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Quantitative Prediction of *Spirulina platensis* Biomass Using UV-Vis Spectrophotometry

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Abstract

Spirulina platensis has become a promising feedstock for the synthesis of several industrially important biomolecules, including proteins, lipids, and carotenoids. However, significant technological obstacles pertaining to optimization, growth monitoring, and biomolecule extraction in *Spirulina* remain despite advancements in industrial-scale microalgae production and biomolecule harvesting. Standard techniques used for microalgal biomass and biomolecules monitoring include FTIR spectroscopy, colorimetric techniques, and manual cell counting. However, these techniques have drawbacks, particularly processing time and handling errors. This paper seeks to establish an operational equation that effectively relates measured absorbance (or optical density, OD) to the dry weight of *Spirulina platensis* microalgae using a UV-Vis spectrophotometer. The wavelengths of 680, 750, and 565 nm were selected based on the absorption spectrum of chlorophyll-a, as well as the wavelengths at which absorbance does not reach its peak. The best results were obtained at a wavelength of 680 nm with the equation $y = 1.2759x + 0.1512$, with an R^2 value of 0.9914. This technique allows for more accurate measurement of *Spirulina platensis* dry weight and total biomass.

1. Introduction

Indonesia is a biodiversity hotspot of considerable magnitude, harboring an extensive array of microalgae species. According to Guiry and Guiry (2026), the country of Indonesia is home to 2,060 species of algae, encompassing both microalgae and macroalgae. These species exhibit high adaptability and flourish across a wide range of ecosystems, including freshwater and seawater environments. Microalgae, which span the spectrum of eukaryotic and prokaryotic organisms and measure between 2 and 50 mm, exhibit remarkable capabilities in photosynthesis (Baweja & Sahoo, 2015; Elisabeth et al., 2021). Microalgae play a critical role in Indonesian inland water ecosystems (rivers, lakes, wetlands) by serving as the foundation of the food chain as primary producers, and oxygenating water through photosynthesis. With a species count exceeding 2,060, they function as vital bioindicators, facilitating the monitoring of water quality, heavy metal bioremediation, and nutrient cycling in diverse habitats (Goher et al., 2016; Rinanti et al., 2022; Mahlangu et al., 2024). Apart from functioning as the primary producer in aquatic ecosystems, the microalga's substantial biomass represents a valuable reservoir of carbon compounds. These compounds have significant applications in the production of biofuels, health supplements, and beauty products. In biotechnological and nutraceutical applications, several common microalgal species are employed. These include *Spirulina platensis/maxima (Arthrospira), Chlorella vulgaris, Dunaliella salina,* and *Haematococcus pluvialis* (Elisabeth et al., 2021; Tiwari et al., 2026).

Spirulina platensis is a microscopic, filamentous cyanobacterium rich in protein. Promoted as a "superfood," it contributes to high energy and contains special pigments called phycocyanin that can be used as dyes. These microalgae can be cultivated in various ways, either outdoors in open tanks or indoors in photobioreactors (Soni et al., 2017). The careful management of microalgal cultures is paramount within

the aquaculture sector to maintain a reliable and high-quality food source. Timely adjustments to process conditions, including light intensity, pH, temperature, and nutrient levels are crucial for preventing costly downtime and production failures (Nyakundi & Cleophas, 2021). Therefore, accurately measuring *Spirulina's* biomass is critical for ecological, commercial, and research purposes (Schagerl et al., 2022). Similar results were highlighted by the existing literature on microalgal biomass and/or estimation (Podevin et al., 2017; Liu et al., 2019).

Researchers have applied several techniques in this regard; however, each has its own sets of merits and demerits as outlined below:

