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Nutritional Composition, Pigment Profiling, and Antioxidant Capacity of Locally Produced Powdered Spirulina from Central Java, Indonesia

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ABSTRACT

Spirulina (*Arthrospira sp*.) is widely recognized as a nutrient-dense microalgal biomass with potential functional properties. This study aimed to characterize the nutritional composition, bioactive pigment profile, and antioxidant activity of locally produced spirulina powder from Central Java (Indonesia), with particular emphasis on its radical scavenging capacity evaluated using the DPPH assay. Spirulina powder was analyzed for proximate composition (moisture, crude protein, crude fat, carbohydrate, and ash), as well as major bioactive pigments including phycobiliproteins, chlorophyll, and β-carotene. Antioxidant activity was determined using the DPPH radical scavenging assay. The spirulina powder contained 56% protein. The bioactive pigment profile was dominated by phycobiliproteins, particularly C-phycocyanin, alongside high levels of chlorophyll and β-carotene. Antioxidant evaluation showed a concentration-dependent DPPH radical scavenging activity, with an IC_{50} value of approximately 396 ppm, indicating moderate direct radical scavenging capacity within the DPPH system. These findings suggest that local spirulina from Central Java possesses limited instantaneous free radical scavenging activity when assessed using a single-mechanism chemical assay. However, its complex nutritional matrix may contribute to antioxidant mechanisms beyond direct radical neutralization, although this was not specifically evaluated in the present study.

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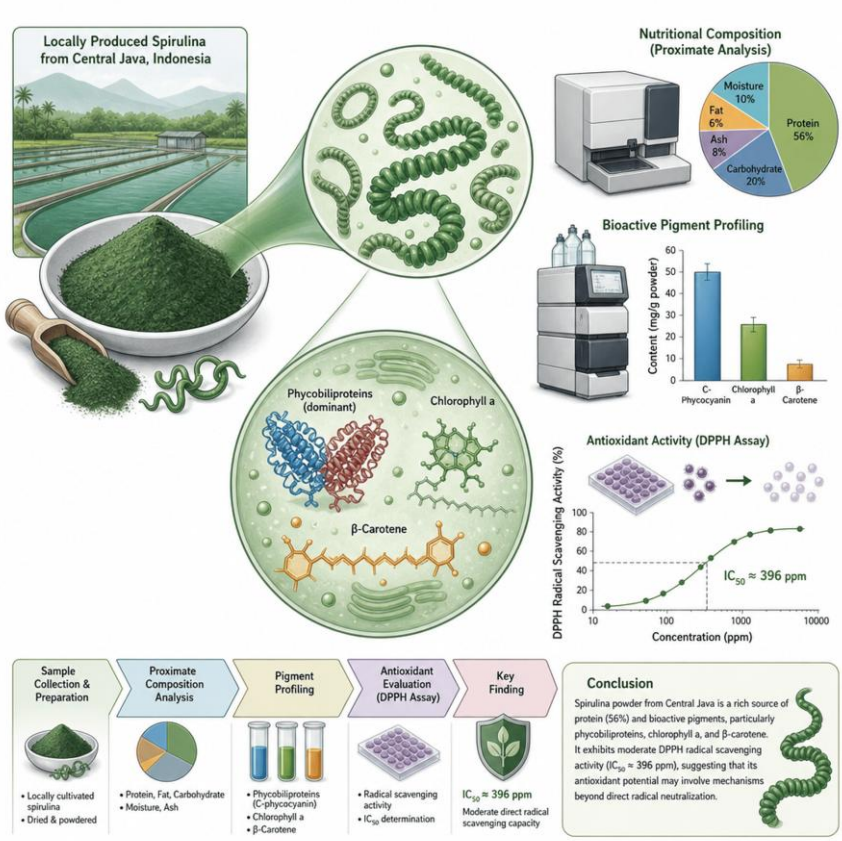
Key Messages:

- This study provides the nutritional, pigment, and antioxidant characterization of locally cultivated spirulina from Central Java, (Indonesia) a source that has been scarcely documented in the scientific literature
- The spirulina powder demonstrated a high protein content and a rich profile of bioactive pigments, confirming its potential as a nutrient-dense biomass derived from a locally adapted cultivation environment
- This study supports the applicability of locally produced spirulina as a functional food ingredient and provides a scientific basis for the valorization of region-specific spirulina production systems in nutrition and health research

