

Nutritional profile and immunomodulatory potential of a polyherbal honey formulation for stunted children

Akrom^{1,2}, Titiek Hidayati³, Arif Budi Setianto⁴, Laela Hayu Nurani⁵, Ikhwan Dahlan Rais⁵, Nurkhasanah⁶, Lukman Hardia⁷, Prasasti Bintarum⁸, Muslih Anwar⁹

¹Pharmacology and Clinical Pharmacy Department, Pharmacy Faculty, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

²Ahmad Dahlan Drug Information and Research Center, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

³Family Medicine Department, Medicine and Health Science Faculty, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia

⁴Pharmaceutical Technology Department, Pharmacy Faculty, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

⁵Biology Pharmacy Department, Pharmacy Faculty, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

⁶Chemistry Department, Pharmacy Faculty, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

⁷Pharmacology and Clinical Pharmacy Department, Pharmacy Faculty, Universitas Pendidikan Muhammadiyah, Sorong, Indonesia

⁸Imogiri 1 Public Health Center, Bantul Residence, Special Region of Yogyakarta, Indonesia

⁹Food Technology and Processing Research Center, National Research and Innovation Agency, Yogyakarta, Indonesia

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ABSTRACT

Stunting remains a major public health challenge in Indonesia and has been linked to immune dysregulation, including increased pro-inflammatory TNF- α and reduced anti-inflammatory IL-4 activity. Nutrient-dense polyherbal supplementation may provide a complementary strategy to support nutritional status and immune function. This experimental study investigated the nutritional composition and immunomodulatory activity of polyherbal honey (PHH) formulated with curcuma xanthorrhiza, black cumin seeds, and honey. Vitamins, minerals, and bioactive constituents were characterized using laboratory assays. The immunomodulatory effects of PHH were subsequently evaluated in HTB-183 cells by measuring IL-4 and TNF- α expression after exposure to different formulation concentrations. The analysis confirmed the presence of vitamins A, C, and E; calcium and potassium; and several bioactive compounds in the PHH formulation. PHH exposure increased IL-4 expression and reduced TNF- α expression relative to untreated cells, although the magnitude of these responses varied across concentrations. These findings demonstrate that PHH contains nutritional and phytochemical constituents with potential immunomodulatory activity. Further preclinical and clinical investigations are required to determine its efficacy and safety as complementary nutritional support for children with stunting.

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Corresponding Author:

Akrom

Pharmacology and Clinical Pharmacy Department, Pharmacy Faculty, Universitas Ahmad Dahlan

Yogyakarta, Indonesia

Email: akrom@pharm.uad.ac.id

1. INTRODUCTION

Stunting is associated with immune system deterioration and delayed cognitive development [1]. According to epidemiological studies, stunting in children is associated with increased inflammatory responses and infection rates [2]. One of the risk factors for stunting is inadequate consumption of macro and micronutrients [3]. Malnutrition has been linked to an increased prevalence of infectious and non-infectious diseases, as well as degenerative processes [4]. It has been demonstrated that stunted children have increased neuroinflammation, leading to delays in brain function development due to malnutrition and oxidative stress [5].

