USD 615.276:615.322:[616.153.455-008.61:578.834]-092.4-07:612.017
https://doi.org/10.26641/2307-0404.2026.2.365902

A.H. Nurhasanah [1],
M. Louisa [2 *],
M. Angelina [3],
A. Estuningtyas [2]

AN EXPLORATORY EFFECT OF *PHALERIA MACROCARPA* FRUIT EXTRACT ON INTERLEUKIN-6 mRNA EXPRESSION IN HUMAN PBMCs INDUCED BY GLUCOSE-ENHANCED SARS-COV-2 SPIKE PROTEIN AND LIPOPOLYSACCHARIDE *IN VITRO*

Faculty of Medicine, Universitas Indonesia, Master's Program in Biomedical Sciences [1]
Faculty of Medicine, Universitas Indonesia, Department of Pharmacology and Therapeutics [2]
Salemba str., 6, Jakarta Pusat, Jakarta, 10430, Indonesia
National Research and Innovation Agency of Indonesia (BRIN) [3]
Research Center for Pharmaceutical Ingredients and Traditional Medicine
KST BJ Habibie, Puspitek, South Tangerang, 15314, Indonesia
Університет Індонезії, медичний факультет, магістерська програма з біомедичних наук [1]
Університет Індонезії, медичний факультет, кафедра фармакології та терапії [2]
вул. Салемба, 6, Джакарта Пусат, Джакарта, 10430, Індонезія
Національне агентство досліджень та інновацій Індонезії (BRIN) [3]
Дослідницький центр фармацевтичних інгредієнтів і традиційної медицини
KST ім. Б.Дж. Хабібі, Пуспітек, Південний Тангеранг, 15314, Індонезія
[*]*e-mail: melva.louisa@gmail.com*

Цитування: ***Медичні перспективи. 2026. Т. 31, № 2. С. 108-119***
Cited: ***Medicni perspektivi. 2026;31(2):108-119***

Key words: *hyperglycemia, inflammation, interleukin-6, peripheral blood mononuclear cells, type-2 diabetes mellitus*
Ключові слова: *гіперглікемія, запалення, інтерлейкін-6, мононуклеарні клітини периферичної крові, цукровий діабет 2 типу*

Abstract. An exploratory effect of *Phaleria macrocarpa* fruit extract on interleukin-6 mRNA expression in human PBMCs induced by glucose-enhanced SARS-CoV-2 spike protein and lipopolysaccharide *in vitro*. Nurhasanah A.H., Louisa M., Angelina M., Estuningtyas A. *Type 2 diabetes mellitus is a significant risk factor for dysregulated inflammatory responses during Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection. Corticosteroids, commonly used as anti-inflammatory agents in diabetic patients, often cause hyperglycemia, making infections and inflammation more difficult to control. These conditions highlight the need for laboratory models that can support preliminary screening of anti-inflammatory drug candidates under elevated-glucose conditions. This exploratory study aimed to establish a human peripheral blood mononuclear cell (PBMC) inflammatory model induced by SARS-CoV-2 and lipopolysaccharide in the presence of high glucose and to assess the modulatory activity of Phaleria macrocarpa fruit ethanol extract on interleukin-6 (IL-6) mRNA expression under combined stimulation. This in vitro study used a human PBMC model induced by the SARS-CoV-2 spike protein and lipopolysaccharide under elevated glucose conditions. PBMCs were isolated from venous blood of a limited number of healthy adult volunteers. The concentrations of glucose, SARS-CoV-2 spike protein, and lipopolysaccharides used in PBMC cultures were selected for their ability to increase IL-6 mRNA expression under the tested conditions. Non-cytotoxic concentrations of Phaleria macrocarpa fruit ethanol extract were identified by maintaining cell viability of at least 80 percent. Combined stimulation with the SARS-CoV-2 spike protein, lipopolysaccharide, and high glucose increased IL-6 mRNA expression. Treatment with Phaleria macrocarpa fruit ethanol extract was comparable with dexamethasone in reducing IL-6 mRNA expression under the combined stimulation condition. These preliminary findings suggest that Phaleria macrocarpa suppresses IL-6 mRNA expression in response to combined stimulation in this in vitro PBMC model. However, our findings should be interpreted as exploratory due to the small number of the PBMC donor pool. Further studies using a larger, sex-balanced donor population, broader cytokine panels, and chemical standardization are required.*

Реферат. Пошукове дослідження впливу екстракту плодів *Phaleria macrocarpa* на експресію мРНК інтерлейкіну-6 у мононуклеарних клітинах периферичної крові людини, індукованих spike-білком SARS-CoV-2 та ліпополісахаридом в умовах підвищеного рівня глюкози *in vitro*. Нурхасанах А.Г., Луїса М., Ангелина М., Естунінгтіяс А. *Цукровий діабет 2-го типу є значущим фактором ризику розвитку порушених запальних реакцій під час інфекції, спричиненої вірусом Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Кортикостероїди, які широко застосовуються як протизапальні засоби в пацієнтів з діабетом, часто*

На умовах ліцензії CC BY 4.0

викликають гіперглікемію, що ускладнює контроль інфекційних і запальних процесів. Ці обставини зумовлюють потребу у створенні лабораторних моделей для попереднього скринінгу потенційних протизапальних лікарських засобів в умовах підвищеної концентрації глюкози. Метою цього пошукового дослідження було створення запальної моделі на основі мононуклеарних клітин периферичної крові людини (peripheral blood mononuclear cell, PBMCs), індукованої SARS-CoV-2 та ліпополісахаридом за умов високого рівня глюкози, а також оцінювання модулювальної активності етанольного екстракту плодів Phaleria macrocarpa щодо експресії мРНК (матрична РНК) інтерлейкіну-6 (interleukin-6, IL-6) за комбінованої стимуляції. У дослідженні in vitro використовували модель людських PBMCs, стимульованих spike-білком SARS-CoV-2 та ліпополісахаридом в умовах підвищеної концентрації глюкози. PBMCs виділяли з венозної крові обмеженої кількості здорових дорослих добровольців. Концентрації глюкози, spike-білка SARS-CoV-2 та ліпополісахариду для культивування PBMCs підбирали на основі їхньої здатності підвищувати експресію мРНК IL-6 за досліджуваних умов. Нетоксичні концентрації етанольного екстракту плодів Phaleria macrocarpa визначали шляхом забезпечення життєздатності клітин на рівні не менше 80%. Комбінована стимуляція spike-білком SARS-CoV-2, ліпополісахаридом та високою концентрацією глюкози спричиняла підвищення експресії мРНК IL-6. Вплив етанольного екстракту плодів Phaleria macrocarpa щодо зниження експресії мРНК IL-6 за умов комбінованої стимуляції був зіставним з ефектом дексаметазону. Отримані попередні результати свідчать, що Phaleria macrocarpa пригнічує експресію мРНК IL-6 у відповідь на комбіновану стимуляцію в цій моделі PBMCs in vitro. Водночас результати слід розглядати як пошукові через невелику кількість донорів PBMCs. Для підтвердження отриманих даних необхідні подальші дослідження із залученням більшої та гендерно збалансованої популяції донорів, ширших панелей цитокінів і проведенням хімічної стандартизації екстракту.

Individuals with type 2 diabetes mellitus (T2DM) face a higher risk of developing severe coronavirus disease 2019 (COVID-19) and have higher mortality rates compared to non-diabetic individuals. Although the exact mechanisms underlying greater severity in diabetic patients are not fully understood, both conditions involve dysregulated immune and inflammatory responses [1, 2]. A key feature of severe COVID-19 is the cytokine storm, which causes vascular hyperpermeability, multiorgan failure, and death. In severe cases, there is an overproduction of proinflammatory cytokines and chemokines, along with a limited induction of interferons (IFN-α, IFN-β, and IFN-γ). Increased interferon production activates nuclear factor kappa light chain enhancer of activated B cells (NF-κB), a crucial mediator of inflammatory responses. When activated, NF-κB translocates to the nucleus, leading to increased production of proinflammatory molecules, including interleukin-6 (IL-6). IL-6 is a key biomarker of COVID-19-related cytokine storms and is inversely associated with immune function impairment [3, 4].

