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Phytochemical Test of Sacha Inchi Oil from Central Java

Mulyono Hadi[a], Adi Permadi[a*], Totok Eka Suharto[b], Mutiara Wilson Putri[b], Herbert Alessandro Panias Gulo[b], Nadin Okta Maema[b], Halimathusyakhdyah[b], Ahmad Lupi[b]

[a]Master of Chemical Engineering Department, Faculty of Industrial Technology, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
[b]Chemical Engineering Department, Faculty of Industrial Technology, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

[*]Corresponding author, Email: adi.permadi@che.uad.ac.id

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ABSTRACT
Sacha inchi oil is a seed-derived oil from the Amazon Rainforest, known for its high nutritional value and bioactive compounds. It contains essential fatty acids such as omega-3 and omega-6, along with tocopherols, polyphenols, carotenoids, and phytosterols, making it beneficial for health applications. Due to its nutritional and therapeutic properties, sacha inchi oil has gained significant attention in the food, cosmetic, and pharmaceutical industries. This study contributed to identify and analyze the bioactive compounds in sacha inchi oil extracted from seeds obtained in Central Java, Indonesia. The extraction process was carried out using a hot pressing method, followed by qualitative phytochemical analysis and LC-HRMS identification. The phytochemical tests confirmed the presence of flavonoids, alkaloids, tannins, phenolics, saponins, steroids, and terpenoids, all of which contribute to antioxidant, anti-inflammatory, anticancer, and antimicrobial properties. However, LC-HRMS analysis did not detect flavonoids, tannins, and saponins, possibly due to their low concentration, matrix effects, or degradation during analysis. These findings highlight sacha inchi oil's potential in nutraceutical, pharmaceutical, and cosmetic industries. Its bioactive compounds suggest its potential use in functional foods, dietary supplements, and therapeutic applications, particularly in preventing oxidative stress-related diseases. Further research is recommended to optimize extraction techniques, improve compound stability, and evaluate its bioavailability and long-term health benefits. The presence of bioactive compounds indicates that sacha inchi oil can be a valuable functional ingredient for health and medical applications, contributing to sustainable and natural health solutions.

KEYWORDS
Antioxidants; Bioactive compounds; Nutraceutical; Phytochemical analysis; Sacha inchi oil

1. INTRODUCTION

Sacha inchi (*Plukenetia volubilis* L.) is an oil-producing plant native to the Amazon Rainforest in Peru. Traditionally, it has been cultivated in San Martín and six other regions of Peru, but it is also grown in some South American countries. However, due to its nutritional benefits and increasing market demand in the food, cosmetics, and pharmaceutical industries, this plant has expanded to other regions, including Southeast Asia [1], [2]. The sacha inchi plant produces green, star-shaped fruits that, when ripe, yield dark brown seeds that are edible. These seeds are rich in oil (35–60%) and protein (27%) and contain thermally unstable compounds with a slightly bitter taste. Sacha inchi oil is characterized by a high content of essential fatty acids, particularly C18:3 ω3 (α-linolenic acid) and C18:2 ω6 (linoleic acid), which together account for approximately 82% of the total oil content. The reported ω6/ω3 ratio is about 0.81 [3], [4].

Several studies have reported that unsaturated ω-6 and, especially, ω-3 fatty acids provide significant health benefits, including the prevention of diseases such as cancer, coronary heart disease, and hypertension. Additionally, a hypocholesterolemic effect has been observed when sacha inchi oil is used as a dietary supplement. The presence of bioactive compounds such as tocopherols, polyphenols, carotenoids, and phytosterols in sacha inchi oil has been documented. Additionally, the amino acid profile of sacha inchi protein fractions shows relatively high levels of cysteine, tyrosine, threonine, and tryptophan compared to other oilseed sources [5], [6].

Phytochemical screening is a simple qualitative method to analyze secondary metabolites in plants by observing color changes when specific reagents are applied. For example, the presence of alkaloids is indicated by a cream or brown-red precipitate with Mayer-Wagner reagent, flavonoids by a pink or red color with ethanol, HCl, and magnesium ribbon, and phenols by greenish-blue coloration with $FeCl_3$ and $K_2Fe(CN)_6$ [7]. Secondary plant metabolites are classified as allelochemicals compounds released by individual plants or species that influence the growth, fitness, behavior, or population of other organisms. These secondary metabolites are derived from primary metabolites (such as carbohydrates, proteins, and fats) and offer various biological benefits. Some of the active compounds commonly found in plants and plant extracts include flavonoids, alkaloids, tannins, phenolics, saponins, steroids, and terpenoids [8], [9].