- Cell counting by hemocytometry is a process that necessitates the expertise of a professional scientist. This method involves the manual enumeration of cells within a hemocytometer under the guidance of a microscope. Although widely utilized, hemocytometer cell counting is restricted to small-scale operations due to its labor-intensive and time-consuming methodology (Malletzidou et al., 2024). Additionally, counting personnel's divergent perspectives may introduce inconsistencies in measurement (Heriberto et al., 2018). This phenomenon is particularly evident in cultures exhibiting cellular clumps (Caprio, 2020).
- To measure cellular components such as proteins, lipids, carbohydrates, chlorophyll, and carotenoids, researchers typically employ established biochemical assays. These standard methods include Soxhlet extraction for lipids, the phenol-sulfuric method for total carbohydrates, and Kjeldahl digestion or colorimetric tests (like Lowry and Bradford assays) for proteins (Richmond & Hu, 2013). A major drawback of these protocols is that they require laborious sample preparation and result in the destruction of the analyte.
- FTIR spectroscopy provides an avenue to chemically characterize microalgae like *Chromochloris zofingiensis, Acutodesmus obliquus, Chlorella sorokiniana* (Bartošová & Blinová, 2015), and *Scenedesmus sp.* (Sudhakar & Premalatha, 2015). Conventionally, this method spatially records the distribution of macro-constituents through characteristic wavenumber peaks. However, subsequent investigations have attempted to move beyond qualitative detection by attempting to quantitatively measure the biomolecules present.

Attaining commercial viability necessitates the development of a streamlined and effective framework. Such a system must prioritize user accessibility, operational speed, stability, and selectivity while minimizing the frequency of required calibrations. The goal is to monitor cell growth in a simple, fast, stable, and selective manner to achieve commercial viability.

Evaluating optical density (OD) is a crucial method for successfully managing microalgae production, allowing operators to monitor population density and culture health in real time (Nielsen & Hansen, 2019; Nagabhushan, 2023). Measuring OD is useful for determining biomass concentration (Salgueiro et al., 2018; Dziosa & Makowska, 2016). Researchers frequently use spectrophotometers to estimate microalgal growth through OD tracking. This non-invasive, indirect method calculates biomass by directly linking OD values to cell density. Because the process easily integrates into automated systems, it is a highly practical tool for monitoring and controlling microalgae cultures. This study examined the ability to estimate *Spirulina platensis* biomass versus optical density using a UV-Vis spectrophotometer at various wavelengths, including 565, 680, and 750 nm. Researchers rely on specific light wavelengths to evaluate *Spirulina platensis* cultures. Specifically, biomass is often quantified using 565 nm wavelength (Santos-Ballardo et al., 2015). To determine the amount of *Spirulina* present, the 680 nm wavelength measures chlorophyll-a absorption, as the two are directly proportional (Hotos et al., 2020). Finally, because pigments do not absorb light at 750 nm, this wavelength is used to assess apparent turbidity through light scattering (Eddiwan et al., 2023).

2. Materials and Methods

2.1. Materials

Fresh, active *Spirulina platensis* culture was acquired from the membrane laboratory collection at BRIN-Limnology and Water Resources Research Center. The strain was axenically maintained under controlled laboratory conditions by inoculating 500 mL of a commercially purchased cyanobacterial starter culture into a 5 L transparent PTE plastic bottle, inoculated to 4 L of modified Zarrouk medium (Chrismadha et al., 2022). *Arthrospira platensis* was cultivated at 27.0 ± 0.5°C temperature and pH 9.4 under constant aeration of ~3 L/min. The culture was illuminated continuously with an 18W full-spectrum LED array (Philips 18W, Holland) providing 2,000–3,000 lux and a photoperiod of 24 h light: 0 h dark. Following three weeks of growth, the wet biomass was harvested from the clear PTE container. *S. platensis* culture was observed microscopically using a 40x objective lens (Olympus CKX53 Inverted Microscope). Observations were made on the first and last days of the experiment.

2.2. Methods

The wet culture was air-dried until a dry culture with a constant weight was obtained. Because standard reference materials were unsuitable for creating a calibration curve between *S. platensis* biomass and spectral response, we used a modified serial dilution technique based on standard addition (Malletzidou et al., 2024). The dried *S. platensis* culture was weighed at 0.01 grams to 0.4 grams, as described in Table 1, and was employed as the stock solution to create ten dilution levels that served as the calibration curve's calibration

standards. Using a volumetric flask, these dilution levels were created by combining volumes of the dried S. platensis culture with distilled water up to 50 mL. All dilution levels were performed in three replicates.