Current therapeutic strategies recommended by the World Health Organization (WHO) for managing hyperinflammation include corticosteroids such as dexamethasone; however, their effectiveness remains limited [5, 6]. Additionally, severe COVID-19 and its steroid-based treatments can negatively impact diabetes management by worsening hyperglycemia through increased insulin resistance and impaired β-cell secretory function. In turn, uncontrolled hyperglycemia may further increase COVID-19 severity [7]. High-dose dexamethasone is also associated with significant adverse effects [7, 8, 9]. These limitations highlight the need for complementary strategies that can reduce hyperinflammation while minimizing risks in patients with metabolic comorbidities.

Natural products remain an important source of potential anti-inflammatory agents [10, 11]. One known natural product is *Phaleria macrocarpa fruit*, which has been reported to possess various bioactive properties, including antioxidant, antidiabetic, antiviral, anti-inflammatory, and immunomodulatory activities [12-15]. However, its anti-inflammatory activity has not been thoroughly evaluated in a human immune cell hyperinflammatory model with increased glucose exposure, a context relevant to diabetes-related vulnerability.

Previous mechanistic studies indicate that the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spike protein can enhance inflammatory responses to lipopolysaccharide (LPS), a well-established toll-like receptor 4 (TLR4) ligand, thereby increasing proinflammatory cellular responses both *in vitro* and *in vivo* [16, 17, 18]. Evidence also suggests that higher glucose exposure may amplify immune cell inflammatory responses. Therefore, a peripheral blood mononuclear cell model that combines increased glucose exposure with stimulation by the spike protein and lipopolysaccharide could serve as a practical platform for early screening of potential anti-inflammatory interventions [19, 20]. Given its known pharmacological properties, *P. macrocarpa* was selected for preliminary evaluation in this model, with IL-6 mRNA expression used as the primary inflammatory marker.

This study aimed to establish an exploratory model of inflammatory peripheral blood mononuclear cells (PBMCs) under high-glucose exposure by sequential stimulation with SARS-CoV-2 spike protein and lipopolysaccharide, and to evaluate whether an ethanol extract of *Phaleria macrocarpa* fruit modulates the induced IL-6 mRNA response.

MATERIALS AND METHODS OF RESEARCH

This study adhered to the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No. KET-1415/UN2.F1/ETIK/PPM.00.02/2025). Written informed consent was obtained for participation and PBMC sample collection.

Plant collection and extract preparation

The dried fruit of *Phaleria macrocarpa* was obtained from a sample collected by the National Research and Innovation Agency (BRIN). The plant determination was done by the Traditional Medicine Raw Material Standardization Laboratory, BRIN (Voucher Number: 6449-244096-1).

Approximately 166.8 g of the powdered sample was sequentially extracted by maceration in 70% ethanol for 4 days at ambient temperature, with periodic shaking. The extract was then concentrated under reduced pressure using a rotary evaporator (Buchi Laboratorium-Technik AG, Flawil, Switzerland) at 45°C until all solvent was removed, yielding the dried crude extract.

Phytochemical analysis

The bioactive compounds of the *Phaleria macrocarpa* fruit extract were analyzed using phytochemical screening. The test procedures involved the examination of alkaloids, phenols, flavonoids, saponins, tannins, and terpenoids [21, 22].

High-performance liquid chromatography (HPLC) analysis

HPLC analysis was performed to confirm the presence of phalerin and mangiferin as markers for *Phaleria macrocarpa* fruit ethanol extracts. The study was conducted using a Waters HPLC 2695 Alliance system equipped with a photodiode array detector (2998). Chromatographic separation was achieved on a Symmetry C18 column. Sample solutions were filtered through a 0.22 μm membrane filter and injected into the column in a 30 μL volume. For phalerin, isocratic separation was performed using acetonitrile and water (63:35, v/v) as the mobile phase at 35°C, a flow rate of 0.9 mL/min, and detection at 220 nm. Mangiferin analysis was conducted using a mobile phase consisting of methanol and 0.5% formic acid (30:70, v/v). The chromatographic conditions were as follows: column temperature, 40°C; flow rate, 1.0 mL/min; and detection at 257 nm [18, 19].

PBMC isolation

Human peripheral blood mononuclear cells were isolated from venous blood of healthy adult volunteers. Eligibility criteria for donors were healthy men or women aged 18-45 years, blood group O, who received at least two doses of the COVID-19 vaccine. Individuals with confirmed diabetes mellitus were excluded. PBMCs were obtained from two healthy female donors. Three experimental replicates were performed and should be interpreted as technical replicates. A blood volume of 15 mL was collected from each donor at each collection.

Whole blood was diluted 1:1 with cold Dulbecco's phosphate-buffered saline (DPBS, Gibco, Cat No. 14190-144) and layered over an equal volume of density-gradient medium (Ficoll-Paque™ PLUS, Cytiva, Cat No. 17144003) [13]. Samples were centrifuged at 2,500 rpm for 30 minutes. The PBMC layer was collected, washed twice with cold Dulbecco's phosphate-buffered saline (DPBS, and centrifuged at 1,200 rpm for an additional 10 minutes at room temperature. The isolated pellets were resuspended in 1 mL of cold DPBS. Cell count was determined using a hemocytometer, and viability was assessed by trypan blue exclusion. Samples with >90% viability were selected for downstream experiments [23].

PBMC culture, glucose conditioning, and hyperinflammatory stimulation

PBMCs were cultured in complete medium consisting of RPMI-1640 (Gibco, Cat No. 11875-093) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich, Cat No. F9665) and 1% penicillin-streptomycin (Sigma-Aldrich, Cat No. P4333). Cells were incubated for 2 h at 37°C in a humidified 5% CO_2 atmosphere. Afterward, adherent cells were separated from the non-adherent ones [24]. Adherent PBMCs were seeded at a density of 1.5×10^6 cells per well in high-glucose medium and incubated overnight.

PBMCs were cultured in high-glucose medium conditioned for 2 hours by supplementing the culture media with glucose at 5.5, 15, 25, or 30 mM. The resulting glucose concentrations in culture supernatants were measured spectrophotometrically at 500 nm using the Glucose GOD FS assay (DiaSys, Cat. No. 1 2500 99 83 021). Based on these measurements and the corresponding IL-6 mRNA response, 15 mM glucose supplementation was selected for subsequent experiments.

To induce a hyperinflammatory condition, adherent PBMCs were subsequently stimulated with SARS-CoV-2 spike protein (RayBiotech, Cat No. 30-01101-10) for 2 hours, followed by lipopolysaccharide (LPS) (Sigma-Aldrich, Cat No. L2880) at 10 ng/mL for 24 hours. Spike protein exposure was maintained during the LPS phase. An overview of the study workflow is presented in Figure 1.

Treatment with Phaleria macrocarpa extract

Adherent PBMCs were treated with varying concentrations of *Phaleria macrocarpa* fruit ethanol extract (12.5, 25, and 50 μg/mL) or dexamethasone (50 mg/mL) for 24 hours. Dexamethasone (Infalabs, B0120206) served as a positive anti-inflammatory

На умовах ліцензії CC BY 4.0

control. Vehicle controls contained dimethylsulfoxide (Sigma-Aldrich, Cat No. D2650) at the same final concentrations as treatment groups. *Phaleria macrocarpa* fruit extracts or dexamethasone were added after sequential PBMC stimulation with SARS-CoV-2 spike protein and lipopolysaccharides (Fig. 1).