Many nutritional supplement formulations have been developed for children with stunting [6]. However, few nutritional supplements on the market are also immunomodulators, particularly those with anti-inflammatory properties derived from medicinal plants. Indonesia, a nation with an equatorial geography, has a range of nutrient-rich medicinal plants that show potential as immunomodulators [7]. Indonesian people have empirically used curcuma xanthorrhiza (CX) and black cumin (BC) seeds as traditional herbal remedies and dietary supplements [8]. CX has historically been used to increase appetite in picky eaters among kids and has been scientifically proven to be an immune booster, anti-inflammatory, and antioxidant [9]. The antioxidant activity of CX has been demonstrated by xanthorhizol, curcumin, and curcuminoid [10]. Xanthorhizol has been reported to exert neuroprotective and antioxidant effects by reducing reactive oxygen species (ROS) generation, attenuating glutamate-induced neurotoxicity, and inhibiting lipid peroxidation in rat brain homogenates. Black cumin (BC), similar to Curcuma xanthorrhiza (CX), is also widely recognized as a medicinal plant and is commonly used as both a culinary spice and a traditional health-promoting ingredient. Experimental evidence indicates that black cumin seed extract and oil possess several pharmacological activities, including anti-inflammatory [11], antibacterial [12], antiviral [13], immunomodulatory [14], antioxidant, and chemopreventive effects [15]. In vivo studies have further demonstrated that BC can enhance immune responses by increasing T lymphocyte counts [16], elevating interferon- γ (IFN- γ) levels [17], and improving macrophage phagocytic activity [14]. Thymoquinone, the principal bioactive constituent of BC, has been associated with the inhibition of cyclooxygenase and lipoxygenase pathways in arachidonic acid metabolism [18]. These biological properties support its potential role as an analgesic, anti-inflammatory, anticancer, antioxidant, anti-infective, and antihistamine agent [12], [19]-[21].

One study reported increased levels of pro-inflammatory cytokines, specifically IL-6 and TNF- α , as well as reactive C protein in stunted children, confirming that they experience an inflammatory response [2]. Immunomodulatory drugs are expected to enhance the host's immune response [22]. Interleukin-4 (IL-4) is an important cytokine with recognized anti-inflammatory functions [23]. This cytokine contributes to the differentiation of naïve helper T cells into the Th2 cell lineage. Once Th2 cells are formed, IL-4 further stimulates these cells to produce additional IL-4, thereby sustaining a positive regulatory feedback mechanism. The major cellular sources of IL-4 include mast cells, Th2 lymphocytes, eosinophils, and basophils. The development of effector T-cell responses, hematopoiesis, inflammation, and antibody generation is all significantly influenced by IL-4 [24]. In contrast to IL-4, TNF- α is a pro-inflammatory cytokine [25]. Malnutrition has been linked to a high level of TNF- α in stunted children [2]. Herbal immunomodulatory supplements are expected to balance the immune response by increasing immune cell activity and anti-inflammatory cytokine levels [26].

It has long been customary to combine two or more medicinal herbs [27] empirically. As a traditional medical preparation for specific uses, Javanese herbal remedies, or "Jamu," combine numerous medicinal herbs [28]. Combining CX and BC extracts in honey as polyherbal honey (PHH) is expected to include a mixture of active polyphenols, flavonoids, and minerals with potential anti-inflammatory and immunomodulatory properties [29]. PHH preparation has been developed to reduce inflammation and enhance immunity. Micronutrients and macronutrients with anti-inflammatory and immunomodulatory properties are considered present in the PHH preparation. This study evaluated the levels of vitamins and minerals, the active component, and the potential immunomodulatory effects of the PHH formulation.

2. METHOD

2.1. Study design

Several laboratory-based experimental procedures were conducted to address the objectives of this study. The PHH preparation was used as the primary test material. Its flavonoid, polyphenol, and mineral contents were quantified, followed by evaluation of its antioxidant and immunomodulatory potential. The PHH preparation was obtained from a traditional herbal medicine home industry that had received authorization from the Indonesian Food and Drug Authority.

Flavonoid content was quantified using the aluminum chloride colorimetric method with 10% AlCl₃ and quercetin standard, and absorbance was measured using a UV-Vis spectrophotometer (Shimadzu 1240). Total polyphenol levels were determined using the Folin-Ciocalteu method with gallic acid as the reference standard and Na₂CO₃ as the reaction reagent, followed by spectrophotometric analysis using the same instrument. Thymoquinone concentration was analyzed by densitometry using purified thymoquinone as the standard compound. The vitamin and mineral profiles of the PHH formulation were measured using a Hitachi 7600-110 Chemistry Auto Analyzer. The immunomodulatory potential of PHH was evaluated using fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies against IL-4 and TNF- α .