Flavonoids are secondary polyphenolic metabolites widely distributed in plants. They exhibit various bioactive effects, including antiviral, anti-inflammatory, anticancer, antidiabetic, anti-aging, and antioxidant properties [10]. Flavonoid compounds contain a carbon structure arranged in a C6-C3-C6 configuration, meaning they consist of two benzene rings connected by a three-carbon aliphatic chain [11]. In plants, flavonoids are generally found in bound/conjugated forms with sugar compounds. Alkaloids are one of the largest groups of secondary metabolites, commonly found in higher plants, and contain nitrogenous base structures with one or two nitrogen atoms in heterocyclic rings [12]. Plants are estimated to produce approximately 12,000 different alkaloids, which can be categorized based on their carbon framework structure. Alkaloid biosynthesis in plants involves multiple catalytic steps mediated by various enzymes [13]. Alkaloids exhibit antibacterial properties by disrupting peptidoglycan synthesis in bacterial cell walls, leading to incomplete cell wall formation and ultimately cell death. Pharmacologically, alkaloids have numerous effects, including anti-inflammatory, antibacterial, hepatoprotective, anticancer, and antioxidant-enhancing properties [14].

Tannins are secondary metabolites with various biological functions, including astringent, antidiarrheal, antibacterial, and antioxidant activities. Tannins are classified into two groups: condensed tannins and hydrolyzable tannins. These compounds play a complex biological role, from protein precipitation to metal chelation. Tannins also act as biological antioxidants, tumor growth inhibitors, and enzyme inhibitors, such as "reverse" transcriptase and DNA topoisomerase [15], [16]. Phenolics are a diverse group of compounds containing hydroxyl (-OH) groups bound directly to aromatic hydrocarbon structures. These compounds are widely found in various plant species and exhibit significant biological activities, including anti-inflammatory, antimicrobial, and antiproliferative effects. Such bioactive properties have generated interest in using these molecules for nutraceutical product formulations [17], [18].

Saponins are natural glycosides composed of a steroid or triterpene aglycone linked to sugar moieties. Their structural diversity, particularly variations in the aglycone core and sugar chain attachments, significantly influences their biological and pharmacological activities. These compounds exhibit a wide range of effects, including antioxidant, antimicrobial, anticancer, anti-inflammatory, insecticidal, nematicidal, and neuroprotective properties [19]. Due to their amphiphilic nature, saponins act as surfactants and form foam when shaken with water. They exhibit antitussive and expectorant effects, contributing to cough relief. Additionally, saponins function as antioxidants, anti-inflammatory agents, analgesics, and inhibitors of dental caries and platelet aggregation. Steroids are organic compounds derived from terpene or squalene degradation and belong to the sterol family of non-hydrolyzable fats. In plants, steroid compounds act as protective agents, repelling certain insects while attracting others [20], [21]. Various types of steroid compounds are used in medicine, including estrogen (used as an ovulation inhibitor in contraceptives), progestins (synthetic steroids for preventing miscarriage and pregnancy testing), glucocorticoids (for treating inflammation, allergies, fever, leukemia, and hypertension), and cardenolides (cardiac steroid glycosides used as diuretics and heart tonics). Several bioactive compound will be beneficial for human health [22].

Terpenoids are hydrogenated and oxidized derivatives of terpenes. They share the same carbon skeleton as isoprene (C_5H_8) and are thus also known as isoprenoid compounds. Terpenoids exert antioxidant effects by scavenging reactive species, such as superoxides, and chelating metal ions (Fe^{2+} and Cu^{2+}). They exhibit antioxidant properties, inhibit lipid peroxidation, and possess hepatoprotective, analgesic, antitumor, antiproliferative, and immunomodulatory effects [23], [24]. Phytochemical evaluations of various plants have shown that leaf extracts from three plant species contain phenolics, flavonoids, steroids, and saponins [25]. Meanwhile, cucumber extract has been found to contain a variety of active compounds, including steroids, terpenoids, alkaloids, phenolics, flavonoids, and saponins [26]. Additionally, phytochemical tests on basil leaves have revealed the presence of flavonoids (identified by the appearance of a black color), alkaloids (indicated by a yellow-brown precipitate), saponins (marked by stable foam formation), and tannins (identified by a green-black coloration) [27], [28].