Interleukin-6 mRNA gene expression

For gene expression analysis, cells were harvested after 24 h, and total RNA was isolated using the Quick-RNA™ Miniprep Plus Kit (Zymo Research, Cat No. R1058). Total RNA was reverse-transcribed into cDNA using the ReverTra Ace® RT-qPCR Master Mix with gDNA remover (Toyobo, Cat No. FSQ-301), following the manufacturer's instructions. Relative mRNA expression was quantified using the comparative method, with β-actin serving as the internal reference gene. Real-time PCR was performed using the SensiFAST SYBR® No-ROX (Biolane, Cat No. BIO-98005) on a MiniOpticon instrument (Bio-Rad). Primer sequence used for Interleukin-6 (IL-6): Forward: CACTCACCTCTTCAGAACGAAT and Reverse: GCTGCTTTCACACATGTTACTC. As a housekeeping gene, β-actin was used, with primer sequences: Forward: ACAGGATGCAGAAGGAGATTAC; Reverse: ATAGAGCCACCAATCCACAC. The data consisted of cycle threshold (CT) values automatically generated by the software. Relative mRNA expression levels were calculated using the Livak ($2^{-\Delta\Delta Ct}$) method [25].

Data analyses

Data are presented as the median and interquartile range (IQR). Differences between groups were tested using the Kruskal-Wallis test, followed by the Mann-Whitney U test. A p-value less than 0.05 was considered significant at the 95% confidence level. Because of the limited number of PBMCs used in this experiment, the analysis was considered preliminary. The experiment was conducted in three replicates and served as a technical control. All inferential statistics should be interpreted with caution and regarded as exploratory. Plots were created using GraphPad Prism version 10.1.1 (GraphPad Software, San Diego, CA, USA).

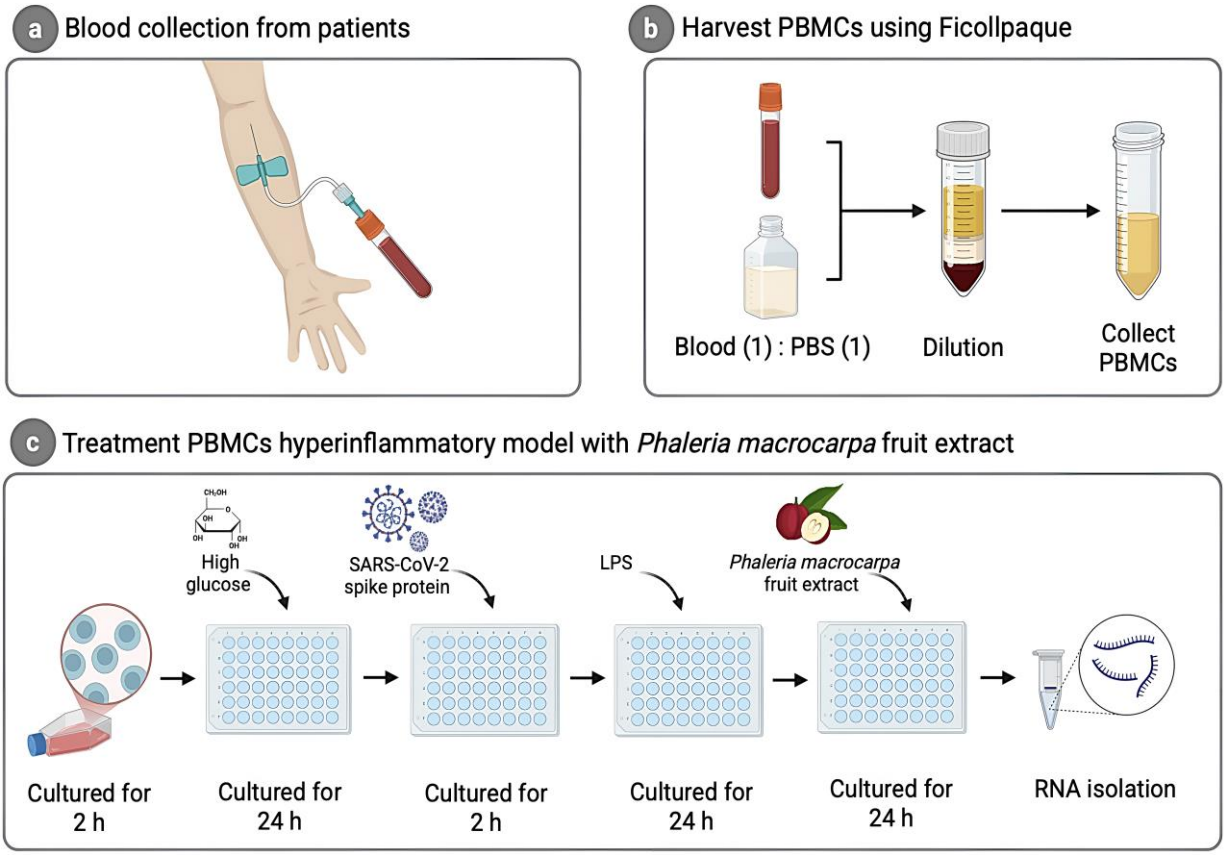


Fig. 1. Overview of the study workflow: (a) Blood samples were collected from patients using standard venipuncture procedures; (b) PBMCs were isolated by density-gradient centrifugation using Ficoll-Paque; and (c) isolated PBMCs were cultured and sequentially stimulated to establish an exploratory inflammatory model. Cells were first cultured under high-glucose conditions, then exposed to the SARS-CoV-2 spike protein and lipopolysaccharide (LPS). PBMCs were then treated with Phaleria macrocarpa fruit extract for 24 h. After treatment, total RNA was isolated for gene expression analysis

RESULTS AND DISCUSSION

Phytochemical profile and extraction yield

The ethanol extraction of 166.8 g of *Phaleria macrocarpa* fruit powder yielded 56.7 g of dried extract, corresponding to an extraction yield of 34%. Qualitative phytochemical screening (Table 1) indicated the presence of alkaloids, phenols, flavonoids, saponins, tannins, and triterpenoids. This broad phytochemical profile is consistent with the reported pharmacological potential of *P. macrocarpa* and supports its evaluation in anti-inflammatory assays in various conditions [26, 27, 28].

References in previous studies indicate that phalerin and mangiferin are the two main components and markers of *Phaleria macrocarpa* extracts [29, 30, 31]. Therefore, we conducted a targeted HPLC analysis using the reference compounds phalerin and mangiferin. The results showed that the *Phaleria macrocarpa* fruit ethanol extract contained phalerin as the main compound at 3710 ng/mL (17.04%) and mangiferin, a bioactive compound with antidiabetic properties, at 82.17 ng/mL (2.93%). Studies have shown that *Phaleria macrocarpa* extracts rich in phalerin and mangiferin exert antidiabetic and anti-inflammatory activities [27, 30, 31, 32], thereby substantiating their use in hyperinflammatory conditions aggravated by hyperglycemia.

Table 1

Phytochemical test results of *Phaleria macrocarpa* fruit ethanol extract

Types of qualitative tests	Results
Alkaloids	+
Phenols	+
Flavonoids	+
Saponins	+
Tannins	+
Triterpenoids	+

Note. (+) shows the presence of the phytochemical constituent.

Selection of a high-glucose culture condition

Glucose concentrations in culture supernatants were evaluated under the specified glucose conditions and are presented in Table 2. Among the four glucose-supplementation conditions tested, the 15 mM glucose-supplemented medium yielded a supernatant glucose concentration of 358 mg/dL, which corresponds to the hyperglycemic range. When peripheral blood mononuclear cells were incubated for 24 hours under 15 mM glucose-supplemented conditions, IL-6 mRNA expression increased by about 1.80-fold compared with control (Fig. 2, a). Accordingly, 15 mM glucose was selected for subsequent experiments because it produced a measurable IL-6 mRNA response and represented exposure above the physiological reference of 5.5 mM.