The immunomodulatory potential of PHH was assessed using HTB-183 cells, also known as the NCI-H661 cell line. Cells were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10%

fetal bovine serum and 1% penicillin–streptomycin, then incubated at 37 °C in a humidified incubator containing 5% CO₂. The culture medium was replaced according to the observed growth condition of the cells. Subculturing was performed when cell confluence reached approximately 90%. Cell growth and morphology were monitored routinely throughout the experiment. Three independent experiments were conducted in parallel to ensure consistency of the observations. Using fluorescence-activated cell sorting (FACS) flow cytometry, we assessed whether the PHH preparation could act as an immunomodulator by measuring the expression of IL-4 and IL-10 in HTB-183 cells.

2.2. Statistical analysis

Descriptive statistics were used to summarize the vitamin, mineral, and other micronutrient contents of the PHH preparation. Differences in IL-4 and TNF- α expression among HTB-183 cell groups exposed to different PHH concentrations were evaluated using one-way analysis of variance (ANOVA). The distributions of IL-4 and TNF- α expression across the treatment concentrations were visualized using box plots.

3. RESULTS AND DISCUSSION

3.1. Vitamin, mineral, and phytochemical bioactive composition of the PHH preparation

The PHH preparation contained several vitamins, minerals, and bioactive compounds in each 5 mL serving. Vitamin A was detected at 1.74 mg, whereas vitamin C and vitamin E were present at 13.29 and 743.95 μ g, respectively. The mineral analysis showed calcium and potassium contents of 18 mg and 10 mg per 5 mL, respectively. Phytochemical characterization demonstrated total polyphenol and flavonoid contents of 160.35 and 239.29 mg, respectively. Thymoquinone was also identified at a concentration of 200.04 mg per 5 mL. These findings characterize PHH as a preparation containing micronutrients and measurable concentrations of polyphenols, flavonoids, and thymoquinone.

The PHH formulation provides a combination of essential macro- and micronutrients, along with several bioactive constituents. Its phytochemical components include polyphenols, flavonoids, and thymoquinone, which have been associated with antioxidant, anti-inflammatory, and immunomodulatory activities. The preparation also contains vitamins A, C, and E, as well as calcium and potassium, which contribute to normal growth and development in children. Vitamin C is water-soluble, whereas vitamins A and E are fat-soluble compounds with antioxidant and immune-regulatory functions. Overall, this nutrient and phytochemical profile indicates that PHH may serve as a complementary herbal preparation to support nutritional adequacy, growth, and recovery in children requiring additional nutritional support.

3.2. Concentration-dependent effects of PHH on IL-4 and TNF- α expression

The immunomodulatory activity of PHH was assessed in HTB-183 cells by examining IL-4 and TNF- α expression following exposure to three concentrations: $\frac{1}{4}$ IC₅₀ (13.695ppm), $\frac{1}{2}$ IC₅₀ (27.39 ppm), and IC₅₀ (54.78 ppm). Untreated cells cultured in growth medium served as the control group. The effect of the PHH formulation on IL-4 expression in HTB-183 cells is presented in Figure 1. As shown, the comparative analysis of intracellular IL-4 (flow-based) levels across the four experimental groups (media, 1/4 IC, 1/2 IC, and IC) demonstrated statistically significant differences. The 1/4 IC group exhibited the highest median IL-4 value, followed by the 1/2 IC group, whereas the media and IC groups showed lower levels. The overall group comparison was significant ($p < 0.05$). Post-hoc analyses indicated that IL-4 expression in the 1/4 IC group was significantly higher than in the media group ($p < 0.01$). Significant differences were also observed between the 1/4 IC and 1/2 IC groups ($p < 0.01$), and between the 1/2 IC and IC groups ($p < 0.001$).

These findings are represented by the corresponding asterisk markers in the figure. These results suggest that partial IC exposure (particularly at 1/4 IC concentration) induces the most pronounced increase in flow14 levels. The pattern observed indicates a differential modulatory effect depending on concentration, highlighting the importance of dose optimization in achieving maximal biological response. The effect of the PHH formulation on TNF- α expression in HTB-183 cells is presented in Figure 2.

