Jurnal Jamu Indonesia (2026) 11(5): 419–425
https://jamu-journal.ipb.ac.id/index.php/JJI/index

ISSN 2407-7178
eISSN 2407-7763

Research Article
Doi: https://doi.org/10.29244/jji.v11i5.578

Analysis of Antioxidant Activity, Total Phenolic and Flavonoid Contents of Ethyl Acetate Extracts from *Carica papaya* L. Seeds and Peels

Analisis Aktivitas Antioksidan, Kadar Fenolik dan Flavonoid Total Ekstrak Etil Asetat Biji dan Kulit *Carica papaya* L.

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ARTICLE INFO

Article history

Received on: 2026-04-31

Revised on: 2026-07-02

Accepted on: 2026-09-04

Keyword:

Carica papaya;
DPPH;
Flavonoids;
Phenolic compounds;
Ultrasound-assisted extraction

Kata kunci:

Carica papaya;
DPPH;
Ekstraksi berbantuan ultrasonic;
Flavonoid;
Senyawa fenolik

ABSTRACT

Papaya seeds and peels are underutilized by-products containing antioxidant-associated phytochemicals. This study compared ethyl acetate extracts of *Carica papaya* L. seeds and peels prepared using matrix-specific ultrasound-assisted extraction conditions. Total phenolic content was determined by the Folin–Ciocalteu method, total flavonoid content by the aluminum chloride colorimetric method, and antioxidant activity by the DPPH assay. Peel extract contained higher total phenolic and flavonoid contents than seed extract, at 12.076 ± 0.091 versus 6.699 ± 0.087 mg GAE/g and 75.983 ± 1.089 versus 39.604 ± 1.169 mg QE/g, respectively. DPPH IC_{50} values were 91.78 ± 0.99 ppm for peel, 815.79 ± 3.77 ppm for seed, and 6.62 ± 0.01 ppm for ascorbic acid. Under the extraction conditions applied, peel showed greater antioxidant-associated responses than seed, although the comparison should not be interpreted as intrinsic tissue superiority.

ABSTRAK

Biji dan kulit pepaya merupakan produk samping yang belum dimanfaatkan secara optimal dan mengandung fitokimia terkait aktivitas antioksidan. Penelitian ini membandingkan ekstrak etil asetat biji dan kulit *Carica papaya* L. yang diperoleh dengan kondisi ekstraksi berbantuan ultrasonik yang disesuaikan untuk masing-masing matriks. Kadar fenolik total ditentukan dengan metode Folin–Ciocalteu, kadar flavonoid total dengan metode kolorimetri aluminium klorida, dan aktivitas antioksidan dengan metode DPPH. Ekstrak kulit memiliki kadar fenolik dan flavonoid total lebih tinggi daripada ekstrak biji, masing-masing 12,076 ± 0,091 berbanding 6,699 ± 0,087 mg GAE/g dan 75,983 ± 1,089 berbanding 39,604 ± 1,169 mg QE/g. Nilai IC_{50} DPPH sebesar 91,78 ± 0,99 ppm untuk kulit, 815,79 ± 3,77 ppm untuk biji, dan 6,62 ± 0,01 ppm untuk asam askorbat. Pada kondisi ekstraksi yang digunakan, kulit menunjukkan respons terkait antioksidan yang lebih tinggi, tanpa menyiratkan keunggulan intrinsik jaringan.

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Citation: Sarina, G., Amir, M., Wardhani, A. D., & Bachri, N. (2026). Analysis of Antioxidant Activity, Total Phenolic and Flavonoid Contents of Ethyl Acetate Extracts from *Carica papaya* L. Seeds and Peels. *Jurnal Jamu Indonesia*, *11*(5), 419–425. https://doi.org/10.29244/jji.v11i5.578

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1. INTRODUCTION

Oxidative stress develops when reactive oxidant production exceeds antioxidant defenses, shifting cellular redox balance toward oxidative damage. Excess reactive oxygen species can damage lipids, proteins, and nucleic acids, whereas lower, regulated oxidant levels also participate in physiological signaling (Juan et al., 2021; Forman & Zhang, 2021). Oxidative stress is implicated in the pathophysiology of numerous chronic disorders, although the biological role of oxidants is context-dependent and antioxidant interventions do not uniformly translate into clinical benefit (Forman & Zhang, 2021). Plant phenolic compounds and flavonoids are therefore widely investigated as antioxidant-associated phytochemicals because many can participate in electron- or hydrogen-donation reactions and other redox-related mechanisms (Rahman et al., 2022). Nevertheless, antioxidant activity measured in a crude extract represents the combined response of multiple constituents and should not be attributed to a single phytochemical class without compound-level evidence.