Table 2

Glucose concentrations in PBMC culture media after supplementation with varying glucose concentrations

Concentrations of glucose supplementation in culture media (mM)	Supernatant glucose concentrations after 24-hour treatment	
	in mM	in mg/dL
5.5	**14.15**	**257.23**
15	**19.73**	**358.60**
25	**28.82**	**524.04**
30	**32.10**	**578.41**

На умовах ліцензії CC BY 4.0

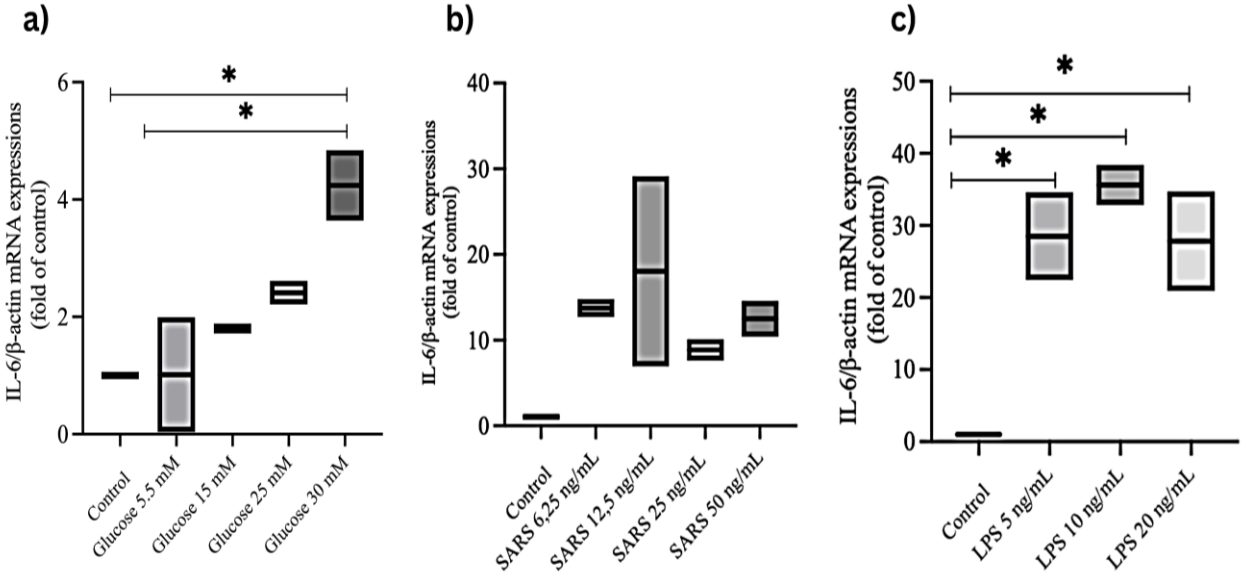
Proinflammatory response to SARS-CoV-2 spike protein and lipopolysaccharides

In the present study, IL-6 mRNA expression was assessed as a marker of the acute proinflammatory response. Stimulation of PBMCs with the SARS-CoV-2 spike protein at 12.5 ng/mL for 24 hours resulted in about an 18-fold increase in IL-6 expression compared with the control group (Fig. 2, b). Thus, the SARS-CoV-2 spike protein at 12.5 ng/mL was selected for sequential stimulation because it elicited the highest IL-6 response in this step.

Mechanistically, the SARS-CoV-2 spike protein activates proinflammatory signaling in immune cells. A study by Olajide et al. showed that high–dose SARS-CoV-2 S1 spike protein significantly increases secretion of TNF-α, IL-6, IL-1, and IL-8, along with upregulation of NF-κB signaling pathways [33]. Similar increases in IL-1 and IL-6 expression have been observed in PBMCs from patients with immune-mediated hearing loss (IMHL) stimulated with the SARS-CoV-2 spike protein at 12 ng/mL [34]. Prior *ex vivo* studies also indicate that the spike protein acts synergistically with low-dose LPS to enhance TNF- and IL-1 production [35]. Additionally, *in silico* studies have reported that the SARS-CoV-2 spike protein can bind not only to the angiotensin–converting enzyme 2 receptor but also to TLR4, a key receptor for lipopolysaccharide, potentially intensifying downstream inflammatory signaling [36]. However, receptor engagement and downstream pathway activation were not directly assessed in the present study; therefore, these mechanisms should be interpreted as literature-supported hypotheses rather than confirmed findings.

To induce a hyperinflammatory state, lipopolysaccharide (LPS) was added to the culture medium to stimulate the innate immune response in PBMCs. LPS, a structural component of Gram-negative bacteria, a potent activator of the innate immune system and widely used in experimental models of inflammation because it mimics cytokine-mediated inflammatory responses. Upon interaction with innate immune cells, particularly monocytes and macrophages, LPS stimulates the production of proinflammatory cytokines and chemokines, thereby contributing to host defense mechanisms [37]. Toll-like receptor 4 (TLR4) is the primary receptor for LPS recognition and signal transduction. Binding of LPS to TLR4 activates NF-κB through recruitment and activation of MyD88, IL-1 receptor–associated kinase (IRAK), TNF receptor-associated factor 6 (TRAF6), and NADPH oxidase (Nox). NF-κB plays a central role in regulating the transcription of genes related to innate immunity and inflammatory responses [24].

Our results showed that PBMCs stimulated with LPS at 10 ng/mL had the greatest increase in IL-6 mRNA expression (Fig. 2, c). These findings align with previous studies showing that LPS stimulates the production of proinflammatory cytokines and chemokines in innate immune cells. Even low concentrations of LPS have been shown to induce IL-6 and TNF-α production [37].



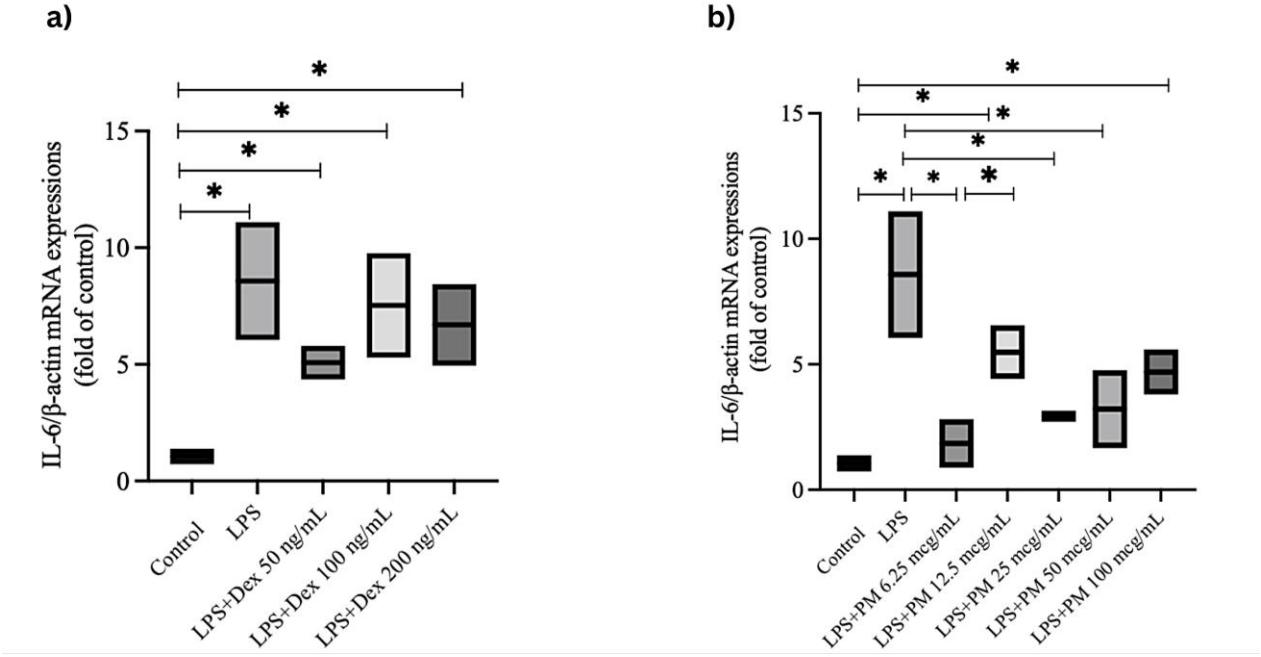
* - p<0.05 between the two groups

Fig. 2. Interleukin-6/actin mRNA expression stimulation with (a) high glucose; (b) SARS-CoV-2 spike protein; (c) lipopolysaccharides. Results are presented as median (interquartile range)

Activity of dexamethasone or Phaleria macrocarpa fruit extract on IL-6 expression in lipopolysaccharide-induced conditions

In the lipopolysaccharide-induced model (10 ng/mL), dexamethasone reduced IL-6 mRNA expression, with the greatest reduction at 50 ng/mL (Fig. 3, a). This effect is consistent with the known ability of glucocorticoids to suppress inflammatory gene transcription [38]. However, glucocorticoid receptor signaling and nuclear factor-κB (NF-κB) activation were not directly measured in this experiment.