This study contributed to identify the phytochemical components present in sacha inchi oil through phytochemical screening and LC-HRMS (Liquid Chromatography-High Resolution Mass Spectrometry) analysis. This research contributes to the growing body of knowledge on the phytochemical profile of sacha inchi oil sourced specifically from Central Java, which has not been widely reported. It highlights potential differences in composition due to geographical origin and supports the use of regional raw materials for nutraceutical applications.

2. MATERIALS AND METHODS

2.1. Materials

This study utilized sacha inchi seeds (*Plukenetia volubilis* L.) sourced from Batang Regency, Central Java, Indonesia, as the primary raw material. The seeds were dark brown in color, approximately 1.5–2 cm in diameter, mature, and harvested during the dry season. Various analytical reagents for phytochemical testing–such as Dragendorff reagent for alkaloids, $AlCl_3$ reagent for flavonoids, and Folin-Ciocalteu reagent for phenolics–were obtained from the Pharmacy Laboratory of Universitas Ahmad Dahlan (UAD), Yogyakarta, Indonesia. The same laboratory also provided the equipment for qualitative analysis, including a UV-Vis spectrophotometer for colorimetric detection. For compound identification, a high-resolution LC-HRMS system (Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ with Thermo Vanquish™ UHPLC) was used at EBM Scitech Laboratory, Bandung Institute of Technology (ITB).

2.2. Research Methods

The phytochemical analysis procedure in this study followed a systematic sequence consisting of three main stages. First, the extraction of sacha inchi oil was carried out using a hot-pressing method, which involved drying and heating the seeds to optimize oil yield. The resulting oil was then allowed to settle and subsequently filtered to obtain a clear sample suitable for analysis. In the second stage, specific chemical reagents were added to the oil samples to detect the presence of various classes of secondary metabolites. These reagents included Dragendorff's reagent for alkaloids, $AlCl_3$ for flavonoids, Folin-Ciocalteu for phenolics, among others, each selected based on its specificity to target compounds. The third and final stage involved observing the resulting color changes or precipitates as qualitative indicators of positive reactions, which were then interpreted to confirm the presence of key phytochemical constituents such as flavonoids, alkaloids, tannins, phenolics, saponins, steroids, and terpenoids.

2.3. Sacha Inchi Seed Extraction

The sacha inchi oil extraction process begins with drying the seeds for 5–7 days to reach the optimum moisture content. Next, the seeds are heated at 60 °C to increase extraction efficiency before undergoing the mechanical pressing process. The hot pressing method is used with a yield ratio of about 3:1, where from 300 g of seeds, about 100 mL of oil can be obtained. The resulting oil is then allowed to stand for 2–3 days to allow for a natural settling process, before finally being filtered using filter paper to separate suspended particles. The stages of sacha inchi oil extraction are shown in Figure 1.

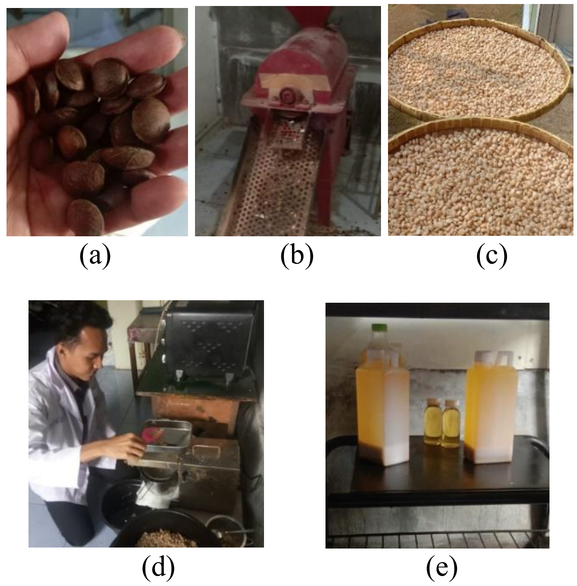


Figure 1. Stages of sacha inchi oil extraction: (a) sacha inchi seeds; (b) seed shelling equipment; (c) seed drying process; (d) seed pressing to obtain oil; (e) sacha inchi oil obtained.