Papaya (*Carica papaya* L.) processing generates peel and seeds that are commonly underutilized despite containing bioactive constituents. Comparative studies show that papaya phenolic composition and antioxidant responses vary with plant part, cultivar, ripening stage, and extraction system. Insanu et al. (2022) found marked differences among polar, semi-polar, and nonpolar extracts of papaya tissues, including high total flavonoid content in the ethyl acetate peel extract, while Jeon et al. (2022) reported distinct antioxidant profiles between seed and peel-pulp fractions. Zhou et al. (2023) further demonstrated tissue- and ripeness-dependent phenolic profiles using LC-ESI-QTOF-MS/MS, and Reyna et al. (2025) showed that the relative bioactive performance of peel and seeds varied among papaya varieties. Thus, neither peel nor seed should be assumed to be universally superior as an antioxidant source.

Extraction conditions are an important source of variation because ultrasound-assisted extraction performance is affected by factors such as solvent type, temperature, extraction time, ultrasound conditions, and liquid-to-solid ratio (Kumar et al., 2021). Papaya-specific studies likewise show that ultrasound-assisted extraction outcomes depend on the matrix and processing conditions: Samaram et al. (2013) evaluated papaya seed oil extraction, Lara-Abia et al. (2022) optimized carotenoid extraction from papaya pulp and peel, Singla et al. (2023) investigated ultrasound-assisted recovery of bioactive compounds from ripe papaya peel, and Robles-Apodaca et al. (2024) demonstrated strong extraction-condition effects on papaya seed polyphenols and antioxidant responses. Ethyl acetate is particularly relevant to the present study because Insanu et al. (2022) directly evaluated semi-polar ethyl acetate extracts of papaya tissues. However, comparative evidence for ethyl acetate extracts of papaya seeds and peels prepared under matrix-specific ultrasound-assisted extraction conditions remains limited.

Accordingly, this study aimed to determine and compare the extraction yield, moisture characteristics, qualitative phytochemical profile, total phenolic content, total flavonoid content, and DPPH radical-scavenging activity of ethyl acetate extracts of *C. papaya* seeds and peels. The comparison was interpreted specifically within the matrix-dependent extraction conditions used for each plant part.

2. METHODS

2.1. Plant Material, Reagents, and Instrumentation

Mature fruits of *C. papaya* were obtained from a papaya plantation in Cilacap, Central Java, Indonesia. Seeds and peels were separated manually and processed independently. The principal analytical instruments were an ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany), a V-730 UV–visible spectrophotometer (JASCO Corporation, Hachioji, Tokyo, Japan), a rotary evaporator (IKA-Werke GmbH & Co. KG, Staufen, Germany), an oven and water bath (Memmert GmbH + Co. KG, Schwabach, Germany), an analytical balance (OHAUS Corporation, Parsippany, NJ, USA), and micropipettes (Mettler-Toledo Rainin, LLC, Oakland, CA, USA). The principal reagents were ethyl acetate ($C_4H_8O_2$), methanol (CH_3OH), Folin–Ciocalteu reagent, gallic acid ($C_7H_6O_5$), quercetin ($C_{15}H_{10}O_7$), aluminum chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid ($C_6H_8O_6$), hydrochloric acid (HCl), iron(III) chloride ($FeCl_3$), sodium chloride (NaCl), chloroform ($CHCl_3$), acetic anhydride, sulfuric acid (H_2SO_4), acetic acid, Mayer's reagent, Dragendorff's reagent, magnesium powder, gelatin, and distilled water.

2.2. Sample Preparation and Ultrasound-Assisted Extraction

One kilogram of each plant part was washed under running water. Seeds were dehydrated at 50 °C for 48 h, whereas peels were dehydrated at 45 °C for 15 h. The dried materials were ground, passed through a No. 40 mesh sieve, and stored in closed containers until extraction. For seeds, 10 g of powder was combined with ethyl acetate at a solid-to-solvent ratio of 1:8 (g/mL) and sonicated for 30 min at 50 °C (Samaram et al., 2013). For peels, 10 g of powder was combined with ethyl acetate at a ratio of 1:5 (g/mL) and sonicated for 20 min at 70 °C using matrix-specific conditions adopted from papaya-peel literature (Lara-Abia et al., 2022; Wani & Uppaluri, 2022). The suspensions were filtered, concentrated using a rotary evaporator at 100 rpm and 50 °C, and further concentrated in a water bath until viscous extracts were obtained.