A Shimadzu UV-1800 UV-visible spectrophotometer (Shimadzu, Japan) was used to conduct spectrophotometric measurements. To determine the wavelength to be chosen in the O.D.-cell density regression model, the absorption spectrum of *Spirulina platensis* in the 400-800 nm range was first examined to identify the absorption peaks. According to the APHA method, the best wavelengths to measure O.D. in microalgae cell density calculations are 750, 664, 647, and 630 nm (APHA AWWA, 2017). Since 750 nm is a wavelength that is significantly longer than the absorbance peak of the chlorophylls in every species we looked at, we chose it for every species. We selected 680 nm and 565 nm for *Spirulina platensis* to target the maximum absorbance of chlorophyll-a and the combined accessory pigments (phycobilin and carotenoids) unique to cyanobacteria, respectively. Distilled water blank was used for baseline comparison. Data analysis was conducted in Microsoft Excel to determine the slope, coefficient of determination (R^2), and standard deviation for both wavelengths.

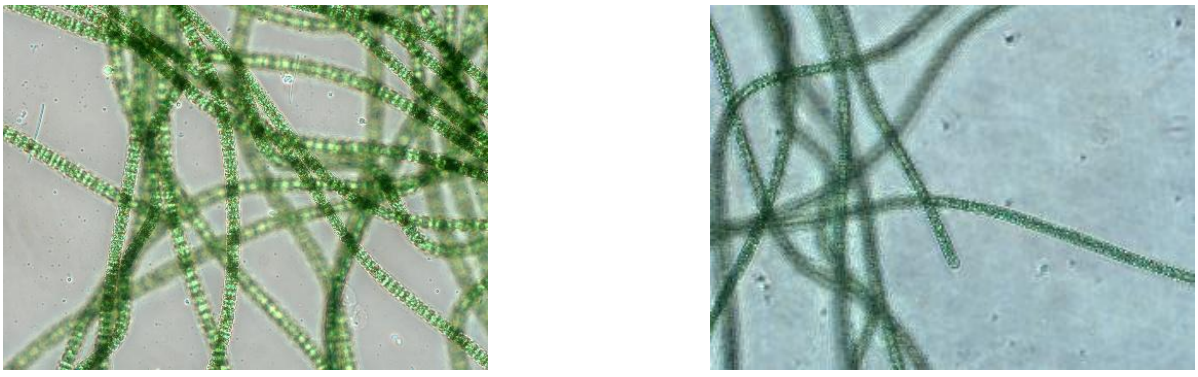
Table 1. Modified serial dilution of *S. platensis* for preparing standard calibration curve

Calibration Standard	Dried *S. platensis* Culture (g)	Distilled Water (mL)
0	0.01	50
1	0.02	50
2	0.03	50
3	0.05	50
4	0.075	50
5	0.1	50
6	0.125	50
7	0.15	50
8	0.2	50
9	0.4	50

3. Results And Discussion

Spirulina platensis has been viewed under the 40x lenses of a microscope before and after cultivation, and *Spirulina* species were identified based on their spiral structure morphology. The images from 40x magnification of *Spirulina platensis* cultured in modified Zarrouk medium are shown in Figure 1.

Microscopic analysis showed a high-density *Spirulina* culture with a single strain before and after cultivation. The *Spirulina* cells exhibited uniformity both prior to and following cultivation. Additionally, the absence of bacteria or other foreign organisms in the culture was noted. This finding suggests that the Spirulina culture utilized in this study was free from contamination (Barth et al., 2025; Tavˇ & Dolinar, 2025).



(a) (b)

Figure 1. Microscopic image under 40X magnification of *Spirulina platensis* culture before (a) and after cultivation (b) in modified Zarrouk medium.

The result of the measurements is tabulated in Table 2. Table 2 presents the correlation results between microalgal biomass (measured as dry weight) and OD at 565 nm wavelengths. The data for 565 nm (in the green/yellow range) were chosen because this wavelength is commonly found to provide the optimum balance for detecting biomass while minimizing error from pigment variability (Wacogne et al., 2024). Besides that, the 565 nm wavelength is primarily due to the scattering of light by the cell volume, not the variable pigment concentration inside the cells, and can provide better sensitivity in certain turbidity ranges (Myers et al., 2013; Nanni, 2023).

Table 2. Obtained absorbance values at 565 nm for the prepared *Spirulina* serial dilutions.