2.4. Qualitative Phytochemical Analysis

Qualitative analysis of phytochemicals was conducted at the Integrated Research and Testing Laboratory (LPTP) of the Faculty of Pharmacy, Universitas Ahmad Dahlan (UAD). Qualitative tests to identify the presence of phytochemical compounds in plant extracts through various specific methods [1]. The flavonoid test was carried out by adding 1% $AlCl_3$ reagent, which showed a positive result if a solid yellow color was formed. The alkaloid test uses Dragendroff reagent, with an indicator of success in the form of a red precipitate. The tannin test is carried out by adding 2% NaCl and 1% gelatin, which will form a precipitate if tannin compounds are present in the sample. Furthermore, the phenolic test uses Folin Ciocalteau reagent and NaOH, which produces a greenish-blue color as a sign of the presence of phenolic compounds. The saponin test is applied by the frothing method, where a stable foam indicates a positive result. In addition, a steroid test was performed using the Libermann-Burchard method, which produced a brown ring if steroids were detected. The terpenoid assay used the Salkowski method, with a yellow color indicator as a sign of the presence of triterpene. These methods were applied to confirm the presence of secondary metabolites in the analyzed plant extracts.

2.5. LC-HRMS Analysis

Phytochemical analysis in this study was also carried out using advanced instrument techniques, namely Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) [1]. The identification of secondary metabolite compounds of sacha inchi oil was carried out at the Inter-University Center of the Bandung Institute of Technology (ITB) using the Thermo High Resolution Mass Spectrometer Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ instrument and the Thermo Binary Pump Scientific™ Vanquish™ UHPLC system. Compound separation was performed using a High-Performance Liquid Chromatography (HPLC)-based gradient method, with the phase motion consisting of MS grade methanol containing 0.1% formic acid as phase B and MS grade water containing 0.1% formic acid as phase A. The separation process took place at a column temperature of 40 °C with a flow rate of 0.3 mL/min. Mass analysis was performed using Electrospray Ionization (ESI) technique in positive mode, with a scanning range of 66.7–1000 m/z.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Composition of Sacha Inchi Oil

Sacha inchi oil is known to contain various bioactive compounds that play a crucial role in its health benefits. Based on Figure 2 phytochemical tests, the detected compounds include flavonoids, alkaloids, tannins, phenolics, saponins, steroids, and terpenoids.