2.3. Extraction Yield and Moisture Content

Extraction yield was expressed as the mass fraction of viscous extract recovered from the dried plant material:

$$\text{Yield (\%)} = (m_e / m_s) \times 100$$

where m_e is the mass of recovered extract (g) and m_s is the mass of dried simplicia used for extraction (g). Moisture content was

determined gravimetrically by drying approximately 1 g of sample at 105 °C to constant mass.

$$\text{Moisture (\%)} = [(W_2 - W_3) / (W_2 - W_1)] \times 100$$

where W_1 is the mass of the empty crucible, W_2 is the mass of the crucible plus sample before drying, and W_3 is the mass of the crucible plus sample after drying.

2.4. Phytochemical Screening

Qualitative phytochemical screening was performed using classical color and precipitation reactions described in standard phytochemical analysis references (Farnsworth, 1966; Harborne, 1998). Alkaloids were assessed using Mayer's and Dragendorff's reagents after extraction with 2 N HCl. Flavonoids were screened using the magnesium–HCl reaction. Tannins were assessed independently using 10% $FeCl_3$ and a gelatin–NaCl precipitation test. Saponins were evaluated from persistent foam after vigorous shaking and acidification. Triterpenoids/steroids were assessed using chloroform, acetic anhydride, and concentrated H_2SO_4, whereas phenolics were screened with 10% $FeCl_3$. Results of reagent-specific tests were recorded separately for each extract.

2.5. Total Phenolic Content

Total phenolic content (TPC) was quantified using the Folin–Ciocalteu assay with gallic acid as the calibration standard (Singleton et al., 1999). Gallic acid standards of 25, 40, 55, 70, 85, 100, and 115 µg/mL were prepared. For each standard, 0.5 mL was mixed with 5.0 mL of 10% Folin–Ciocalteu reagent, allowed to react for 5 min, then combined with 4.0 mL of 1 M Na_2CO_3 and incubated for 30 min at room temperature in the dark. Absorbance was measured at 750 nm according to the adopted reference procedure (Hidayatullah et al., 2024). For sample analysis, 25 mg of extract was dissolved in 5.0 mL methanol to obtain a 5000 µg/mL solution. Three analytical replicate solutions were processed under the same reaction conditions. The experimental gallic acid calibration relationship was:

$$y = 0.0066105x + 0.044781 \quad (R^2 = 0.99919)$$

$$\text{TPC (mg GAE/g)} = c \times V \times \text{DF} / (1000 \times m)$$

where c is the gallic-acid-equivalent concentration obtained from the calibration equation (µg/mL), V is the total volume of the prepared extract solution (mL), DF is the dilution factor, and m is the mass of extract used (g). The factor 1000 converts µg to mg. For the preparation used here, V = 5.0 mL, m = 0.025 g, and DF = 1.

2.6. Total Flavonoid Content

Total flavonoid content (TFC) was determined using the $AlCl_3$ colorimetric assay with quercetin as the calibration standard (Chang et al., 2002). Quercetin standards of 40, 55, 70, 85, 100, 115, and 130 µg/mL were prepared. An aliquot of 0.5 mL of each standard was mixed with 0.5 mL of 10% $AlCl_3$ and 4.0 mL of 5% acetic acid, incubated for 30 min at room temperature in the dark, and measured at 410 nm according to the adopted reference procedure (Hidayatullah et al., 2024). For sample determination, 10 mg of extract was dissolved in 5.0 mL methanol to obtain 2000 µg/mL and subsequently diluted to 1500 µg/mL. Three analytical replicate solutions were analyzed. The experimental quercetin calibration relationship was:

$$y = 0.0066198x - 0.018194 \quad (R^2 = 0.99972)$$

$$\text{TFC (mg QE/g)} = c \times V \times \text{DF} / (1000 \times m)$$

where c is the quercetin-equivalent concentration obtained from the calibration equation (µg/mL), V is the total volume of the original extract solution (mL), DF is the dilution factor, and m is the mass of extract used (g). The factor 1000 converts µg to mg. For the preparation used here, V = 5.0 mL, m = 0.010 g, and DF = 2000/1500 = 1.3333.

