Bioactive Properties of Commercially Cultivated *Spirulina platensis* in Indonesia: Evaluation of Anticancer and Antioxidant Potentials

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Abstract

Spirulina platensis is a commercially cultivated cyanobacterium known for its rich bioactive compounds, including phenolics, flavonoids, and phycocyanin, which contribute to its antioxidant and anticancer properties. While previous studies focused on laboratory-grown strains, this study evaluates the total flavonoid and phenolic content, antioxidant activity, and in vitro anticancer effects of ethanol extracts from two commercial manufacturers (MA and MB). Ethanoicl extracts were prepared via maceration, and total phenolics and flavonoids were analyzed using Folin–Ciocalteu and $AlCl_3$ colorimetric assays. Antioxidant activities were assessed using DPPH and FRAP, and anticancer effects were evaluated via MTT assay on A549, HeLa cells, and Chang cells. The antioxidant potential was assessed using DPPH and FRAP assays, while cytotoxicity against A549 lung and HeLa cervical cancer cells was determined via MTT assays. Results indicated that MB exhibited higher flavonoid (12.52 ± 0.23 mg QE.g^{-1} DW) and phenolic content (19.10 ± 0.66 mg GAE/g DW) than MA, correlating with stronger antioxidant activity (FRAP: 16.55 ± 0.10 µmol TE.g^{-1} DW vs. 11.10 ± 0.29 µmol TE.g^{-1} DW). DPPH inhibition reached 80.33% (MA) and 77.77% (MB). MB demonstrated greater anticancer activity, with 28.31% inhibition in A549 cells at 100 ppm, while MA's 20.60%, and 33.85% inhibition in HeLa cells at 50 ppm. Both extracts displayed preferential toxicity toward cancer cells over normal Chang cells, supporting their potential as safer alternatives to chemotherapy. This study confirms the dual therapeutic potential of commercially cultivated S. platensis as an antioxidant and anticancer agent, highlighting its applications in pharmaceutical and nutraceutical industries. Further research is recommended to isolate and identify the bioactive compounds responsible for its cytotoxic effects.

Keywords: *A549 cancer cell line, doxorubicin, HeLa cell, microalgae, scaling up*

Introduction

Cancer continues to pose a significant worldwide health challenge, with an estimated 18.1 million new cases and 9.6 million deaths reported in 2018 (WHO, 2025). In Indonesia, cancer incidence reached 396,000 cases, resulting in 234,000 fatalities in 2020 (GLOBOCAN, 2020). Among various cancer types, cervical and lung cancers are particularly concerning. Cervical cancer is the fourth most common malignancy among women worldwide, with 604,000 new cases and 342,000 deaths recorded in 2020 (WHO, 2025). In Indonesia, it ranks as the second most diagnosed cancer in women, with 36,633 cases in 2020, primarily linked to persistent Human Papillomavirus (HPV) infections (Della Fera *et al.,* 2021; Karim *et al*., 2021). Lung cancer is the third most prevalent cancer globally, accounting for 2.21 million cases and reaches 1.80 million deaths in 2020 (WHO, 2025). Its pathogenesis is multifactorial, influenced by genetic predisposition and environmental exposures such as smoking and carcinogens (Oemiati, 2015).

Traditional cancer treatments, including surgery, radiotherapy, and chemotherapy, remain standard; however, these methods often face challenges such as non-selectivity, severe cytotoxicity to normal cells, and adverse side effects (Denny *et al*., 2015; Pratama and Nuwarda, 2018). Consequently, there is a growing interest in natural remedies, including marine product-based options like *Spirulina platensis* (Sirait *et al*., 2019). *S. platensis*, a

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cyanobacterium widely cultivated in Indonesia, is recognized for its rich composition of proteins, vitamins, minerals, and bioactive molecules such as carotenoids, flavonoids, C-phycocyanin (C-PC), and gamma-linolenic acid (GLA) (Jung *et al*., 2019; Ilhamdy *et al*., 2021). Notably, its high protein concentration, ranging from 60-71%, is easily absorbed and digested by the human body due to its soft cell wall.

Emerging evidence highlights the diverse health benefits of *S. platensis*, including its antioxidant, immunomodulatory, and anticancer properties (Afriani *et al*., 2015). Its bioactive compounds, particularly flavonoids and phenolics, play an important role in mitigating oxidative stress, a key factor in carcinogenesis (Pizzino *et al*., 2017). Studies on *S. platensis* extracts further demonstrate their potential to inhibit cancer cell growth, induce apoptosis, and selectively target malignant cells while sparing normal ones, indicating a promising therapeutic index (Subramaniam *et al*., 2021).