GRAPHICAL ABSTRACT



INTRODUCTION

Global demand for protein continues to rise in parallel with population growth and shifts in dietary consumption patterns. The global protein market was valued at approximately USD 38 billion in 2019 and is projected to grow at an annual rate of 9.1%, exceeding USD 70 billion by 2027. Although animal-based proteins still dominate overall consumption, a substantial shift toward alternative protein sources has emerged, driven by sustainability concerns, competitive pricing, and the growing adoption of vegetarian and flexitarian diets. While strategies to minimize anti-nutritional factors remain an important technological consideration, market-driven preferences for clean-label and hypoallergenic protein sources have independently contributed to the growing prominence of plant-based and non-animal proteins in global food diversification (1).

In addition to its high protein content and balanced essential amino acid profile, spirulina is rich in unsaturated fatty acids, vitamins, minerals, and bioactive pigments such as phycocyanin and β-carotene, which function as natural antioxidants (2). Beyond their role as protein suppliers, the nutritional relevance of alternative food sources is increasingly evaluated based on the presence of bioactive components, including pigments with antioxidant properties, which may contribute to oxidative stability and potential health-related functions (3). Spirulina occupies a strategic position as an alternative protein source that has attracted increasing scientific and commercial attention. These characteristics position spirulina not merely as a substitute for animal protein, but also as a functional superfood with broad health-promoting potential.

Spirulina production is relatively more efficient than conventional animal farming, offering tangible contributions to the development of sustainable food systems. Consequently, characterization of the nutritional and bioactive pigments of spirulina, particularly that produced locally in Indonesia, is highly relevant both academically and practically in supporting future food security. This study aims to characterize the nutritional composition, bioactive pigments, and antioxidant capacity of locally produced powdered Spirulina from Central Java, Indonesia.

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METHODS

Sample Source

Dried spirulina (*Arthrospira platensis* or *Arthrospira maxima*) powder was obtained from a cultivation system located near a natural spring (umbul) in Klaten, Central Java, Indonesia. The study was conducted in 2025 at the Nutrition Biochemistry Laboratory, IPB University; and the Testing Laboratory - Center for Testing and Standardization of Postharvest Agricultural Instrument (Laboratorium Penguji - Balai Besar Pengujian Standar Instrumen Pascapanen Pertanian), Bogor, Indonesia.

Proximate Analysis

Proximate composition was determined according to Indonesian National Standards (SNI 01-2891-1992). Crude fat content was determined by Soxhlet extraction using n-hexane as the solvent. After extraction, the solvent was evaporated and the extracted lipid fraction was dried at 105 °C to constant weight. Crude fat content was expressed as a percentage of sample weight. Protein content was determined using the Kjeldahl method. Samples were digested with H_2SO_4 in the presence of a catalyst, followed by distillation with NaOH. The released ammonia was captured in H_3BO_3 and titrated with standardized HCl. Nitrogen content was converted to crude protein using a conversion factor of 6.25.

Moisture (water) content was determined gravimetrically by drying a known weight of sample in an oven at 105 °C until a constant weight was achieved. Ash content was analyzed by incinerating a known weight of sample in a muffle furnace at 550 °C until complete ashing, and expressed as a percentage of the residue relative to the initial sample weight. Carbohydrate content was calculated by difference, subtracting the percentages of moisture, ash, protein, and fat from 100%.

Pigment Analysis

Phycocyanin content in spirulina was determined using a spectrophotometric method following ultrasound-assisted extraction. Briefly, spirulina biomass was accurately weighed and suspended in distilled water at a ratio of approximately 1.5% (w/v). The suspension was subjected to ultrasonic extraction using a bath sonicator operating at approximately 40 kHz for 15-20 minutes. During sonication, the extraction temperature was maintained at 25 ± 2 °C to minimize thermal degradation of the pigments. Following ultrasonication, the extract was centrifuged at 4,000 rpm for 10 minutes to separate insoluble residues. The resulting blue-green supernatant was carefully collected and analyzed using a UV-Vis spectrophotometer. Absorbance measurements were recorded at wavelengths of 615 nm and 652 nm. Phycocyanin concentration was calculated using the equations proposed by Bennett and Bogorad (4).