2.7. DPPH Radical-Scavenging Assay

DPPH radical-scavenging activity was measured using a 0.1 mM DPPH solution prepared by dissolving 3.9 mg DPPH in methanol to 100 mL. The analytical wavelength was set at 517 nm according to the papaya-specific reference procedure (Oktavia & Hidayah, 2022), while the assay principle followed Brand-Williams et al. (1995). Ascorbic acid was tested at 4–10 ppm, seed extract at 500–2000 ppm, and peel extract at 100–700 ppm. For each concentration, 1.0 mL test solution was mixed with 3.0 mL DPPH solution and incubated for 30 min in the dark before absorbance measurement.

$$\text{DPPH inhibition (\%)} = [(A_0 - A_s) / A_0] \times 100$$

where A_0 is the absorbance of the DPPH control and A_s is the absorbance of the sample–DPPH reaction mixture.

IC_{50} was calculated from y = a ln(C) + b as $IC_{50} = \exp[(50 - b)/a]$. The mean concentration–response models were y = 29.1807 ln(C) − 5.1511 for ascorbic acid (R^2 = 0.99916), y = 15.4738 ln(C) − 53.7416 for seed extract (R^2 = 0.99915), and y = 9.6695 ln(C) + 6.2899 for peel extract (R^2 = 0.99890).

2.8. Statistical Analysis

Quantitative results are reported as mean ± standard deviation from three analytical replicates. Seed and peel total phenolic and flavonoid contents were compared using Welch's independent-samples t-test. Replicate-derived IC_{50} values for ascorbic acid, seed extract, and peel extract were compared by one-way analysis of variance, followed by pairwise Welch t-tests with Bonferroni adjustment. Statistical significance was set at $p < 0.05$, and η^2 was used as an effect-size estimate. Because replicates originated from analytical preparations/absorbance series rather than independent extraction batches, inferential statistics were interpreted primarily as measures of analytical repeatability.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield, Moisture Content, and Phytochemical Profile

As shown in **Table 1**, ethyl acetate extraction yielded 5.57% extract from papaya seeds and 8.20% from papaya peels, while the moisture contents of dried seed and peel simplicia were 6.66% and

8.00%, respectively, and those of the corresponding extracts were 17.82% and 14.42%. The higher peel yield observed under the present conditions may reflect differences in matrix composition and extractable semi-polar constituents rather than an inherent difference between plant parts. Similar variability has been reported in studies showing that papaya phytochemical recovery depends on solvent polarity, extraction conditions, and tissue type (Kumar et al., 2021; Singla et al., 2023; Robles-Apodaca et al., 2024). The qualitative phytochemical screening in **Table 2** showed that both extracts contained flavonoids and saponins, while the seed extract showed a triterpenoid reaction and the peel extract a steroid reaction. Phenolics were qualitatively detected only in the peel extract, whereas tannin reactions differed according to reagent. Comparable tissue- and solvent-dependent variations have been reported in papaya fractions (Insanu et al., 2022; Zhou et al., 2023). The negative qualitative phenolic reaction in the seed extract should not be interpreted as complete absence of phenolic-reactive constituents because the quantitative Folin–Ciocalteu assay detected measurable phenolic content.

3.2. Total Phenolic and Flavonoid Contents

As shown in **Figure 1A–B**, the peel extract contained higher total phenolic and flavonoid contents than the seed extract. Total phenolic content was 12.076 ± 0.091 mg GAE/g in peel and 6.699 ± 0.087 mg GAE/g in seed, with a significant difference by Welch's independent-samples t-test ($t = -73.38$, $p < 0.001$). Total flavonoid content followed the same pattern, reaching 75.983 ± 1.089 and 39.604 ± 1.169 mg QE/g, respectively ($t = -39.41$, $p < 0.001$). However, direct peel–seed comparisons vary among studies; Jeon et al. (2022) and Reyna et al. (2025) reported stronger phenolic or antioxidant performance in seeds under other experimental conditions. Therefore, the higher values observed here should be interpreted as specific to the material and extraction conditions used.