Treatment with *Phaleria macrocarpa* fruit extracts at several concentrations after LPS stimulation at 10 ng/mL generally reduced IL-6 mRNA expression compared with the stimulated control (Fig. 3, b). However, the pattern was not entirely consistent. This variability may be attributable to the complex and heterogeneous chemical composition of herbal extracts, in which observed effects may result from the combined interactions of multiple constituents [39]. Therefore, these findings should be interpreted as a preliminary indication of an IL-6 mRNA-suppressive signal, suggesting that *Phaleria macrocarpa* fruit extract contains components that may attenuate LPS-induced IL-6 upregulation.



* - p<0.05 between the two groups

Fig. 3. Interleukin-6/β-actin mRNA expression after stimulations with (a) lipopolysaccharides 10 ng/mL and dexamethasone 50, 100, or 200 ng/mL; (b) lipopolysaccharides 10 ng/mL and *Phaleria macrocarpa* (PM) fruit extract 6.25, 12.5, 25, 50, or 100 μg/mL. Results are presented in median (interquartile range)

Cell viability assessment and determination of non-cytotoxic conditions

To confirm that changes in inflammatory markers were unaffected by the treatment's cytotoxic effects, cell viability was evaluated under the designated inflammatory stimulation and treatment conditions. The stimulatory agents (glucose, SARS-CoV-2 spike protein, and lipopolysaccharides) and the treatment agents (dexamethasone and *Phaleria macrocarpa* fruit extract) were assessed for their effects on PBMC viability. None of the treatments showed a significant reduction in cell viability compared with the control cells, and the average cell viability remained above 80% (Fig. 4).

The results suggest that the stimulation treatments used in subsequent analysis were unlikely to account for the observed effects on IL-6, indicating that these effects were unlikely to result from a significant loss of viable cells.

Suppression of IL-6 mRNA expression by *Phaleria macrocarpa* extracts in the combined spike protein and lipopolysaccharide model under high-glucose conditions

Figure 5 addresses the central objective of establishing an inflammatory model that incorporates both infection-associated stimuli and a high-glucose condition, and then applying it to the early screening of drug candidates.

На умовах ліцензії CC BY 4.0

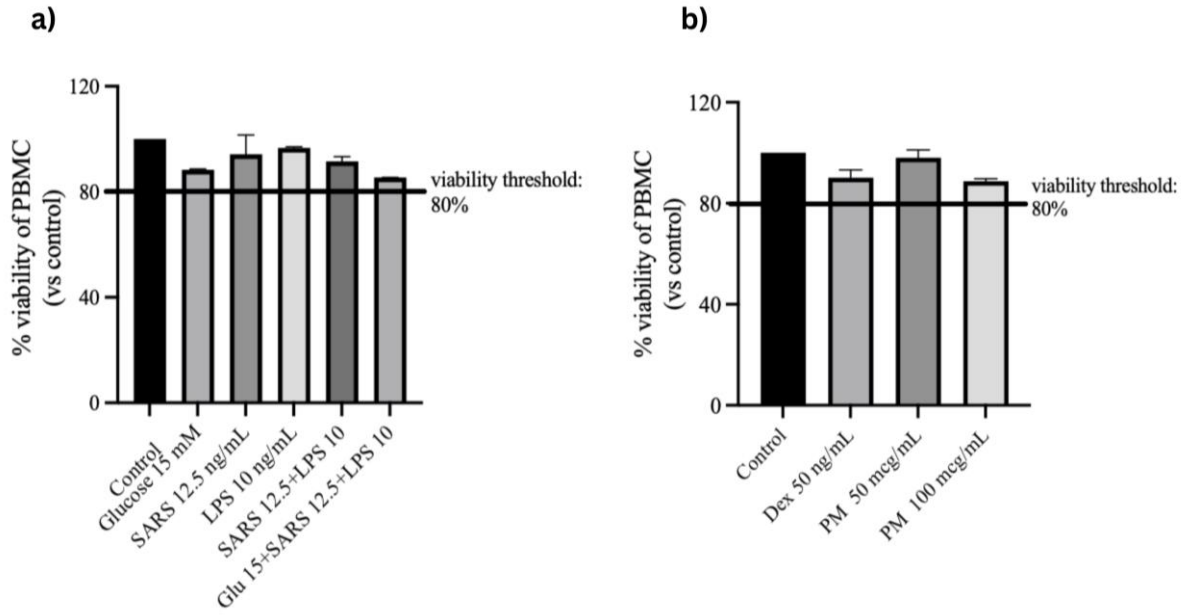


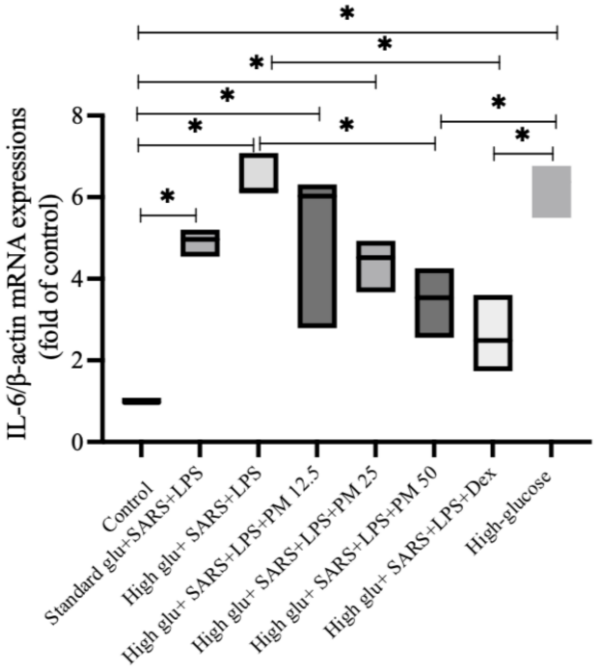
Fig. 4. Percentage of viable PBMC after treatment with stimulatory or treatment agents. (a) stimulatory agents: glucose 15 mM, SARS-CoV-2 spike protein 12.5 ng/mL; lipopolysaccharide 10 ng/mL; combination of SARS-CoV-2 spike protein 12.5 ng/mL and LPS 10 ng/mL; or combination of glucose 15 mM, SARS-CoV-2 spike protein 12.5 ng/mL, and LPS 10 ng/mL; (b) treatment agents: dexamethasone 50 ng/mL, *Phaleria macrocarpa* (PM) fruit extract 50 μg/mL, or *Phaleria macrocarpa (*PM) fruit extract 50 μg/mL. Results are presented as median (interquartile range)

Hyperglycemia is a common feature of poorly controlled diabetes, prompting investigation into how immune cells pre-exposed to elevated glucose levels respond to inflammatory stimuli. A previous study modeled this clinical context by pre-incubating PBMCs with graded glucose concentrations (5.5, 8, 16, and 24 mM) for 48 h, followed by stimulation with the TLR3 agonist poly(I: C) (20 μg/mL) for an additional 24 h. Hyperglycemia has been reported to increase proinflammatory cytokine production while impairing type I interferon generation and signaling, mechanisms that may exacerbate inflammatory responses and weaken antimicrobial defenses in diabetes [40].