Spirulina platensis has garnered attention for its potential therapeutic applications, particularly in oncology. Flavonoids present in *S. platensis* have been shown to interfere with the HPV E6 oncoprotein, thereby restoring p53-mediated apoptosis in cervical cancer cells (Cherry *et al*., 2013). Additionally, phycocyanin, another bioactive compound in *S. platensis*, has been reported to induce apoptosis and suppress tumor progression in various cancer cell lines, such as A549 lung cancer cells (Muszynska *et al*., 2018).

Despite promising findings, most studies focus on laboratory-cultivated *S. platensis*, leaving a gap in research on commercially cultivated variants. Concurrent investigations into its anticancer effects on lung (A549) and cervical (HeLa) cancer cell lines remain limited. Nutritionally, *S. platensis* biomass contains ~47% protein by dry mass, surpassing soybeans (36–40%) (Ramírez-Rodrigues *et al*., 2021). However, large-scale cultivation and processing are costlier than traditional crops, potentially limiting commercial viability. Still, its high protein content may drive further cultivation efforts. Additionally, *S. platensis* is a valuable source of antioxidant peptides, with microbial fermentation emerging as an effective strategy to enhance their utilization (Wei *et al*., 2024).

Addressing the global burden of noncommunicable diseases (NCDs), including cancer, aligns with international efforts such as the WHO Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013–2030. This plan emphasizes the need for research into alternative therapeutic agents with improved safety profiles to reduce NCD-related morbidity and mortality (WHO, 2013).

Therefore, this study aims to evaluate the total flavonoid and phenolic content, antioxidant activity, and in vitro anticancer effects of *S. platensis* ethanol extracts from two commercial manufacturers, utilizing FRAP, DPPH, and MTT assays. The goal is to determine its potential as a dual-action therapeutic agent against cervical (HeLa) and lung (A549) cancer cells. This research contributes to the broader exploration of natural alternatives for cancer therapy by providing insights into the bioactivity of *S. platensis* sourced from commercial cultivation, thereby expanding its potential applications in pharmaceutical and nutraceutical fields.

Materials and Methods

Spirulina platensis samples were sourced from two manufacturers in Central Java, Indonesia: Manufacturer A (MA) and Manufacturer B (MB). MA employed a continuous cultivation system to maximize yield, while MB used controlled open-pond cultivation. Both samples were harvested per manufacturer protocols and prepared for analysis. Antioxidant assay reagents, including DPPH (1,1-diphenyl-2-picrylhydrazyl), TPTZ (2,4,6- tri(2-pyridyl)-s-triazine), and Trolox were obtained from Sigma Aldrich, while the $K_2S_2O_8$, $FeCl_3{\cdot}6H_2O$, and $FeSO_4{\cdot}7H_2O$, reagents were obtained from E. Merck.

Extraction of Spirulina (Modified from Safithri et al., 2022)

Spirulina extract was prepared using ethanol as a polar solvent at a 1:20 (b/v) ratio. Five grams of dried *Spirulina* was dissolved in 100 mL ethanol and extracted via maceration in a water bath for 3 × 24 hours, with filtration every 24 h. The filtrate was stored at 4 °C, while the residue was re-extracted with fresh ethanol. The combined filtrates were evaporated using a rotary evaporator at 40–48 °C and stored in vials under cold conditions for antioxidant and anticancer analyses against A549, HeLa and Chang cells.

Total phenolic test (Safithri et al., 2020)

To determine the total phenolic content, 20 µL of the *Spirulina* extract solution was mixed with 120 µL of 10% Folin–Ciocalteu reagent in a 96-well microplate and incubated in the dark for 5 min. Subsequently, 80 µL of 7.5% sodium carbonate (Na_2CO_3) was added, and the mixture was incubated for 30 min in the dark. Absorbance was measured at 750 nm using a microplate reader. A standard curve was constructed using gallic acid solutions ranging from 0 to 300 ppm, yielding the equation y = 0.002x - 0.0214 with an R^2 = 0.9942. Sample absorbance values were applied to this equation to determine phenolic concentrations, expressed as milligrams of

gallic acid equivalents per gram of dry weight (mg GAE/g DW). All measurements were conducted in triplicate to ensure reproducibility.