3.3. DPPH Radical-Scavenging Activity

The concentration–response profiles in **Figure 2A–B** show increasing DPPH inhibition with increasing concentration for ascorbic acid and both papaya extracts. The IC_{50} values in **Figure 2C** were 6.62 ± 0.01 ppm for ascorbic acid, 91.78 ± 0.99 ppm for peel, and 815.79 ± 3.77 ppm for seed. Within the analytical replicate series, the overall comparison was significant by one-way analysis of variance ($F = 117{,}289.53$, $p < 0.001$), and pairwise Welch tests with Bonferroni adjustment indicated differences among all groups.

Table 1. Extraction yield and moisture characteristics of papaya seed and peel samples

Sample	Moisture (%)	Simplicia (g)	Extract (g)	Yield (%)
Seed simplicia/extract	6.66 / 17.82	214.23	11.95	5.57
Peel simplicia/extract	8.00 / 14.42	266.50	21.86	8.20

Table 2. Qualitative phytochemical profile of ethyl acetate extracts of papaya seed and peel

Phytochemical test	Seed extract	Peel extract
Alkaloids	–	–
Flavonoids	+	+
Tannins ($FeCl_3$ 10%)	–	+
Tannins (gelatin 1%)	–	–
Saponins	+	+
Triterpenoids/steroids	+ (triterpenoid)	+ (steroid)
Phenolics	–	+

Note: + = detected; – = not detected.

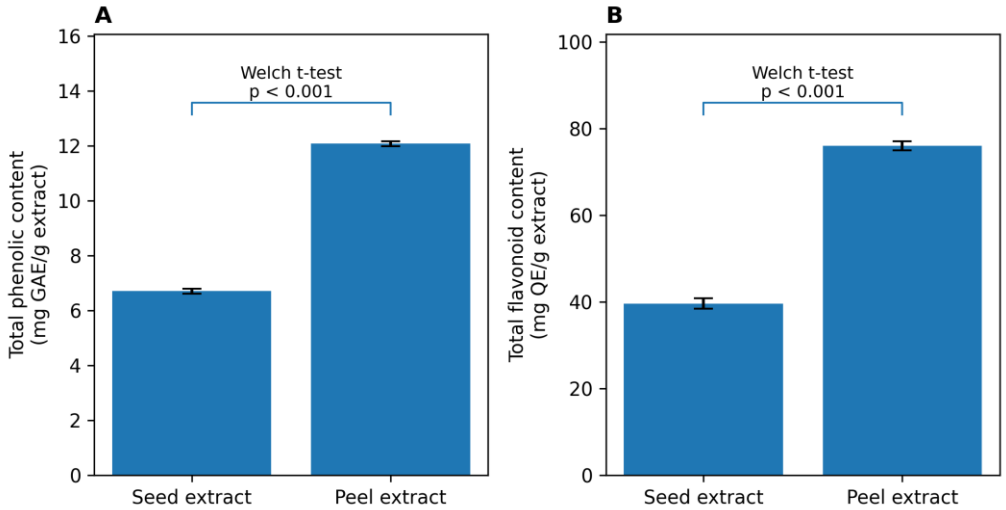

Figure 1. Total phenolic and flavonoid contents of papaya seed and peel ethyl acetate extracts. Bars represent mean ± SD (n = 3 analytical replicates); p values are from Welch's t-test.