Figure 2 illustrates the effect of PHH on intracellular TNF- α expression in HTB-183 cells across four experimental groups (media, 1/4 IC, 1/2 IC, and IC). TNF- α levels were highest in the media group and progressively decreased with increasing PHH concentration. The IC group demonstrated the lowest median TNF- α expression, indicating a marked suppressive effect at the highest concentration. Statistical analysis revealed significant differences among groups. Compared with the media control, TNF- α expression was significantly reduced in the 1/4 IC ($p < 0.05$), 1/2 IC ($p < 0.01$), and IC groups ($p < 0.01$). Furthermore, significant reductions were observed between 1/4 IC and IC, as well as between 1/2 IC and IC groups ($p < 0.01$). These findings indicate a concentration-dependent inhibitory effect of PHH on TNF- α expression, supporting its anti-inflammatory potential in HTB-183 cells.

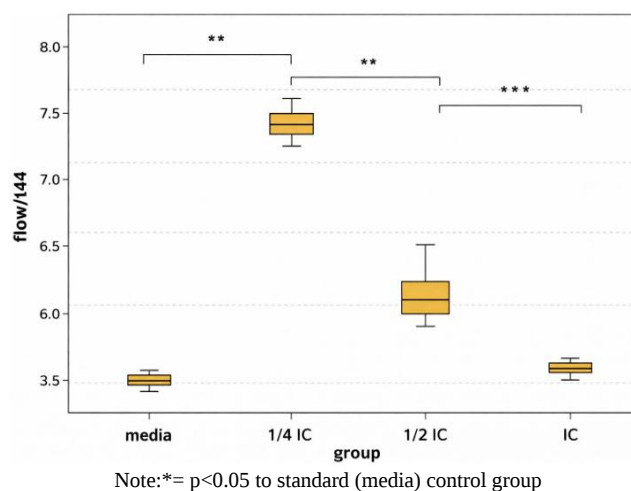


Figure 1. Boxplot image of IL-4 expression examination in HTB-183 cells after induction with PHH preparations at concentrations according to IC50, 1/2IC50, 1/4IC50, and solvent as a control

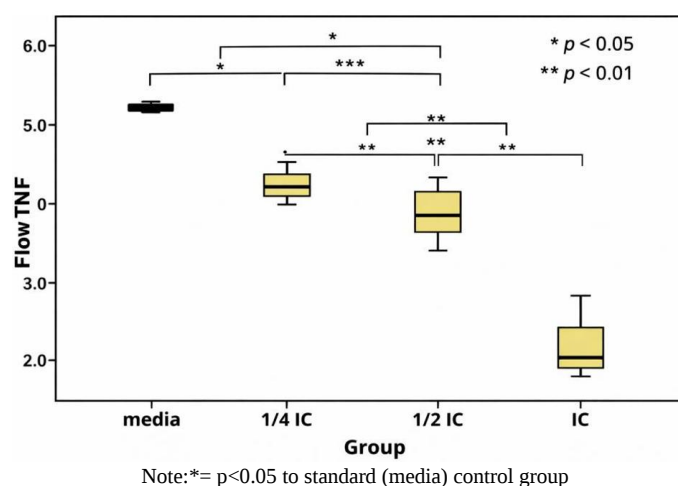


Figure 2. Boxplot image of TNF- α expression examination in HTB-183 cells after induction with PHH preparations with concentrations according to IC50, 1/2IC50, 1/4IC50, and solvent as a control

The problem of chronic malnutrition in children under five years old in Indonesia is still a serious public health problem in Indonesia and the world. The supplementary feeding program has been provided to children at risk of stunting, but has not effectively reduced the incidence of stunting in Indonesia. The target of reducing the incidence of stunting to below 14% remains far from expectations. As an agricultural country with a rich diversity of medicinal plants, it is necessary to develop polyherbal preparations as nutritional supplements to prevent stunting. Many medicinal plants contain micro and macronutrients as well as minerals and vitamins. Honey, CX, and BC have been used empirically as health supplements to increase appetite, but this has not been scientifically proven. For physical development, sexual maturity, neuromotor development, immune system health, and immune system function, specific micronutrients are necessary [30]. Malnourished children frequently co-occur with infectious diseases and micronutrient deficiencies, and these two conditions often interact in intricate ways to promote acute inflammation, which is linked to the activation of macrophages, natural killer cells, and the complement system, leading to the production of cytokines [31]. In addition to poor nutrition, chronic, low-grade inflammatory exposure during fetal and postnatal life significantly contributes to child stunting [32]. The clinical syndrome of malnutrition is partly due to an unchecked inflammatory response driven by an imbalance between pro-inflammatory and anti-inflammatory mediators [33].