Cell Density (gram/Liter)	Optical Density (OD_{565})	Standard Deviation (SD)
0.2520	0.287	0.00289
0.5000	0.488	0.00321
0.7540	0.779	0.00755
1.0040	0.885	0.00723
1.5060	1.210	0.00351
2.0000	1.372	0.00971
2.5060	1.551	0.00252
3.0120	1.666	0.00436
4.0000	1.922	0.00058

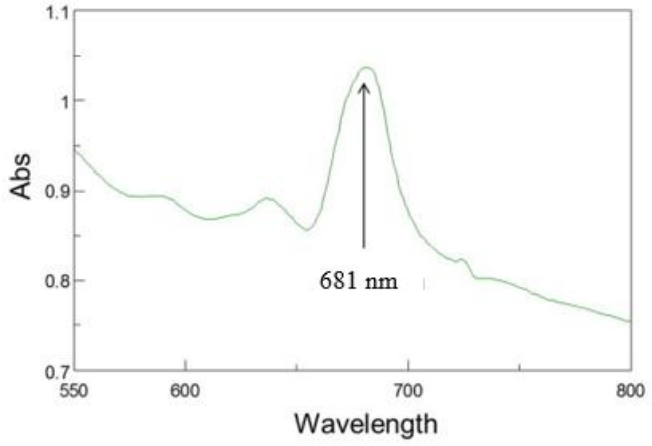


Figure 2. Pattern of light absorbance for a solution with *Spirulina platensis* screened between 400 and 800 nm.

Figure 2 shows the absorption spectrum of the *Spirulina platensis* microalgae culture in the 400–800 nm range, which exhibits a prominent peak at approximately 680 nm. These results indicate that the absorption peak corresponds to the composition of cell pigments and represents the maximum absorbance of chlorophyll a. Photosynthetic organisms, such as *Spirulina platensis*, have accessory pigments in their chloroplasts that are essential for photosynthesis because they capture light. Important accessory pigments include chlorophyll (chlorophyll a and c for cyanobacteria such as *S. platensis*), carotenoids, and phycobilins (Hotos et al., 2020; Dziosa & Makowska, 2016). In addition to capturing light, these pigments give organisms their specific colors.

The absorbance of all dilution levels at all wavelengths (565; 680; and 750 nm) were used to create a standard curve and determine the experimental parameters, including the detection and upper limits and the linear curve. Figure 3 presents the optical density measurement results.

Figure 3 shows that the optical density (OD_{750}) reading begins to reach saturation at a dry weight of *Spirulina* greater than 2 g/L. This is evident from the absorbance value, which approaches a linear line. Therefore, it is not advised to measure samples with an absorbance greater than 3.0 for accurate quantitative measurements since they may be more prone to error (Mori et al., 2021; Phansi et al., 2022). Therefore, data above 2 g/L is excluded from the standard curve.

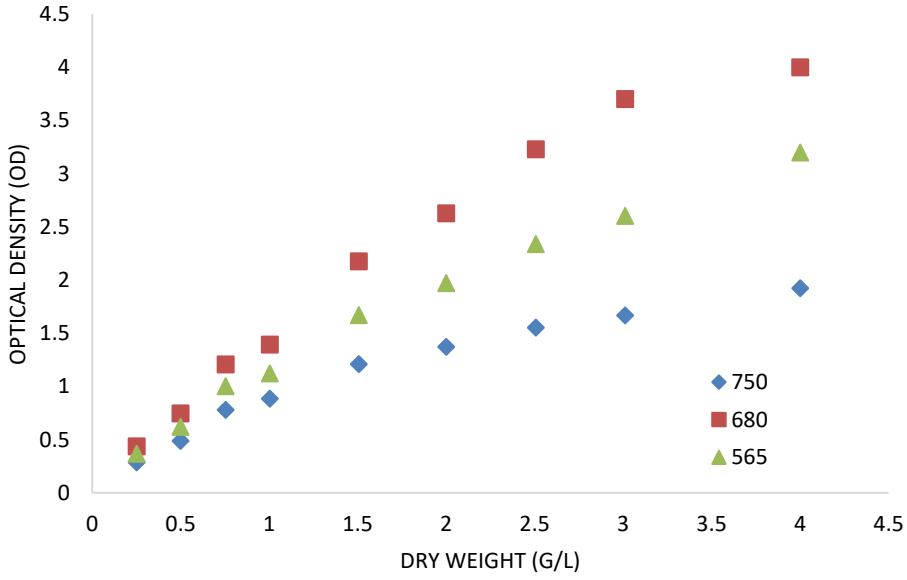


Figure 3. Measured optical density at various dry weight concentrations.