Total flavonoid content of Spirulina extract (Safithri et al., 2020)

For the total flavonoid content assay, 20 µL of the *Spirulina* extract solution was combined with 20 µL of 10% aluminum chloride ($AlCl_3$), 20 µL of 1 M potassium acetate (CH_3COOK), and 180 µL of distilled water in a 96-well microplate. The mixture was incubated at room temperature for 30 minutes. Absorbance was then measured at 415 nm using a microplate reader. Quercetin solutions (100–400 µM) were used to construct the standard curve, yielding the equation y = 0.023x - 0.0693 and an R^2 value of 0.982. Sample absorbance values were applied to this equation to calculate flavonoid concentrations, expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE.g^{-1} DW). To ensure reproducibility all measurements were performed in triplicate. The result was calculated as mg QE.g^{-1} DW extract using:

$$C = \frac{c \times V}{m} \times FP$$

Note: C= Total flavonoid content (mg QE.g^{-1} DW extract); c= Sample flavonoid concentration from the standard curve (mg.L^{-1}); V= Sample extraction volume (L); m= Sample mass (g); FP= Dilution factor.

FRAP antioxidant activity test

The FRAP reagent was freshly prepared by mixing 1 mM TPTZ solution (in 40 mM HCl), 20 mM $FeCl_3$ solution (in distilled water), and 0.3 M acetate buffer (pH 3.6) in a ratio of 1:1:10 (v/v/v). In a 96-well microplate, 10 µL of the *Spirulina* ethanol extract was combined with 300 µL of the FRAP reagent. The mixture was incubated at room temperature for 30 minutes in the dark. Absorbance was measured at 595 nm using a microplate reader. A standard curve was constructed using Trolox solutions at concentrations of 150, 200, 250, 300, 350, and 400 ppm, yielding the equation y = 0.0015x + 0.0347 and R^2= 0.99. Sample absorbance values were applied to this equation to calculate FRAP values, expressed as milligrams of Trolox equivalent antioxidant capacity per gram of dry weight (mg TE.g^{-1} DW). All assays were conducted in triplicate to ensure reproducibility.

DPPH antioxidant activity test (Safithri et al., 2020)

The antioxidant activity of *Spirulina platensis* extract was evaluated using the DPPH radical scavenging assay. A 250 µM DPPH solution was freshly prepared by dissolving DPPH in ethanol. In a 96-well microplate, 100 µL of *Spirulina* extract at various concentrations was mixed with 100 µL of the DPPH solution. The mixture was incubated at 37 °C for 30 min in the dark. Absorbance was measured using a microplate reader at 517 nm. A standard curve was constructed using ascorbic acid solutions at concentrations of 0, 5, 25, 30, 40, 50, and 75 ppm, yielding the equation $y = ax + b$. Sample absorbance values were applied to this equation to calculate the antioxidant capacity, expressed as milligrams of Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) per gram of dry weight (mg AAE.g^{-1} DW). All assays were conducted in triplicate to ensure reproducibility.

Cancer cell assay (Misgiati et al., 2024)

HeLa cells, sourced from the Primate Research Center (PSSP) at IPB University, were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. When the cells have covered the entire surface, the adherent monolayer was rinsed with PBS to remove residual medium. Trypsin-EDTA was then added to detach the cells, and subsequently the flask was incubated at 37 °C until cells were fully detached. The cell suspension was transferred to a centrifuge tube and centrifuged at a relative centrifugal force for 5 minutes. The resulting pellet was resuspended in fresh DMEM. Cells were seeded into a 96-well plate at a density of 5,000 cells per well and then incubated at 37 °C with 5% CO_2 for 24 h to allow for proper adherence and morphology development.

The MTT assay was performed to evaluate the anticancer activity of the *Spirulina* extract against A549 lung cancer cells and Chang cells. A549 cells were seeded into a 96-well plate at a density of 5,000 cells per well and incubated at 37 °C with 5% CO_2 for 24 h. Post-incubation, 50 µL of *Spirulina* extract, diluted in DMEM to concentrations of 0, 1, 10, 100, 200, 400, 600, 800, and 1000 ppm, was added to each well. Then the plate was incubated for an additional 48 hours. Subsequently, 10 µL of MTT reagent (final concentration 0.5 mg.mL^{-1}) was added to each well, and the plate was incubated at 37 °C for 4 h. Formazan crystals obtained by metabolically active cells were dissolved by adding 100µL of solubilization solution, and the absorbance was measured at 570 nm using a microplate reader. Doxorubicin served as a positive control, and untreated cells were used as a negative control. To ensure reproducibility all assays were conducted in triplicate. The percentage of cell inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100\%$$

Data analysis

Data were analyzed descriptively using Microsoft Excel and GraphPad, presented as graphs and tables with standard deviation.