Chlorophyll a was extracted using an ultrasound-assisted extraction (UAE) method. Spirulina powder (1 g) were mixed with 16 mL of 96% ethanol (solvent-to-material ratio 16:1, v/w) in a 50 mL centrifuge tube. The mixture was subjected to ultrasonication at 40 kHz for 2.5 h at 52 °C, with temperature maintained within ±2 °C. After extraction, the samples were centrifuged at 6,000 rpm for 15 min, and the clear supernatant was collected for analysis. Absorbance was measured at 663 nm and 645 nm using a UV-Vis spectrophotometer with ethanol as the blank and a 1 cm quartz cuvette. Chlorophyll a content was calculated using standard equations for ethanol extracts and expressed as mg/g fresh weight (5).

β-carotene content was determined by high-performance liquid chromatography (HPLC) following acetone extraction. The extraction procedure was conducted under dark conditions to prevent photodegradation of carotenoids. The extract was filtered prior to injection into the HPLC system equipped with a UV–Vis detector, and β-carotene was monitored at 450 nm. Quantification was performed based on retention time and peak area comparison with an authentic β-carotene standard.

Antioxidant Activity Assay

The antioxidant activity of spirulina was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. This method is based on the ability of antioxidant compounds present in the sample to donate hydrogen atoms or electrons to the stable DPPH radical, resulting in a decrease in absorbance. Spirulina powder was dispersed in 96% ethanol and sonicated for 15 min to facilitate the release of soluble antioxidant compounds. The suspension was then centrifuged at 4000 rpm, and the

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resulting supernatant was used for DPPH radical scavenging assay. The supernatant at various concentrations were mixed with a DPPH solution and incubated under dark conditions. The reduction of the DPPH radical was monitored by measuring the absorbance at the 516 nm wavelength using Multi-Mode Microplate Readers. Antioxidant activity was expressed as the IC_{50} value (ppm), defined as the concentration required to inhibit 50% of DPPH radicals (6). Phycocyanin is primarily water-soluble, ethanol was selected to ensure compatibility with the DPPH assay system.

RESULTS

Proximate Composition of Spirulina

Proximate analysis of spirulina cultivated in Klaten revealed a high protein content, with an average value of 55.80%, while total lipid content was relatively low at 5.24%. Carbohydrate content, calculated by difference, accounted for 27.08%, whereas ash content was 5.61%. The measured moisture content was approximately 6.27%. The total energy value of spirulina was 378.68 kcal/100 g, with lipids contributing 47.16 kcal/100 g to the overall energy content (Table 1).

Table 1. Proximate composition of spirulina cultivated in Klaten, Central Java, Indonesia

Nutrient	Content*	Unit
Protein	55.80 ± 0.97	%
Carbohydrate	27.08 ± 0.62	%
Moister content (Water)	6.27± 0.11	%
Ash	5.61 ± 0.14	%
Total fat	5.24 ± 0.10	%
Total energy	378.68 ± 0.52	kcal/100 g
Energy from fat	47.16 ± 0.89	kcal/100 g

Note: *Values are expressed as mean ± SD from duplicate analyse

Pigment Content

The pigment analysis of powdered spirulina demonstrated substantial variation in the concentrations of individual pigments (Table 2). Phycocyanin was identified as the most abundant pigment, with a concentration of 4,692.00 mg/100 g of spirulina powder, accounting for the largest proportion of the total pigment content measured in this study.

Chlorophyll was detected at a markedly lower concentration, reaching 931.70 mg/100 g, which represented approximately one-fifth of the phycocyanin level. In contrast, β-carotene was present at the lowest concentration among the pigments analyzed, with a measured value of 227.66 mg/100 g of spirulina powder. The quantitative differences among these pigments are summarized in Table 2.