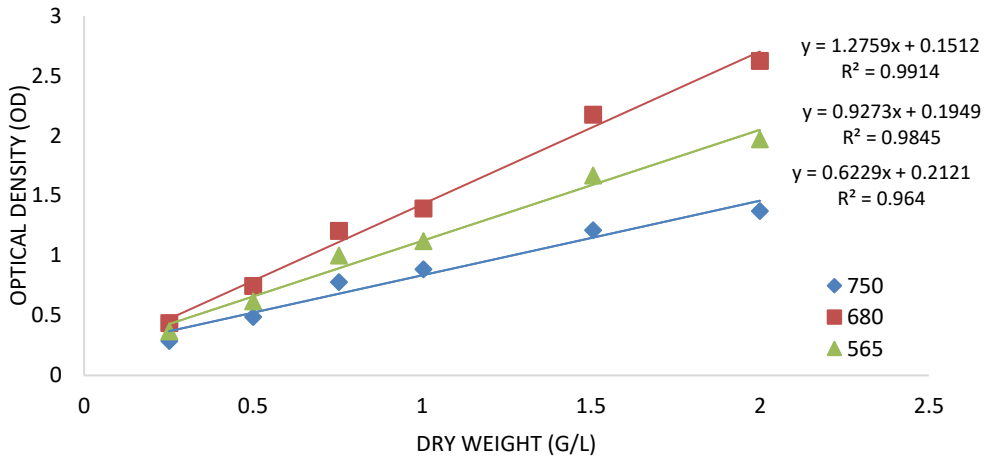


Figure 4. Standard curve for biomass of *Spirulina* using spectrophotometer at 565 nm, 680 nm and 750 nm.

Figure 4 shows that there is a change in absorbance value for every change in *Spirulina's* dry weight at all positive wavelengths. This indicates that a change in *Spirulina's* weight will correspond to a change in absorbance value. The correlation coefficient (R^2) varies between 0.96 and 0.99 (close to 1). The high R^2 value indicates that the linear model is a good representation of the variation of the two variables. The highest R^2 value was found when *Spirulina* was observed at a wavelength of 680 nm. Therefore, from the result of this study, cell weight measurements can be more accurately performed using a 680 nm wavelength spectrophotometer.

The results of this study are similar to those of a previous study that used Photopette® (Yap et al., 2018). Another study by Hotos et al (2020) mentioned using a wavelength of 680 nm to estimate cell density in *Nephroselmis* (green microalgae) and *Amphidium* (dinoflagellates). In several studies, the 680 nm wavelength was used to measure cell concentration while being sensitive to pigment content (chlorophyll) (Pahija & Hui, 2019; Katam et al., 2022). Chlorophyll demonstrates a direct proportionality to growth (cell weight) under conditions unaffected by external factors, such as light stress, temperature changes, or the nutrient content of the medium (Hotos et al., 2020; Fakhri et al., 2021; Eddiwan et al., 2023).

The utilization of the OD 680 wavelength can be contemplated in the assessment of cell weight, contingent upon the substantiation that these factors do not contribute to the chlorophyll content in algae cells (Grif et al., 2011; AlFadhly et al., 2022; Eddiwan et al., 2023). However, its application is constrained to the period of logarithmic or exponential growth (Ferreira & Sant, 2017; Nielsen & Hansen, 2019). Following the entry of cells into the stationary phase, there is an accumulation of metabolites, leading to a decrease in chlorophyll content despite an increase in cell weight (Aparicio et al., 2022).

The limit of detection (LOD) for this UV-Vis spectrophotometer measurement is derived from the equation below, accounting for both the variance in blank readings and empirical data:

$$\mathrm{LOD} = \frac{3 \times \mathrm{SD}_{\mathrm{blank}}}{\mathrm{slope}_{\mathrm{standard\ curve}}}$$

It is imperative to ascertain the LOD of a UV-Vis spectrophotometer when undertaking calculations pertaining to algae growth, as this parameter delineates the minimum cell concentration that can be reliably discerned from the blank (i.e., the medium). Absent a clearly delineated LOD, it becomes unfeasible to differentiate between the onset of genuine growth and baseline noise, resulting in imprecise growth rates (Agberien & Örmeci, 2020; Malhotra & Ormeci, 2023).