These results are in line with the increasing scientific attention given to medicinal plant-based preparations. Phenolic and flavonoid constituents are considered key compounds that contribute to the antioxidant capacity of *Curcuma xanthorrhiza* extracts and black cumin seed oil [34], [35]. Several traditional medical systems, including phytotherapy, Jamu as traditional Javanese medicine, Traditional Chinese

Medicine, and Ayurveda, generally apply medicinal plants as multicomponent formulations rather than as single-herb preparations [36]. The therapeutic relevance of such herbal combinations has been associated with possible synergistic effects, particularly in chronic and complex diseases, including diabetes mellitus, cancer, hypertension, and infectious diseases such as SARS-CoV-2 infection, severe acute respiratory syndrome (SARS), tuberculosis, HIV/AIDS, and malaria [24], [37].

The findings showed that PHH preparations increased IL-4 and reduced TNF- α expression at IC50-equivalent doses. Compared with the solvent control group, the treatment group showed higher IL-4 expression. TNF- α expression was lowest in the IC50 group and highest in the 1/4 IC50 concentration group. TNF- α was a pro-inflammatory cytokine, and IL-4 was an anti-inflammatory cytokine. The pathophysiology of various inflammatory and autoimmune diseases has been linked to TNF- α , which is a critical regulator of inflammatory responses [2], [38]. To maintain immune balance, IL-4 and TNF- α are crucial. TNF- α is a physiologically important part of a typical immune response. TNF- α can regulate the immune system, but excessive or improper synthesis of TNF- α can be damaging and may result in disease. The fundamental etiology of growth retardation includes poor nutrition and chronic inflammation mediated by pro-inflammatory cytokines (TNF- α). According to research findings, PHH preparations can reduce excessive inflammation by upregulating the anti-inflammatory cytokine IL-4 and downregulating the pro-inflammatory cytokine TNF- α [18], [25], [39].

The immunomodulatory potential of PHH may be associated with its micronutrient and bioactive compound profile. The formulation analysis indicated the presence of minerals, thymoquinone, polyphenols, flavonoids, and vitamins A, C, and E. Vitamins and minerals are essential micronutrients involved in maintaining physiological homeostasis, supporting immune regulation, contributing to brain and bone development, and facilitating metabolic processes [40]–[43]. Vitamin A comprises a group of fat-soluble compounds, including retinol, retinal, and retinoic acid. This vitamin is obtained from dietary sources in the form of retinyl esters and provitamin A carotenoids derived from plants. Components of PHH, including honey and black cumin seed oil, may contribute to its vitamin and bioactive compound content. Vitamin A has been implicated in the regulation of various immune mediators, including pathways related to both pro-inflammatory and anti-inflammatory responses [44], [45]. Retinoic acid can modulate T-cell responses and plays an important role in maintaining intestinal immune tolerance toward commensal bacteria and food antigens. In cooperation with Toll-like receptor signaling, retinoic acid produced by dendritic cells may promote IL-10-producing T cells and support the suppressive activity of Foxp3⁺ regulatory T cells [46]. Accumulating evidence also suggests that vitamin A contributes to mucosal protection, including the maintenance of T-lymphocyte function. In a rat model of colitis, vitamin A demonstrated anti-inflammatory and protective effects, possibly through preservation of mitochondrial function [47]. Vitamins C and E are also involved in immune regulation and antioxidant defense [48]. Deficiency of these vitamins has been associated with increased pro-inflammatory cytokine levels. Vitamin C, in particular, may directly influence cellular inflammatory signaling by downregulating pro-inflammatory mediator expression and attenuating inflammatory responses. One of the major pathways affected by ascorbic acid is the NF- κ B/TNF signaling pathway [49].