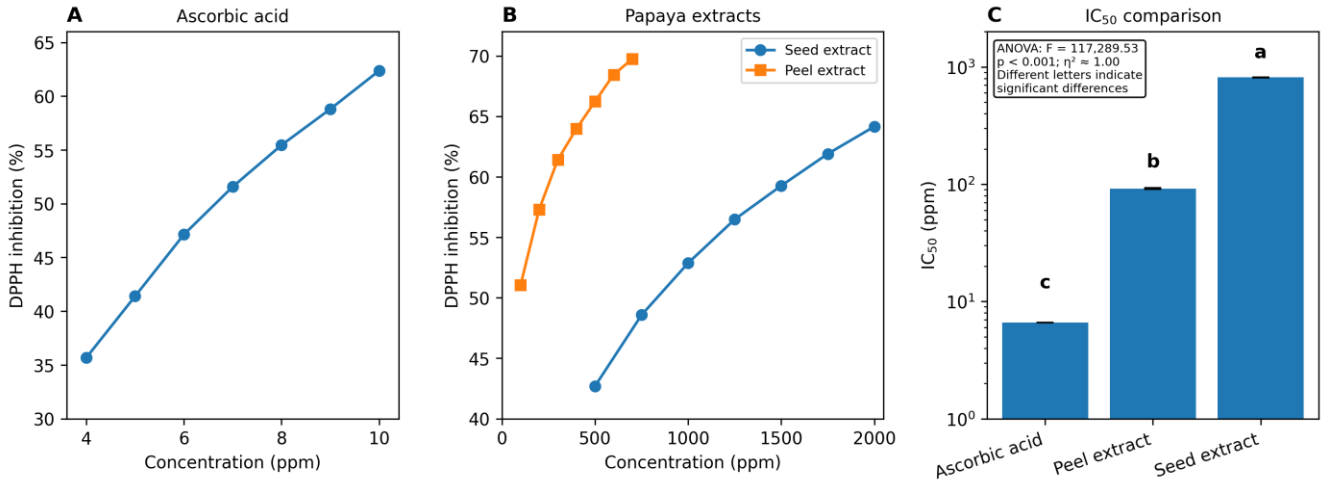

Figure 2. DPPH radical-scavenging activity and IC_{50} comparison. (A) Ascorbic acid concentration–response. (B) Papaya seed and peel concentration–response. (C) IC_{50} values as mean ± SD from three analytical replicate series.

Vasundra and Dutta (2024) likewise reported a lower DPPH IC_{50} for peel than seed under their conditions, whereas Jeon et al. (2022) and Reyna et al. (2025) reported stronger antioxidant responses in seed under other extraction systems. Thus, the present data support higher DPPH-scavenging potency of peel within this experimental system, not universal superiority of peel over seed. Because the IC_{50} replicates were derived from analytical absorbance series rather than independent extraction batches, these statistics primarily describe analytical repeatability.

3.4. Relationship Between Phytochemical Content and Antioxidant Activity

Across **Figures 1** and **2**, the peel extract showed higher total phenolic and flavonoid contents together with a lower DPPH IC_{50} than the seed extract, suggesting that phenolic- and flavonoid-associated constituents may contribute to its stronger radical-scavenging response. However, no direct correlation analysis or compound-level identification was performed, so this relationship should be interpreted as an association rather than causation. Insanu et al. (2022) showed that phytochemical content and antioxidant responses vary across papaya fractions and solvent systems, while Zhou et al. (2023) demonstrated tissue-dependent phenolic profiles. The lower IC_{50} of the peel extract may therefore reflect the combined effects of multiple extractable constituents rather than phenolics or flavonoids alone.

4. CONCLUSION

Under the matrix-specific ultrasound-assisted extraction conditions applied, the ethyl acetate peel extract showed higher total phenolic and flavonoid contents and a lower DPPH IC_{50} than the seed extract. Peel and seed extracts contained 12.076 ± 0.091 and 6.699 ± 0.087 mg GAE/g total phenolics, 75.983 ± 1.089 and 39.604 ± 1.169 mg QE/g total flavonoids, and DPPH IC_{50} values of 91.78 ± 0.99 and 815.79 ± 3.77 ppm, respectively. These findings support the antioxidant-associated potential of papaya peel under the conditions examined but do not establish intrinsic superiority of peel over seed because the two matrices were extracted under different conditions. Future work should employ independently replicated extraction batches and chromatographic characterization.

AUTHOR CONTRIBUTIONS

Conceptualization, G.S. and M.A.; methodology, G.S., M.A., and A.D.W.; formal analysis, G.S. and A.D.W.; investigation, G.S. and W.C.P.; resources, G.S. and M.A.; data curation, G.S. and W.C.P.; visualization, G.S. and A.D.W.; writing—original draft preparation, G.S.; writing—review and editing, M.A., A.D.W., and W.C.P.; supervision, M.A.; project administration, G.S. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. This study did not involve humans or animals.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

FUNDING

This research received no external funding.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Faculty of Health Sciences, Universitas Esa Unggul, for institutional and laboratory support during this study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

Not applicable. This research received no external funding.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, grammar, and readability of the text. After using this tool, the authors carefully reviewed, edited, and verified the entire content to ensure that it accurately represents their own data, analysis, and interpretation. The authors take full responsibility for the integrity, accuracy, and originality of the manuscript.

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