Additionally, hyperglycemia has been reported to predispose monocytes/macrophages to a proinflammatory phenotype by increasing oxidative stress and enhancing NF-κB signaling, thereby intensifying responses to toll-like receptor 4 (TLR4) ligands, including lipopolysaccharide [40]. The combined use of high glucose, the SARS-CoV-2 spike protein, and lipopolysaccharide is therefore a biologically plausible experimental model of hyperglycemia, in which chronic metabolic stress may lower the threshold for cytokine production. However, the model should not be considered a full replication of diabetes-associated COVID-19 hyperinflammation.

Consistent with the above concept, increasing *in vitro* glucose concentrations from 5 to 25 mmol/L had only marginal effects on cytokine release after stimulation with *M. tuberculosis* lysate, LPS, or *Candida albicans*. In contrast, exposure to 40 mmol/L glucose markedly increased production of TNF-α, IL-1β, IL-6, and IL-10. Notably, IL-6 and IL-1β increased in a dose-dependent manner, whereas T-cell–derived cytokines showed greater variability. Collectively, these findings support the concept that substantial hyperglycemia can amplify inflammatory signaling in immune cells [41].

Stimulation with the SARS-CoV-2 spike protein in combination with LPS under high-glucose conditions resulted in elevated IL-6 expression compared with PBMCs maintained in control medium. These findings suggest that elevated glucose may enhance IL-6 mRNA responses to infectious or inflammatory stimuli in this experimental system. The observed response is consistent with the previous literature on spike protein-LPS interaction [42]. However, because nuclear factor kappa B (NF-κB), TLR4, MAPK, and related pathways were not directly measured, the mechanism cannot be confirmed by the present data. Treatment with the *Phaleria macrocarpa* fruit ethanol extract resulted in concentration-dependent suppression of IL-6 expression in the inflammatory PBMC model. This attenuation was comparable to that of dexamethasone, the positive control, thereby substantiating the preliminary data indicating that *Phaleria macrocarpa* fruit extract may inhibit IL-6 expression under the combined condition.



* - p<0.05 between the two groups

Fig. 5. Effect of *Phaleria macrocarpa* fruit extract on IL-6/β-actin mRNA expression in PBMCs induced with SARS-CoV-2 spike protein and LPS under high-glucose conditions. Treatments: High glucose (15 mM); SARS+LPS: SARS-CoV-2 spike protein 12.5 ng/mL + lipopolysaccharides 10 ng/mL; High glu+SARS+LPS: glucose 15 mM + SARS-CoV-2 spike protein 12.5 ng/mL + lipopolysaccharides 10 ng/mL; High glu+SARS+LPS+PM 12.5: glucose 15 mM + SARS-CoV-2 spike protein 12.5 ng/mL + lipopolysaccharides 10 ng/mL + *Phaleria macrocarpa* fruit extract 12.5 µg/mL; High glu+SARS+LPS+PM 25: glucose 15 mM + SARS-CoV-2 spike protein 12.5 ng/mL + lipopolysaccharides 10 ng/mL + *Phaleria macrocarpa* fruit extract 25 µg/mL; High glu+SARS+LPS+PM 50: glucose 15 mM + SARS-CoV-2 spike protein 12.5 ng/mL + lipopolysaccharides 10 ng/mL + *Phaleria macrocarpa* fruit extract 50 µg/mL; High glu+SARS+LPS+Dex: glucose 15 mM + SARS-CoV-2 spike protein 12.5 ng/mL + lipopolysaccharides 10 ng/mL + dexamethasone 50 ng/mL. Results are presented as the median (interquartile range)

Taken together, these results (Fig. 5) demonstrate that the ethanol extract reduced IL-6 mRNA expression induced by the SARS-CoV-2 spike protein and LPS under high-glucose conditions. This finding aligns with prior reports that *P. macrocarpa* contains bioactive constituents, such as alkaloids, flavonoids, phenolics, sterols, and terpenoids, which may inhibit IL-6 production and therefore possess potential anti-inflammatory activity relevant to inflammation associated with viral infections and hyperglycemia [43-46]. However, the present study did not identify which constituent was responsible for the observed effect and did not assess IL-6 at the protein level.

Our findings support the feasibility of a peripheral blood mononuclear cell inflammatory platform incorporating high-glucose exposure. SARS-CoV-2 spike protein and lipopolysaccharide, when combined with high-glucose exposure, enhanced inflammatory responses. Additionally, initial evidence suggests that *Phaleria macrocarpa* fruit ethanol extract may reduce IL-6 mRNA expression in this system. Dexamethasone, used as a control, produced a similar suppression of IL-6 mRNA expression, consistent with its known anti-inflammatory pharmacology [38]. However, comparisons with dexamethasone should be interpreted with caution because only IL-6 mRNA expression was measured, and no protein-level or pathway-specific validation was performed.

The present study remains an early-stage exploratory experiment because its primary endpoint is a single messenger of RNA marker. To improve mechanistic and translational relevance, subsequent studies should validate effects at the protein level, such as IL-6 concentrations in the supernatant; incorporate additional inflammatory mediators, including TNF-α, IL-1β, interferon responses, and oxidative stress markers; and improve the interpretability of extracts through analytical standardization and/or fractionation.

The fruit of *Phaleria macrocarpa* contains phenolic and flavonoid compounds, including mangiferin and phalerin, that have been reported to

На умовах ліцензії CC BY 4.0

influence inflammatory signaling and oxidative stress in preclinical studies [12, 29, 31]. The current study did not identify a single active constituent. Therefore, any proposed involvement of upstream pathways, such as NF-κB or mitogen-activated protein kinase cascades, remains hypothetical and should be tested directly in future work using pathway-specific assays [47]. Chemical standardization, such as high-performance liquid chromatography profiling, will be crucial for ensuring repeatability and facilitating future translation.

From a translational standpoint, the use of primary human immune cells offers advantages over immortalized cell lines; however, inter-donor variability and limited biological replicates pose significant limitations. This study has several limitations. First, PBMCs were obtained from only two healthy female donors; therefore, donor-to-donor variability, sex-related immune differences, and generalizability could not be adequately assessed. Second, IL-6 mRNA expression was the sole inflammatory endpoint, and no other inflammatory markers, such as TNF-α, IL-1β, or interferons, were measured. Finally, the findings are based on an *in vitro* model and should not be interpreted as evidence of clinical efficacy in COVID-19, diabetes, or diabetes-associated hyperinflammation. Subsequent research should involve a broader, more heterogeneous donor population, protein-level multiplex cytokine assays, and an expanded endpoint panel and functional pathways.

CONCLUSIONS

1. This exploratory study established an in vitro inflammatory model using human peripheral blood mononuclear cells exposed to elevated glucose levels, subsequently stimulated with the Severe Acute Respiratory Syndrome Coronavirus-2 spike protein and lipopolysaccharides, with interleukin-6 mRNA expression as the primary marker of effect.
2. Phaleria macrocarpa fruit ethanol extract reduced the interleukin-6 mRNA expression at non-cytotoxic concentrations under the tested conditions. Due to the limited number of PBMC donors, this data should be considered preliminary.
3. A subsequent study using a larger, sex-balanced donor population, broader anti-inflammatory endpoints, and chemical standardization is needed before this cell-based platform can be applied to screen anti-inflammatory candidates under conditions of elevated glucose exposure.

Contributors:

Nurhasanah A.H. – data curation, formal analysis, writing original draft;

Louisa M. – conceptualization, methodology, supervision, review & editing;

Angelina M. – validation, supervision, review & editing;

Estuningtyas A. – validation, review & editing.