Table 2. Pigment content of spirulina (mg/100 g spirulina powder)

Pigments	Content (mg/100 g wet basis)
Phycocyanin	4,692.00
β-carotene	227.66
Chlorophyll	931.70

Antioxidant Activity

Spirulina powder exhibited a concentration-dependent DPPH radical scavenging activity over the tested concentration range (62.5-1000 ppm). As summarized in Table 3, the percentage of inhibition increased progressively with increasing concentration, indicating a consistent dose-response pattern. At 1000 ppm, spirulina showed a DPPH inhibition of 63.80 ± 6.68%, which decreased to 53.28 ± 6.10% at 500 ppm and 45.42 ± 3.08% at 250 ppm. Lower inhibition values were observed at 125 ppm (40.67 ± 4.29%) and 62.5 ppm (38.99 ± 5.30%). The variability between independent measurements remained moderate across all concentrations, as reflected by the standard deviation values. Based on linear interpolation of the dose-response curve, the IC_{50} value of spirulina was estimated to be approximately 396 ppm, falling within the concentration range tested. The dose-response relationship showed good linearity around the IC_{50}

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region, supporting the robustness of the IC_{50} estimation.

Table 3. Antioxidant activity of spirulina powder

Concentration (ppm)	Spirulina (% inhibition)
1000	63.80 ± 6.68
500	53.28 ± 6.10
250	45.42 ± 3.08
125	40.67 ± 4.29
62.5	38.99 ± 5.3

DISCUSSION

Spirulina (*Arthrospira sp.*) is widely recognized as a microalgal biomass with a high nutritional value and a distinctive profile of bioactive constituents. In the present study, the spirulina powder used was characterized by a protein content of 56%. Protein concentration in spirulina is known to vary substantially depending on cultivation-related factors, including nitrogen availability, salinity, light intensity, temperature, culture medium composition, and the growth phase at harvest. Consistent with previous reports, the protein content observed in this study falls within the commonly reported range of 55-70% across Spirulina samples originating from different geographical and production systems (**7**).

The protein content contributes to the growing interest in spirulina as a potential alternative protein source (**8**). Importantly, the protein fraction of spirulina does not function solely as a reservoir of amino acids but also plays a structural role that is closely linked to its bioactive composition. However, the present study was limited to compositional characterization and did not evaluate its role in specific nutritional interventions.

Proteins constitute the primary structural matrix of phycobiliproteins, including phycocyanin and allophycocyanin, which serve as the principal photosynthetic pigments responsible for light energy capture in spirulina. As a result, variations in protein content are biologically associated with differences in pigment composition and abundance. In particular, C-phycocyanin, one of the dominant pigments in spirulina, represents a substantial proportion of the total protein fraction. This relationship indicates that spirulina protein reflects not only quantitative nutritional value but also the integrity of a functional biological system supporting pigment-associated physiological activity. Consequently, differences in protein levels reported across studies are closely related to variations in phycobiliprotein synthesis capacity arising from distinct cultivation conditions (2,9).

The lipid content of the spirulina powder examined in this study was aligning with values reported in other investigations (approximately 5-7%). In contrast, the carbohydrate content was slightly higher (27%) than that reported in some previous studies (15-25%) (7,10). Variability in carbohydrate levels has been attributed to environmental stress during cultivation, under which spirulina tends to redirect metabolic flux toward the synthesis of storage polysaccharides (7). However, environmental parameters of the present cultivation site were not specifically evaluated, and therefore no site-specific interpretation could be made. Beyond their role as energy reserves, spirulina carbohydrates have been widely reported to exhibit biological activities relevant to human health, including immunomodulatory and anti-inflammatory effects (11,12). Among these, calcium-spirulan, a sulfated polysaccharide containing calcium ions, has been identified as a bioactive compound with antiviral and anticoagulant properties (13).

Although quantitatively minor, the lipid fraction of spirulina is biologically meaningful. Lipids in spirulina are dominated by polyunsaturated fatty acids, particularly γ-linolenic acid and linoleic acid, as well as structurally complex glycolipids with documented bioactivity (14). This composition suggests that spirulina lipids function primarily as functional components rather than caloric substrates. Supporting this view, a previous in vivo studies have demonstrated that spirulina lipid fractions can improve serum lipid profiles by reducing triglycerides, total cholesterol, and LDL-cholesterol while increasing HDL-cholesterol. These effects have been linked to improved hepatic lipid metabolism and modulation of the gut microbiota, indicating that the lipid fraction of spirulina exerts biological activities that extend beyond conventional energy provision.