The prevalence of childhood stunting has been linked to inadequate mineral consumption [50], [51]. Mineral deficiencies typically arise in children under five due to inadequate dietary mineral intake, increased nutrient requirements from fast growth, and the emergence of infectious illnesses. The study's findings demonstrated that PHH preparations contained significant amounts of calcium (Ca) and potassium (K). Calcium is an element that primarily affects bone health, skeletal structure, and rigidity. Phosphorus is found naturally in many foods and can also be taken as a supplement. It has several functions in the body. It is a crucial component of cell membranes, bones, and teeth. It maintains a normal blood pH range and helps activate enzymes. Although the precise mechanism is unclear, inorganic phosphorus has also been linked to the modulation of inflammatory cytokines [33]. Elevated inflammatory cytokine levels have been linked to hypophosphatemia in sepsis patients. Phosphorus showed a negative correlation with inflammatory cytokines in patients with chronic kidney disease, indicating the role of phosphorus in inflammation [52]. Elevated pro-inflammatory cytokine levels and delayed bone development are associated with low blood calcium and phosphorus levels. Higher levels of the cytokines IL-1 and TNF- α were observed in the group that consumed insufficient calcium and potassium [2].

Thymoquinone, an active compound of BC, has been shown to increase immune response via phagocytic activity and cytokine secretion (IFN- γ) by macrophages [53]. According to a laboratory experimental report, thymoquinone increases the effector activity of macrophages by activating Toll-like receptor 4 (TLR-4) [54]. Thymoquinone has also been shown to increase the proliferation and differentiation of CD4Th into Th1 and Th2 [55]. Thymoquinone exerted antioxidant effects by stimulating aryl hydrocarbon receptor (AhR) signaling, promoting Nrf2 activation, and enhancing glutathione S-transferase production [56], [57]. Furthermore, the thymoquinone concentration in the present formulation exceeded the 2.7% reported in black cumin seed oil (BCSO) [58].

Research indicates that PHH formulations contain flavonoids and polyphenols. PHH formulations contain polyphenols and flavonoids from BC oil, CX extract, or honey. Polyphenols and flavonoids have immunomodulatory, anti-inflammatory, and antioxidant properties [59]. Polyphenols and flavonoids have been shown to improve the composition of digestive tract microbes by increasing beneficial microbes, decreasing harmful microbes, and acting as immunomodulators and antioxidants. Curcumin is an active compound in the curcuminoid family. Curcuminoids are yellow polyphenolic constituents that contribute to the biological activity of *Curcuma xanthorrhiza* (CX). Among these compounds, curcumin has been widely reported to exhibit several pharmacological properties, including antidyslipidemic, antioxidant, anti-inflammatory, antiviral, and antifungal activities [60]. Curcumin has also been associated with the inhibition of atherosclerotic plaque formation [61], cancer chemoprevention, and hepatoprotective effects. In contrast to thymoquinone, curcumin has been described as an antagonist of Toll-like receptor 4 (TLR-4) [62]. In addition to curcuminoids, CX contains xanthorrhizol (XNT), another bioactive compound with potential metabolic and cardiovascular benefits. Previous studies have shown that XNT can reduce serum free fatty acid and triglyceride levels in obese mice fed a high-fat diet. XNT has also been linked to cardiovascular protection through antihyperglycemic activity, vasorelaxant effects, and inhibition of LDL oxidation [63]. The antioxidant capacity of polyphenols may be influenced by structural changes, the ratio between free phenolic forms and glycosides, the formation of iron-phenol chelates, and interactions between phenolic acids and other components in the food matrix [64]. Overall, previous *in vitro* and *in vivo* studies suggest that black cumin seed oil and *C. xanthorrhiza* may contribute to the reduction of oxidative stress and modulation of immune responses [65], [66]. Based on the study's findings and the literature review, the research team is optimistic that PHH has potential as an herbal immunomodulatory supplement for treating and preventing stunting.

