0.25-0.98 $\mu\text{g/ml}$) (data from covert calculation). Previous studies showed that these polyphenols had anti-inflammatory activity on LPS-induced Raw264.7 macrophage models. At a concentration of 40 $\mu\text{g/ml}$, genistein has been reported to significantly inhibit the activity of inflammatory mediators such as PGE_2 , $\text{TNF-}\alpha$, and $\text{IL-1}\beta$ [36]. This is similar to a study on ferulic acid, which reported that a concentration of 0.012-0.31 $\mu\text{g/ml}$ of ferulic acid significantly decreased inflammatory activities via NO, $\text{IL-1}\beta$, and IL-6 levels without

cytotoxicity [37]. Furthermore, studies have revealed that p-coumaric acid, within the concentration range of 10-100 $\mu\text{g/ml}$, exhibits anti-inflammatory properties by notably suppressing COX-2, iNOS, $\text{TNF-}\alpha$, and $\text{IL1}\beta$ mRNA expression in a dose-dependent fashion, particularly when compared to LPS-treated Raw264.7 macrophage cells [38]. Furthermore, the study on myricetin, at the concentration 3.98-7.96 $\mu\text{g/ml}$, also significantly inhibited the production of pro-inflammatory cytokines via the decreasing of the mRNA expression level of pro-inflammatory factors ($\text{TNF-}\alpha$, IL-6, $\text{IL-1}\beta$, COX-2 and iNOS) [39, 40]. The anti-inflammatory properties of 100 μM gallic acid and 10-20 μM syringic acid have been reported [41, 42]. However, mixed usage of these polyphenols on anti-inflammatory effects showed no evidence.

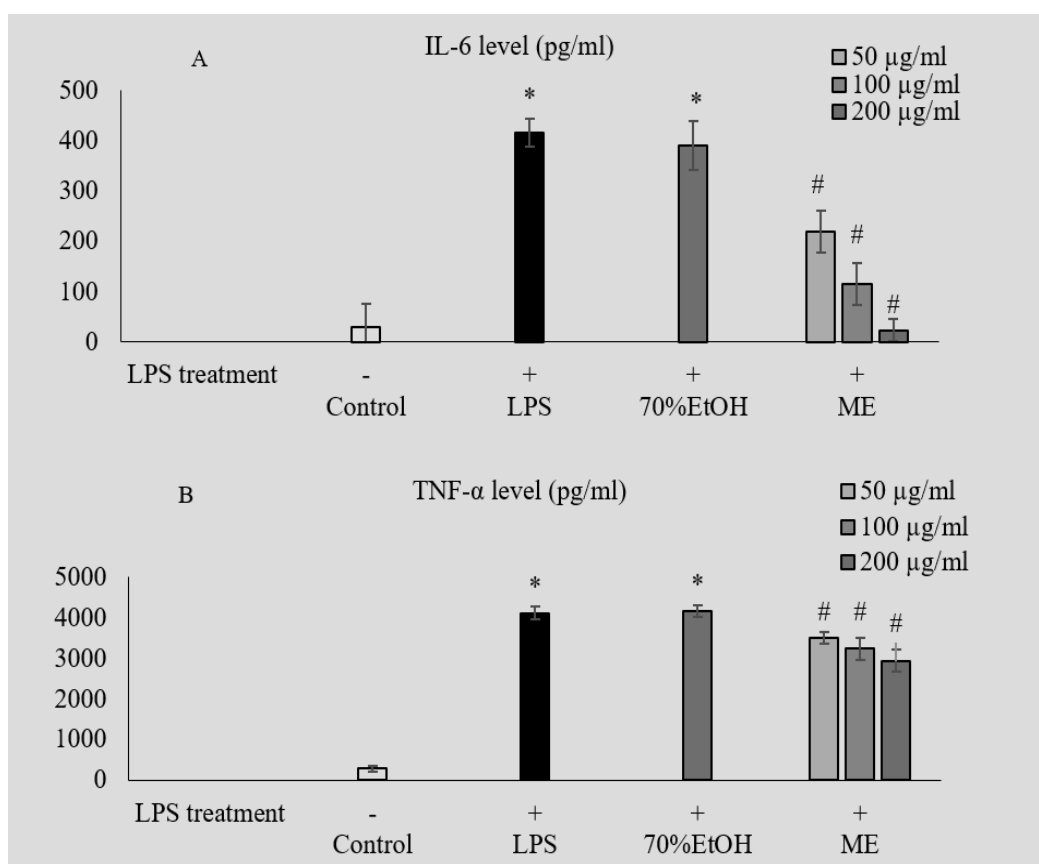


Figure 6. IL-6 (A) and TNF- α (B) cytokine levels were evaluated in RAW 264.7 macrophages following stimulation with lipopolysaccharide for 24 hours. Experimental conditions included Control (Untreated), LPS (treated with LPS at 100 ng/ml), and 70%EtOH (treated with 70% ethanol, serving as the high-value solvent extraction control). Results are presented as mean \pm standard deviation (n=3); * denotes significance at $P < 0.05$ compared to the control, while # indicates significance at $P < 0.05$ compared to the LPS-treated group.

In summary, the results of this study demonstrated that freeze-dried moringa pod extract with bioactive polyphenols could reduce inflammation in LPS-induced Raw264.7 macrophage cells via the suppression of NO, IL-6 and TNF- α levels. However, bioactive absorption, metabolism, and mechanism need further clarification.

CONCLUSION

Freeze-dried moringa pod extract contains various bioactive compounds such as genistein synthetic, trans-ferulic acid, p-Coumaric acid, myricetin, gallic acid, and syringic acid. These compounds have been shown to possess antioxidant properties and exhibit anti-inflammatory effects. The results of this study indicate the functional potential, especially the antioxidant and anti-inflammatory properties, of freeze-dried moringa pods. These raise the local remedy plant to become a new functional ingredient in product development.

List of Abbreviations: DPPH - 2,2 diphenyl-1-picrylhydrazyl; Trolox - 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; DMEM - Dulbecco's Modified Eagle's Medium; FBS - fetal bovine serum; HPLC - High-Performance Liquid Chromatography; MTS - 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NO – Nitric oxide; IL-6 – Interleukin-6; IL-8 – Interleukin 8.

Authors Contributions: KH conceptualized the study, conducted the experiments, and wrote the original study. SB conceptualized the study, provided supervision and review, and revised the original script.

Completing Interest: The author declares no conflict of interest.

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