Moisture content represents a critical parameter influencing the physicochemical stability and

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shelf-life of spirulina biomass. Reported moisture levels in spirulina vary widely depending on post-harvest handling and drying processes. In fresh biomass, moisture content may exceed 70% on a wet basis, whereas in dried spirulina it can be reduced to approximately 6-7% (15). In the present study, the spirulina powder exhibited a moisture content of 6%, indicating that the biomass was in a stable, well-dried condition suitable for storage and subsequent analyses. The measured moisture content (6.27%) complies with the maximum moisture limit specified in the Indonesian National Standard for dried Spirulina (SNI 8468:2018).

Mineral content, estimated through ash determination, was found to fall within the range commonly reported for spirulina cultivated in different geographical regions, typically 3-11% (7). This finding suggests that the mineral profile of the sample is comparable to that of spirulina produced under diverse cultivation systems and does not deviate from expected compositional characteristics.

Chlorophylls and phycobiliproteins in spirulina are known to be susceptible to degradation during drying, storage, and light exposure, which contributes to the variability in pigment concentrations reported in the literature (16). In addition, when comparing pigment concentrations across studies, differences in reporting basis (wet or dry weight) should be taken into account, as they may influence numerical equivalence.

In the present study, the phycobiliprotein fraction comprised C-phycocyanin, allophycocyanin, and phycoerythrin, with a total phycobiliprotein content of approximately 7,780 mg/100 g (wet basis). Among these pigments, C-phycocyanin was the dominant component (4,692 mg/100 g), followed by allophycocyanin (2,770 mg/100 g) and phycoerythrin (910 mg/100 g). Phycocyanin is a water-soluble phycobiliprotein, and its recovery is strongly influenced by solvent polarity. Previous studies have demonstrated that polar extraction systems, particularly phosphate buffer, yield higher C-phycocyanin concentrations compared to less polar solvent systems (17). In the present study, water-based extraction was employed for phycobiliprotein analysis, which is consistent with the known physicochemical properties of these pigments.

The phycocyanin content observed in this study corresponds to approximately 4.7% on a wet-weight basis, or close to 5% on a dry-weight basis, which falls within the reported range of 1-14% dry weight for spirulina phycocyanin (18). The other studies have reported substantially higher phycocyanin levels, ranging from approximately 6.3% to 25% of dry weight, depending on cultivation and processing conditions (19,20). These variations highlight the strong influence of cultivation environment and post-harvest processing on phycobiliprotein accumulation.

Chlorophyll content in the present spirulina sample reached 932 mg/100 g on a wet basis, equivalent to approximately 995 mg/100 g on a dry-weight basis. This value lies within the range reported in several previous studies. For example, another study reported chlorophyll concentrations of 270-470 mg/100 g dry weight in commercial spirulina products, while a laboratory-cultivated sample in the same study exhibited a higher chlorophyll content of 1,080 mg/100 g dry weight (19). Lower chlorophyll levels have also been reported in other Indonesian spirulina samples, such as 334 mg/100 g wet weight (21).

The β-carotene content of the spirulina powder analyzed in this study (227.66 mg/100 g) was relatively high and approached the upper limit of values reported in the literature. β-Carotene concentrations in spirulina from various regions, including Japan, Africa (Lake Chad), and other countries, have been reported to range from 34 to 234 mg/100 g (10,22,23).

The elevated β-carotene content observed in this study may be interpreted as a physiological response of spirulina cells to cultivation-related environmental conditions. In spirulina, β-carotene functions as a secondary carotenoid pigment involved in cellular protection against high light intensity and environmental stress. Unlike protein synthesis, which supports primary structural and metabolic functions, β-carotene biosynthesis is often enhanced under more challenging conditions, such as increased salinity, light intensity, and temperature. Experimental evidence indicates that β-carotene production tends to peak at higher salinity levels than those optimal for protein accumulation and may also increase under elevated nitrate concentrations. These observations suggest a metabolic shift from structural biomass synthesis toward the formation of protective pigments as part of cellular adaptive mechanisms (24). β-Carotene is widely recognized for its antioxidant properties and its role as a provitamin A compound.