4. CONCLUSION

PHH is a rich source of essential vitamins, minerals, and other micronutrients. PHH preparations increase IL-4 and decrease TNF- α expression. HTB-183 cell. The results of this research show that PHH preparation can be used as a nutritional supplement and an immunomodulatory agent for stunted children. It is necessary to carry out the following research stage: testing preparations on test animals.

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AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Akrom	✓	✓	✓	✓	✓	✓		✓	✓	✓			✓	
Titiek Hidayati	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	
Arif Budi Setianto		✓		✓	✓	✓	✓		✓	✓				
Laela Hayu Nurani	✓	✓		✓	✓	✓		✓	✓	✓		✓		
Ikhwan Dahlan Rais		✓			✓	✓	✓	✓	✓	✓				
Nurkhasanah	✓			✓	✓					✓		✓		
Lukman Hardia		✓	✓	✓	✓			✓	✓	✓	✓			
Prasasti Bintarum						✓	✓	✓	✓	✓				
Muslih Anwar	✓	✓		✓						✓		✓	✓	✓

C : Conceptualization

M : Methodology

So : Software

Va : Validation

Fo : Formal analysis

I : Investigation

R : Resources

D : Data Curation

O : Writing - Original Draft

E : Writing - Review & Editing

Vi : Visualization

Su : Supervision

P : Project administration

Fu : Funding acquisition

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, [A], upon reasonable request.

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BIOGRAPHIES OF AUTHORS



Akrom    is a lecturer in the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan, Indonesia. His research interests include immunopharmacology, biomolecular pharmacoepidemiology, evidence-based medicine, and phytomedicine. His academic work also covers chemopreventive immunopharmacology, health technology assessment, medication safety, and medication-error studies in hospital and primary health care settings. In addition, he is involved in developing pharmaceutical care intervention models for chronic diseases, including asthma, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, and HIV/AIDS. His research also includes studies on smoking behavior as a risk factor for chronic noncommunicable diseases. He can be contacted at email: akrom@pharm.uad.ac.id.



Titiek Hidayati    is a lecturer at Universitas Muhammadiyah Yogyakarta and serves as an academic staff member in the Faculty of Medicine and Health Sciences. She is also affiliated with the Department of Public Health and Family Medicine, with expertise in epidemiology. She completed her medical professional education and master's degree at the Faculty of Medicine, Universitas Gadjah Mada. She later pursued doctoral studies at the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, which included an academic sandwich program at National Cheng Kung University. In addition, she completed specialist training in family medicine through the specialist medical education program at the Faculty of Medicine, Universitas Padjadjaran, Bandung. She can be contacted at email: titiekakrom@gmail.com.



Arif Budi Setianto    is a researcher in pharmaceutical formulation and solid-state and crystallographic analysis. His work focuses on drug-drug and drug-coformer interactions in pharmaceutical formulations, including their effects on solubility, stability, phase transformations, manufacturability, and the performance of solid dosage forms. His research also examines analytical approaches for identifying and characterizing changes that occur during pharmaceutical manufacturing. Particular attention is given to phase transformations arising from common unit operations, their underlying mechanisms, strategies for anticipation and prevention, and their implications for product quality. He can be contacted at email: arif.setianto@pharm.uad.ac.id.



Laela Hayu Nurani    is a professor at the Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia. Her principal field of expertise is pharmaceutical biology, with particular emphasis on pharmacognosy, phytochemistry, phytotherapy, and the development of natural products for pharmaceutical and health applications. Her research focuses on the isolation, identification, chemical characterization, and standardization of bioactive compounds from medicinal plants. She also investigates the biological activity and molecular mechanisms of natural products, particularly their immunomodulatory and anticancer potential. Her doctoral research examined the isolation and identification of active constituents from pasak bumi (*Eurycoma longifolia*) and evaluated their anticancer activity through in vitro and in vivo approaches. She completed her Bachelor of Pharmacy, Pharmacist Professional Education, Master of Pharmacy, and Doctoral Program in Pharmacy at Universitas Gadjah Mada. Her teaching and academic supervision cover pharmacognosy, phytochemistry, phytotherapy, natural-product development, cancer and carcinogenesis, and research methodology. She has published original research and review articles on the chemical analysis and biological evaluation of natural products relevant to medicines and health. She can be contacted at email: laela.farmasi@pharm.uad.ac.id.