The blank measurements with seven repetitions had a standard deviation of 0.001. The limit of detection for dry mass using a spectrophotometer and the aforementioned equation was therefore as follows.

for 565 nm:

$$\mathrm{LOD} = \frac{3 \times 0.001\ \mathrm{OD}}{(0.9273\ \mathrm{OD/gram\ per\ Liter})}$$

$$\mathrm{LOD} = 0.003\ \mathrm{gram\ per\ Liter}$$

for 680 nm:

$$\mathrm{LOD} = \frac{3 \times 0.001\ \mathrm{OD}}{(1.2759\ \mathrm{OD/gram\ per\ Liter})}$$

$$\mathrm{LOD} = 0.002\ \mathrm{gram\ per\ Liter}$$

for 750 nm:

$$\mathrm{LOD} = \frac{3 \times 0.001\ \mathrm{OD}}{(0.6229\ \mathrm{OD/gram\ per\ Liter})}$$

$$\mathrm{LOD} = 0.005\ \mathrm{gram\ per\ Liter}$$

Our research results show that the 680 nm wavelength produces the smallest LOD value compared to the 565 nm and 750 nm wavelengths. This value indicates that sample measurements at the 680 nm wavelength are more sensitive and capable of measuring smaller cell weights (Taleuzzaman, 2018). The lowest cell weight measurable at 680 nm wavelength is 0.002 grams

per liter. If the cell sample weight is below this value, the measurement cannot be trusted.

The case below perfectly demonstrates the application of this for *Spirulina platensis* biomass calculation. The measured absorbance of the untested sample at 565 nm is 0.818. Using the linear regression analysis formula y = 0.9273x + 0.1949 and rearranging it to x = (y - 0.1949) / 0.9273, the calculated biomass is 0.641 grams per Liter. The measured biomass of the sample clocks at 0.641 grams per Liter. Similar calculation approach is used for 680 and 750 nm. In instance of known total culture volume (e.g., six 120 Liter culture barrels), the total expected dry biomass for the *Spirulina* harvest can be calculated. For example, if the total volume is 720 Liters (6 x 120 Liters), multiplying by 0.641 g/L yields a total dry weight of 461 grams. The wet biomass of the harvested microalgae can be calculated using the typical 90% moisture content of algal cells, which is ~10 times greater than the dry mass.

Although Optical Density (OD) is a widely used, fast, and cost-effective method for monitoring microalgae growth, this method has weaknesses in predicting dry weight accurately. Specifically, changes in environmental conditions (e.g., nutrient limitation, high emission light, or aging) can cause cells to change their size, shape, and internal structure (Hotos et al., 2020; Malhotra & Ormeci, 2023). Additionally, microalgae growth can reach a stationary phase, which also complicates accurate prediction (Aparicio et al., 2022). Consequently, there is an imperative for an alternative method that can be integrated with OD to predict the weight of microalgae biomass with speed, precision, and reliability, obviating the necessity for frequent calibration regulations.

4. Conclusion

A simple linear model describes the close relationship between cell density and absorbance values as a function of the diversity of spectrophotometer types and wavelengths used. A spectrophotometer with an optical density (OD) of 680 nm is a practical device for estimating the cell density of cyanobacteria, such as *Spirulina platensis*. The dry mass and anticipated total biomass of a *Spirulina* harvest can be measured in a matter of seconds using the technique outlined above. The experiment is inexpensive and simple to carry out. However, due to the shortcomings of this approach, more study is needed to find a different technique that can be combined with OD to quickly, accurately, and reliably forecast the weight of microalgae biomass, eliminating the need for regular calibration regulations.

5. Conflict of Interest

Each author has declared that there is no conflict of interest in the writing or submission of this manuscript.

6. Acknowledgement

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7. Author Contribution

All authors have made significant contributions to this work. **FSL** conceptualized and designed the study, conducted data analysis, and drafted the manuscript. **AZ** provided critical revisions and experiments. **AS** assisted in the interpretation of results and contributed to the final manuscript revision. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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