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Approximately 6 µg of dietary β-carotene is considered equivalent to 1 µg of retinol in terms of vitamin A activity (25).

Based on the DPPH radical scavenging assay, the IC_{50} value of the spirulina powder analyzed in this study was approximately 396.33 ppm (Table 3). The DPPH assay reflects a single electron-transfer mechanism and therefore captures only one aspect of antioxidant behavior. Variability in reported IC_{50} values across studies may partly arise from differences in sample preparation and extraction strategies (26). Similar ranges of antioxidant activity have been reported in previous studies, although many of those investigations employed solvent-extracted spirulina rather than whole powder preparations. For example, another study reported an IC_{50} value of 449 ppm for ethanol extracts of Moroccan spirulina, whereas the corresponding aqueous fraction exhibited a markedly higher IC_{50} of 4,148 ppm. In the same study, antioxidant activity measured by the ABTS assay exceeded 5,800 ppm, further illustrating the relatively weak radical scavenging performance of spirulina in single-mechanism chemical assays (27).

Comparable findings were reported by another study, who demonstrated that spirulina extracts prepared using water or hexane also exhibited IC_{50} values exceeding 400 ppm, indicating limited antioxidant efficiency in the DPPH system (28). In contrast, Fidiyani et al. observed substantially lower IC_{50} values (approximately 65-97 ppm) when spirulina was extracted using semi-polar solvents, highlighting the strong influence of extraction polarity on the apparent antioxidant activity (29). Collectively, these observations suggest that higher IC_{50} values are characteristic of whole biomass or polar fractions, whereas selective extraction with semi-polar solvents enriches compounds with stronger radical scavenging properties.

The relatively high IC_{50} value obtained in the present study should therefore be interpreted as an indication of limited direct radical scavenging capacity, rather than as evidence of low overall antioxidant potential. Biological antioxidant activity cannot be adequately characterized by instantaneous radical scavenging alone. The DPPH assay specifically reflects a single reaction mechanism, namely electron or hydrogen atom transfer to a stable free radical, and thus captures only a narrow aspect of oxidative stress mitigation (30). Similar findings have been reported in a plant-based extract, where higher total phytochemical content does not necessarily correspond to stronger antioxidant activity (DPPH) (31), suggesting that compound structure, maturity, and synergistic interactions may influence antioxidant behavior in ways that are not fully captured by the DPPH assay.

In biological systems, antioxidant protection is mediated through multiple complementary mechanisms beyond direct radical neutralization. These include the interruption of oxidative chain reactions, particularly during lipid peroxidation, as well as the chelation of pro-oxidant transition metal ions such as iron and copper. By sequestering these metal ions, antioxidants can suppress the generation of reactive oxygen species at an early stage, thereby reducing oxidative burden indirectly. Within this context, nutrients play a fundamental role in maintaining redox homeostasis. Rather than functioning solely as radical scavengers, nutrients contribute to antioxidant defense as structural components, enzyme cofactors, and modulators of endogenous antioxidant systems.

Accordingly, the antioxidant contribution of spirulina powder should not be interpreted solely through DPPH-derived IC_{50} values. As a single electron-transfer based assay, DPPH captures only one mechanistic aspect of antioxidant activity and may underestimate the broader functional relevance of complex bioactive matrices (32).

CONCLUSION

This study provides compositional and antioxidant characterization of locally produced Spirulina (Arthrospira sp.) powder from Central Java, Indonesia. The powder was characterized by 56% protein content, moderate lipid levels, low moisture content, and appreciable concentrations of functional pigments, including phycobiliproteins, chlorophyll, and β-carotene. DPPH analysis indicated a measurable radical scavenging activity, with an IC_{50} value of approximately 396 ppm under the applied assay conditions. These findings contribute baseline data on the nutritional composition and chemical antioxidant capacity of locally produced spirulina powder.

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DECLARATION OF THE USE OF AI

The authors declare that no artificial intelligence (AI), AI-assisted technologies, or large language models (LLMs) were used in the conception of the study, data analysis, or the drafting, writing, and editing of this manuscript. The only exception is the graphical abstract, which was created using the design platform Illustrae (https://illustrae.co/). The authors take full responsibility for the content and accuracy of the graphical abstract and the entire manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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