Ikhwah Dahlan Rais    is an assistant professor and devoted lecturer at Universitas Ahmad Dahlan's Faculty of Pharmacy in Yogyakarta, Indonesia. At Prince of Songkla University in Thailand, he finished his Ph.D. studies in pharmacognosy and pharmaceutical botany. His main areas of interest in study are phytochemicals, traditional herbal medicine, and anti-diabetic formulations. He specialises in pharmaceutical biology. He has actively authored and reviewed international articles in ethnopharmacology, toxicity assessments, and natural product developments throughout his academic career, making a significant contribution to worldwide scientific study. He can be contacted at email: ichwan.rais@uad.ac.id.



Nurkhasanah    is a professor at the Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia. Her academic expertise encompasses pharmaceutical chemistry, natural-product drug development, and the scientific assessment of halal compliance in medicines, pharmaceutical ingredients, and herbal medicinal products. Her research applies chemical, biochemical, and molecular approaches to identify active constituents from natural sources and evaluate their biological activity. Her principal research themes include antioxidant, immunomodulatory, anti-inflammatory, and anticancer mechanisms; the development and standardization of herbal products; and chemistry-based approaches to the authentication and halal assurance of pharmaceutical materials and products. She completed her Bachelor of Pharmacy, Pharmacist Professional Education, and Master of Pharmacy at the Faculty of Pharmacy, Universitas Gadjah Mada. She obtained her doctoral degree in Biochemistry from Universiti Kebangsaan Malaysia in 2009. Her doctoral and subsequent research has contributed to the understanding of natural bioactive compounds and their molecular effects, including investigations of eurycomanone and other plant-derived compounds in cancer-related models. She can be contacted at email: nurkhasanah.mahfudh@uad.ac.id.



Lukman Hardia    is a lecturer at the Faculty of Pharmacy, Universitas Pendidikan Muhammadiyah Sorong, Indonesia. His research interests include immunopharmacology, clinical pharmacy, development and evaluation of natural medicines, and the use of Indonesian medicinal plants as a source of phytopharmaceutical candidates. In addition, she also pays attention to research related to stunting, especially in the aspects of prevention and control through pharmaceutical approaches, the use of supplements and micronutrients, and the development of evidence-based interventions that support maternal and child health. He can be contacted at email: lukman@unimudasorong.ac.id.



Prasasti Bintarum    is a primary care dentist at Puskesmas Imogiri I, Bantul, Special Region of Yogyakarta, Indonesia. Her professional work focuses on the provision of comprehensive oral health services at the primary care level, including preventive, promotive, and curative dental care. She is also actively involved in community empowerment programs, particularly initiatives aimed at improving health awareness, self-care capacity, and quality of life among older adults. She completed both her undergraduate dental education and professional dentist program at the Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia. She can be contacted at email: prasastibintarum24@gmail.com.



Muslih Anwar    is a researcher at the Research Center for Food Technology and Processing, National Research and Innovation Agency of the Republic of Indonesia (BRIN), Gunungkidul, Yogyakarta. He contributes to research on smart and sustainable food packaging and the development of natural-polymer materials for food applications. His expertise includes food-related polymers, natural and bio-based polymers, active and intelligent packaging, and the chemical modification of packaging materials. His research addresses the isolation, characterization, and functionalization of polysaccharides such as chitosan, cellulose, and starch. He also investigates antimicrobial and responsive packaging systems designed to improve food quality, safety, and shelf life. His broader scientific interests include green chemistry, organic synthesis, biomass-derived materials, polymer composites, and the sustainable utilization of agricultural and food-processing by-products. He completed both his undergraduate and master's education in Chemistry at the Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada. He can be contacted at email: muslih.anwar@brin.go.id.