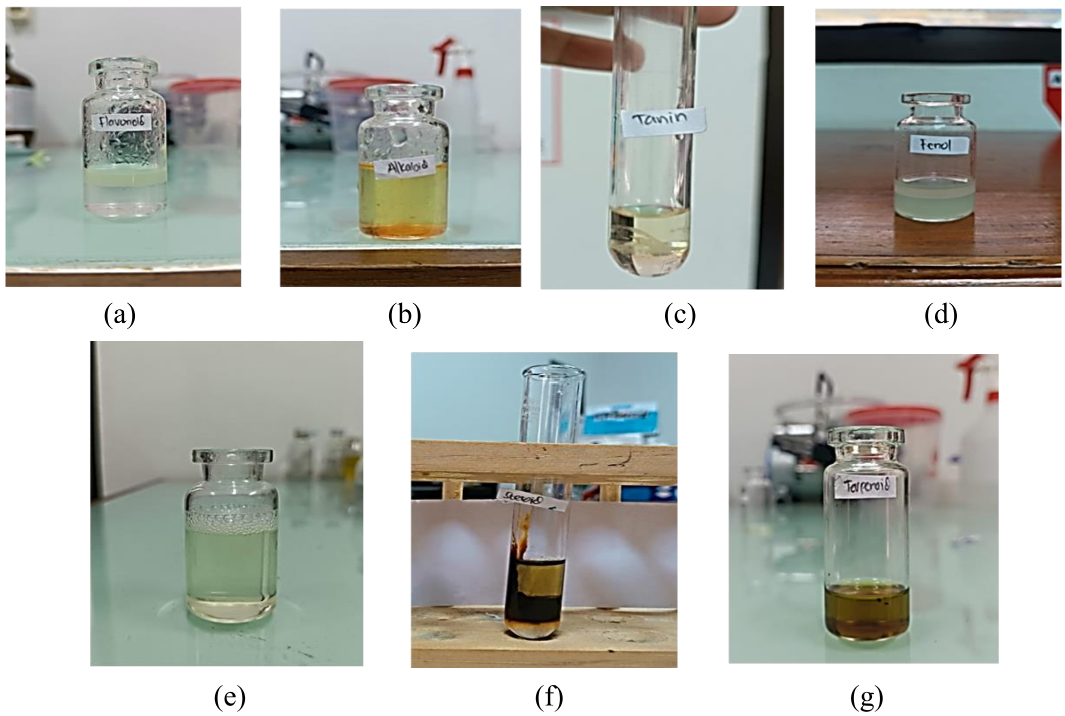


Figure 2. Phytochemical test results of sacha inchi oil (a) flavonoid (b) alkaloid (c) tannin (d) phenolic (e) saponin (f) steroids (g) terpenoids.

Qualitative tests in Table 1 indicated characteristic color changes, confirming the presence of these metabolites. However, LC-HRMS analysis did not detect flavonoids, tannins, and saponins. This discrepancy may be due to differences in sensitivity between methods or potential degradation of compounds during analysis [29].

Table 1. Phytochemical test results of sacha inchi oil.

Compound	Color if the result is positive	Color observed	Information
Flavonoids	Yellow intensive	Yellow	Positive
Alkaloids	Red precipitate	Red precipitate	Positive
Tannin	Green	Green	Positive
Phenolic	Turquoise blue	Turquoise blue	Positive
Saponin	Foam	Foam	Positive
Steroids	Brown rings	Brown rings	Positive
Terpenoids	Yellow	Yellow	Positive

The results presented in Figure 1 indicate that sacha inchi oil contains a variety of phytochemical compounds with potential biological activities. The colorimetric changes observed in the qualitative tests confirm the presence of key secondary metabolites that contribute to the oil's medicinal properties. These findings suggest that sacha inchi oil could be a valuable source of natural antioxidants, anti-inflammatory agents, and bioactive compounds beneficial for health applications. However, further research is required to quantify these compounds more precisely and evaluate their efficacy in pharmacological and nutraceutical applications.

3.2. Bioactive Compounds in Sacha Inchi Oil

3.2.1. Flavonoids

Flavonoids are polyphenolic compounds with various biological activities, including antioxidant, anti-inflammatory, and anticancer properties. These compounds help neutralize free radicals and protect cells from oxidative stress. In qualitative tests, flavonoids were indicated by a yellow color change. However, LC-HRMS analysis did not detect flavonoids, possibly due to their low concentration or structural modifications during analysis [30], [31]. Flavonoids are compound polyphenols in general consists of two rings aromatics linked by chains three carbon can form ring heterocyclic. Examples of flavonoids include quercetin, kaempferol, and catechin. Flavonoids interact with enzymes and receptors in the body, providing protective effects against degenerative diseases such as diabetes, cancer, and cardiovascular disorders [32]. The effects matrix, which contains various components in the sample, can interfere with detection and make it difficult to identify flavonoids using LC-HRMS. Additionally, improper sample preparation and unoptimized LC-HRMS parameters, particularly in ionization and fragmentation, may cause flavonoids to degrade or undergo chemical changes during analysis, reducing their detection effectiveness. Further studies are required to understand the mechanisms by which flavonoids in sacha inchi oil exert their biological effects.

3.2.2. Alkaloids

Alkaloids are nitrogen-containing organic compounds often associated with pharmacological properties such as antibacterial, analgesic, and neuroprotective effects [33]. Their presence was confirmed through the Dragendorff test, which produced a red precipitate. LC-HRMS analysis identified Militarinone A in Figure 3 as one of the alkaloids present in sacha inchi oil.