Funding. This research was funded by the Master Thesis Research Grant of the Ministry of Higher Education, Science and Technology, Republic of Indonesia 2025 (Contract no. PKS-597/UN2/RST/HKP.05.00/2025) and Program Center of the Health Research Organization, National Research and Innovation Agency (Decision of the Head of The Health Research Organization, BRIN, No. 72/III.9/HK/2025).

Conflict of interests. The authors declare no conflict of interest.

REFERENCES

1. Carbajal J, Ballon-Salcedo C, Uribe-Cavero L, Saravia G, Cuadros-Aguilar S, Lopez M, et al. Risk factors associated with the mortality of COVID-19 in patients with type 2 diabetes mellitus. World Acad Sci J. 2024;6:62. doi: https://doi.org/10.3892/wasj.2024.277

2. Sharma P, Behl T, Sharma N, Singh S, Grewal AS, Albarrati A, et al. COVID-19 and diabetes: Association intensify risk factors for morbidity and mortality. Biomed Pharmacother. 2022;151:113089.
doi: https://doi.org/10.1016/j.biopha.2022.113089

3. Zhou Q, Zhang L, Dong Y, Wang Y, Zhang B, Zhou S, et al. The role of SARS-CoV-2-mediated NF-κB activation in COVID-19 patients. Hypertension Res. 2024;47:375-84.
doi: https://doi.org/10.1038/s41440-023-01460-2

4. Hiti L, Markovič T, Lainscak M, Farkaš Lainščak J, Pal E, Mlinarič-Raščan I. The immunopathogenesis of a cytokine storm: The key mechanisms underlying severe COVID-19. Cytokine Growth Factor Rev. 2025;82:1-17. doi: https://doi.org/10.1016/j.cytogfr.2024.12.003

5. Sharun K, Tiwari R, Dhama J, Dhama K. Dexamethasone to combat cytokine storm in COVID-19: Clinical trials and preliminary evidence. Int J Surgery. 2020;82:179-81.
doi: https://doi.org/10.1016/j.ijsu.2020.08.038

6. Tuba S, Aprianto MR. Effectivity of Dexamethasone Fighting Cytokine Storm in Severe COVID-19 Patients; Does it still reliable used for COVID-19? Formosa J Sci Tech. 2022;1:157-64.
doi: https://doi.org/10.55927/fjst.v1i3.835

7. Landstra CP, de Koning EJP. COVID-19 and Diabetes: Understanding the Interrelationship and Risks for a Severe Course. Front Endocrinol (Lausanne). 2021 Jun 17;12:649525.
doi: https://doi.org/10.3389/fendo.2021.649525

8. RECOVERY Collaborative Group. Dexamethasone in Hospitalized Patients with COVID-19. New Engl J Med. 2021;384:693-704.
doi: https://doi.org/10.1056/NEJMoa2021436

9. Arcani R, Cauchois R, Suchon P, Jean R, Jarrot P, Gomes De Pinho Q, et al. Factors associated with dexamethasone efficacy in COVID-19. A retrospective investigative cohort study. J Med Virol. 2022;94:3169-75. doi: https://doi.org/10.1002/jmv.27712

10. Ghasemian M, Owlia S, Owlia MB. Review of Anti-Inflammatory Herbal Medicines. Adv Pharmacol Sci. 2016;2016:1-11.
doi: https://doi.org/10.1155/2016/9130979

11. Gupta M, Singh N, Gulati M, Gupta R, Sudhakar K, Kapoor B. Herbal bioactives in treatment of inflammation: An overview. South Afr J Botany. 2021;143:205-25. doi: https://doi.org/10.1016/j.sajb.2021.07.027

12. Hendra R, Ahmad S, Oskoueian E, Sukari A, Shukor MY. Antioxidant, Anti-inflammatory and Cytotoxicity of Phaleria macrocarpa (Boerl.) Scheff Fruit. BMC Complement Altern Med. 2011;11:110.
doi: https://doi.org/10.1186/1472-6882-11-110

13. Ahmad R, Khairul Nizam Mazlan M, Firdaus Abdul Aziz A, Mohd Gazzali A, Amir Rawa MS, Wahab HA. Phaleria macrocarpa (Scheff.) Boerl.: An updated review of pharmacological effects, toxicity studies, and separation techniques. Saudi Pharm J. 2023;31:874-88.
doi: https://doi.org/10.1016/j.jsps.2023.04.006

14. Lestari I, Lindarto D, Ilyas S, Widyawati T, Mustofa M, Jusuf N, et al. Effect of Phaleria Macrocarpa (Scheff.) Boerl Leaf Ethanol Extract on Serum IL-6 and TNF-α Levels in Diabetic Rats. Med Archiv. 2023;77:254. doi: https://doi.org/10.5455/medarh.2023.77.254-257

15. Heriatmo NL, Estuningtyas A, Soetikno V, Poerwaningsih EH, Kusmardi K. Attenuation of TNF-α and Iron Levels in Renal Hemosiderosis by Phaleria macrocarpa (Scheff.) Boerl Extract in a Rat Iron Overload Model. Indones J Pharm. 2024;35(3):481-90. doi: https://doi.org/10.22146/ijp.6811

16. Petruk G, Puthia M, Petrlova J, Samsudin F, Strömdahl A-C, Cerps S, et al. SARS-CoV-2 spike protein binds to bacterial lipopolysaccharide and boosts proinflammatory activity. J Mol Cell Biol. 2021;12:916-32. doi: https://doi.org/10.1093/jmcb/mjaa067

17. Hansur L, Louisa M, Ernawaty B, Wuyung PE, Zaini J, Fadillah F, et al. Histopathology assay of the lung after intratracheal injection of SARS-CoV-2 spike protein recombinant in mice: A preliminary study. AIP Conf Proc. 2024;3080:090002.
doi: https://doi.org/10.1063/5.0199399

18. Hansur L, Louisa M, Wuyung PE, Fadilah F. Daphnoretin from Carthamus tinctorius as a Potential Inflammatory Inhibitor in COVID-19 by Binding to Toll-like Receptor-4: An in silico Molecular Docking Study. Open Access Maced J Med Sci. 2022;10:220-7. doi: https://doi.org/10.3889/oamjms.2022.7961

19. Melgaço JG, Azamor T, Silva AMV, Linhares JHR, dos Santos TP, Mendes YS, et al. Two-Step In Vitro Model to Evaluate the Cellular Immune Response to SARS-CoV-2. Cells. 2021;10:2206.
doi: https://doi.org/10.3390/cells10092206

20. Nichols JH, Smith AM, Jonsson CB. The Intersection of SARS-CoV-2 and Diabetes. Microorganisms. 2025;13:1390.
doi: https://doi.org/10.3390/microorganisms13061390

21. Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 2020;12:1-10. doi: https://doi.org/10.4103/jpbs.JPBS_175_19

22. Rizki M, Harahap U, Sitorus P. Phytochemical Screening Of Phaleria Macrocarpa (Scheff.) Boerl.) And Antibacterial Activity Test Of Ethanol Extract Against Staphylococcus Aureus Bacteria. Int J Sci Tech Management. 2023;4:422-7.
doi: https://doi.org/10.46729/ijstm.v4i2.781

23. Čolić M, Bekić M, Tomić S, Đokić J, Radojević D, Šavikin K, et al. Immunomodulatory Properties of Pomegranate Peel Extract in a Model of Human Peripheral Blood Mononuclear Cell Culture. Pharmaceutics. 2022;14:1140.
doi: https://doi.org/10.3390/pharmaceutics14061140

24. Ngkelo A, Meja K, Yeadon M, Adcock I, Kirkham PA. LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and G iα dependent PI-3kinase signalling. J Inflamm. 2012 Jan 12;9(1):1.
doi: https://doi.org/10.1186/1476-9255-9-1