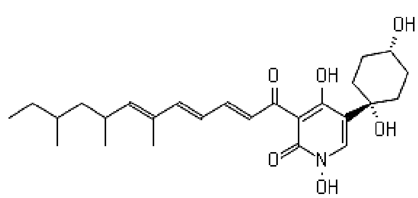


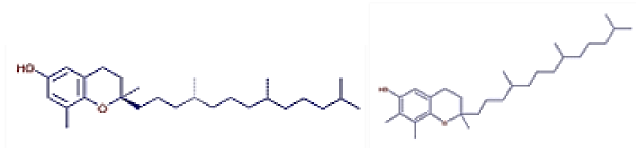
Figure 3. Structure compound militarinone A.

Alkaloids have potential applications in the treatment of microbial infections and inflammation. Additionally, some alkaloids exhibit stimulatory effects on the central nervous system, contributing to increased endurance and immune function [34].

3.2.3. Phenolics and Tannins

Phenolic compounds and tannins are well known for their strong antioxidant effects. These compounds protect cells from damage caused by free radicals and have therapeutic potential for inflammatory and cardiovascular conditions [35], [36]. Although tannins were detected in qualitative tests, they were not identified in LC-HRMS, possibly due to their polymeric nature, which makes ionization difficult in this method.

D-δ-Tocopherol and γ-Tocopherol in Figure 4 are forms of vitamin E that play a role in preventing lipid oxidation and enhancing immune function. These compounds are also essential for maintaining skin health and preventing premature aging [37].



(a) **(b)**

Figure 4. Structure compounds (a) D-Tocopherol and (b) γ-Tocopherol.

3.2.4. Steroids and Terpenoids

Steroids and terpenoids are secondary metabolites with significant pharmacological benefits. The steroid detected in sacha inchi oil by LC-HRMS in figure 5 is cytostenone, which is known for its role in metabolism and anti-inflammatory activities [38]. Meanwhile, terpenoids such as mandenol exhibit antioxidant and antibacterial properties, making them valuable for pharmaceutical and cosmetic applications.

Cytostenone (Figure 5) is commonly used in the pharmaceutical industry as a precursor for steroid hormones and anti-inflammatory drugs. Meanwhile, mandenol has antimicrobial properties that inhibit the growth of pathogenic bacteria and promote wound healing [39]. The presence of these compounds suggests that Sacha Inchi oil has potential as an ingredient in nutraceuticals and pharmaceuticals, particularly for anti-inflammatory and antimicrobial treatments [40].

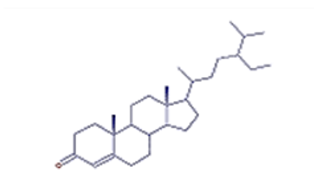


Figure 5. Structure compound cytostenone.

Furthermore, the presence of antioxidants, antimicrobials, and anti-inflammatory compounds in this oil makes it suitable for cosmetic formulations. Its ability to protect against oxidative stress and microbial infections highlights its potential in skincare and therapeutic applications. Future research should focus on optimizing extraction methods and conducting clinical trials to validate the efficacy of these bioactive compounds for commercial use [41].

4. CONCLUSION

This study aimed to identify the phytochemical constituents of sacha inchi (*Plukenetia volubilis* L.) oil obtained from Central Java using qualitative tests and LC-HRMS analysis. The results confirmed the presence of several bioactive compounds, including flavonoids, alkaloids, tannins, phenolics, saponins, steroids, and terpenoids through qualitative methods. However, some compounds were not detected in LC-HRMS, likely due to low concentrations or analytical limitations. These findings highlight the potential of sacha inchi oil as a natural source of functional compounds with antioxidant, anti-inflammatory, and therapeutic properties. Further research is recommended to optimize extraction techniques and assess the pharmacological efficacy of individual compounds for nutraceutical and pharmaceutical applications.

AUTHOR CONTRIBUTION

Mulyono Hadi: Writing (review & editing), writing (original draft), formal analysis. **Adi Permadi**: Writing (review & editing), writing (original draft), investigation, formal analysis. **Totok Eka Suharto**: Investigation, writing (review & editing), supervision, conceptualization. **Mutiara Wilson Putri**: Writing (review & editing), data curation, methodology. **Herbert Alessandro Panias Gulo**: investigation, validation. **Nadin Okta Maema**: visualization, project administration. **Halimathusyakhdyah**: resources, funding acquisition. **Ahmad Lupi**: Conceptualization, methodology, validation, supervision.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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