25. Livak K, Schmittgen T. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-8. doi: https://doi.org/10.1006/meth.2001.1262

26. Agustin E, Insanu M, Mauludin R. Improvement in Pharmacological Activity of Mahkota Dewa (Phaleria macrocarpa (Schef. Boerl)) Seed Extracts in Nanoemulsion Dosage Form: In vitro and In vivo Studies. Pharm Nanotechnol. 2024;12:90-7.
doi: https://doi.org/10.2174/2211738511666230602100045

27. Shofiah A, Christina YI, Dwijayanti DR, Soewondo A, Widodo N, Djati MS. Exploring the inflam_matory pathway modulation of Phaleria macrocarpa: evidence from in vitro and in silico studies. Pharmacia. 2025;72:1-14. doi: https://doi.org/10.3897/pharmacia.72.e153095

28. Rizki M, Harahap U, Sitorus P. Phytochemical Screening of Phaleria macrocarpa (Scheff.) Boerl.) and Antibacterial Activity Test of Ethanol Extract Against Staphylococcus aureus Bacteria. Int J Sci Technol Management. 2023;4:422-7.
doi: https://doi.org/10.46729/ijstm.v4i2.781

29. Oshimi S, Zaima K, Matsuno Y, Hirasawa Y, Iizuka T, Studiawan H, et al. Studies on the constituents from the fruits of Phaleria macrocarpa. J Nat Med. 2008;62:207-10. doi: https://doi.org/10.1007/s11418-007-0209-9

30. Ali RB, Atangwho IJ, Kaur N, Abraika OS, Ahmad M, Mahmud R, et al. Bioassay-Guided Antidiabetic Study of Phaleria macrocarpa Fruit Extract. Molecules. 2012;17:4986-5002.
doi: https://doi.org/10.3390/molecules17054986

31. Altaf R, Asmawi M Bin, Dewa A, Sadikun A, Umar M. Phytochemistry and medicinal properties of Phaleria macrocarpa (Scheff.) Boerl. extracts. Pharmacogn Rev. 2013;7:73.
doi: https://doi.org/10.4103/0973-7847.112853

На умовах ліцензії CC BY 4.0

32. Lestari IC, Ghufron M, Herwiyanti S, Ari Sumiwi YA. The effects of ethanolic extract of Phaleria macrocarpa (Scheff.) Boerl leaf on macrophage phagocytic activity in diabetic rat model. J Med Sci. 2018;50:140-9. doi: https://doi.org/10.19106/JMedSci005002201802

33. Olajide OA, Iwuanyanwu VU, Lepiarz-Raba I, Al-Hindawi AA, Aderogba MA, Sharp HL, et al. Garcinia kola and garcinoic acid suppress SARS-CoV-2 spike glycoprotein S1-induced hyper-inflammation in human PBMCs through inhibition of NF-κB activation. Phytother Res. 2021;35:6963-73. doi: https://doi.org/10.1002/ptr.7315

34. Pathak S, Tan N, Vambutas A. A pilot study on the effect of SARS-CoV-2 spike protein on IL-1β-mediated inflammation in peripheral blood immune cells from AIED patients. Mol Med. 2025;31:174.
doi: https://doi.org/10.1186/s10020-025-01227-0

35. Tumpara S, Gründing AR, Sivaraman K, Wrenger S, Olejnicka B, Welte T, et al. Boosted Pro-Inflammatory Activity in Human PBMCs by Lipopolysaccharide and SARS-CoV-2 Spike Protein Is Regulated by α-1 Antitrypsin. Int J Mol Sci. 2021;22:7941.
doi: https://doi.org/10.3390/ijms22157941

36. Bhattacharya M, Sharma AR, Mallick B, Sharma G, Lee S-S, Chakraborty C. Immunoinformatics approach to understand molecular interaction between multi-epitopic regions of SARS-CoV-2 spike-protein with TLR4/MD-2 complex. Infect Genet Evol. 2020;85:104587.
doi: https://doi.org/10.1016/j.meegid.2020.104587

37. Chaiwut R, Kasinrerk W. Very low concentration of lipopolysaccharide can induce the production of various cytokines and chemokines in human primary monocytes. BMC Res Notes. 2022 Feb 10;15(1):42.
doi: https://doi.org/10.1186/s13104-022-05941-4

38. Świerczek A, Jusko WJ. Pharmacokinetic/Pharmacodynamic Modeling of Dexamethasone Anti-Inflammatory and Immunomodulatory Effects in LPS-Challenged Rats: A Model for Cytokine Release Syndromes. J Pharmacol Exp Ther. 2023;384:455-72.
doi: https://doi.org/10.1124/jpet.122.001477

39. Kiyomi A, Matsuda A, Nara M, Yamazaki K, Imai S, Sugiura M. Immunological Differences in Human Peripheral Blood Mononuclear Cells Treated with Traditional Japanese Herbal Medicines Hochuekkito, Juzentaihoto, and Ninjin'yoeito from Different Pharmaceutical Companies. Evidence-Based Compl Altern Med. 2021;2021:7605057. doi: https://doi.org/10.1155/2021/7605057

40. Hu R, Xia CQ, Butfiloski E, Clare-Salzler M. Effect of high glucose on cytokine production by human peripheral blood immune cells and type I interferon signaling in monocytes: Implications for the role of hyperglycemia in the diabetes inflammatory process and host defense against infection. Clin Immunol. 2018;195:139-48.
doi: https://doi.org/10.1016/j.clim.2018.06.003

41. Lachmandas E, Vrieling F, Wilson LG, Joosten SA, Netea MG, Ottenhoff TH, et al. The effect of hyperglycaemia on in vitro cytokine production and macrophage infection with mycobacterium tuberculosis. PloS One. 2015 Feb 9;10(2):e0117941.
doi: https://doi.org/10.1371/journal.pone.0117941

42. Petruk G, Puthia M, Petrlova J, Samsudin F, Strömdahl AC, Cerps S, et al. SARS-CoV-2 spike protein binds to bacterial lipopolysaccharide and boosts pro-inflammatory activity. J Mol Cell Biol. 2020;12:916-32. doi: https://doi.org/10.1093/jmcb/mjaa067

43. Ahmad R, Khairul Nizam Mazlan M, Firdaus Abdul Aziz A, Mohd Gazzali A, Amir Rawa MS, Wahab HA. Phaleria macrocarpa (Scheff.) Boerl.: An updated review of pharmacological effects, toxicity studies, and separation techniques. Saudi Pharm J. 2023;31:874-88.
doi: https://doi.org/10.1016/j.jsps.2023.04.006

44. Lestari IC, Ghufron M, Herwiyanti S, Ari Sumiwi YA. The effects of ethanolic extract of Phaleria macrocarpa (Scheff.) Boerl leaf on macrophage phagocytic activity in diabetic rat model. J Med Sci. 2018;50:140-9. doi: https://doi.org/10.19106/jmedsci005002201802

45. Kusmardi K, Situmorang NY, Zuraidah E, Estuningtyas A, Tedjo A. The effect of mahkota dewa (Phaleria macrocarpa) leaf extract on the Mucin 1 expression in mice colonic epithelial cells induced by dextran sodium sulfate (DSS). Pharmacogn J. 2021;13:1509-15.
doi: https://doi.org/10.5530/pj.2021.13.192

46. Ali R, Atangwho I, Kaur N, Abraika O. Bioassay-Guided Antidiabetic Study of Phaleria macrocarpa Fruit Extract. Molecules. 2012;17:4986-5002.
doi: https://doi.org/10.3390/molecules17054986

47. Millrine D, Jenkins RH, Hughes STO, Jones SA. Making sense of IL-6 signalling cues in pathophysiology. FEBS Lett. 2022;596:567-88.
doi: https://doi.org/10.1002/1873-3468.14201

Стаття надійшла до редакції 24.02.2026;
затверджена до публікації 19.05.2026