Result and Discussion

Total flavonoid content

The total flavonoid content of *S. platensis* ethanol extracts was quantified using the aluminum chloride colorimetric method, with quercetin as the standard. At 5000 ppm, MB exhibited the highest flavonoid content (12.52±0.23 mg QE.g^{-1} DW extract), followed by MA (9.23±0.12 mg QE.g^{-1} DW extract) (Figure 1). The higher flavonoid concentration in MB suggests potential differences in bioactivity. Flavonoids, as secondary metabolites, have been reported to exhibit cytotoxic effects against cancer cells, promoting apoptosis (Abolateb *et al*., 2018). Notably, they may interfere with the HPV E6 oncogene, restoring p53-mediated cell cycle inhibition (Cherry *et al*., 2013).

The determination of total flavonoid content using aluminum chloride colorimetric assay ($AlCl_3$) method relies on the flavonoid standard employed in the quantitation process (Shraim *et al*., 2021). Quercetin was used as the reference due to its structural similarity and antioxidant properties. The flavonoid content difference of MA and MB *Spirulina* extracts was possibly due to variations in environmental factor, growth condition, and post-harvest handling (Rojsanga *et al*., 2020). Given their established antioxidant and anticancer potential, these findings reinforce the relevance of *S. platensis* in natural cancer therapies.

Total phenolic content

The total phenolic content of *S. platensis* ethanol extracts was determined using the Folin-Ciocalteu method, with gallic acid as the standard. At 5000 ppm, MA contained 18.915±0.46 mg GAE/g DW, while MB had a slightly higher 19.096±0.66 mg GAE.g^{-1} DW (Figure 1). These values suggest that *S. platensis* exhibits significant phenolic content, with minor variations likely due to differences in secondary metabolite profiles and agro-biophysical conditions (Nurcholis *et al*., 2016). Phenolic compounds, characterized by aromatic rings with hydroxyl groups, act as antioxidants by scavenging reactive oxygen species (Diniyah and Lee, 2020).

The extraction efficiency of phenolic compounds depends on factors such as solvent type, extraction method, duration, and particle size. Higher phenolic content correlates with greater antioxidant activity, reinforcing the potential therapeutic applications of *S. platensis*. A 70% ethanolic extract increased lymphocyte proliferation, with the highest viability observed at 20 ppm (124.89±1.84%), suggesting an immune-enhancing effect. Phenolic compounds in *S. platensis* may further support immune function by stimulating IL-2 production and CD4+ cell activity (Andrianto *et al*., 2024). These findings highlight the potential of *S. platensis* as a dual-action therapeutic agent, contributing to the broader exploration of natural cancer treatments through its antioxidant and immunomodulatory properties.

Antioxidant activity with FRAP assay

The antioxidant activity of *S. platensis* ethanolic extract was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay, with Trolox as the standard. At 5000 ppm, MB exhibited the highest antioxidant activity (16.55±0.10 µmol TE.g^{-1} DW extract), while MA had a lower value (11.1±0.29 µmol TE.g^{-1} DW extract) (Figure 2). This suggests that MB has a greater ability to mitigate oxidative stress, potentially due to its higher phenolic and flavonoid content.

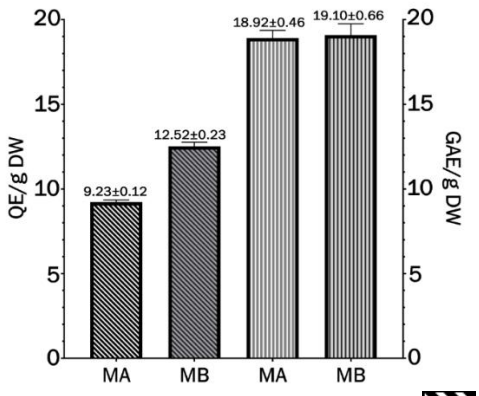

Figure 1. Total phenolic and total flavonoid content of *Spirulina platensis* extract. = total flavonoid ; = total phenolic

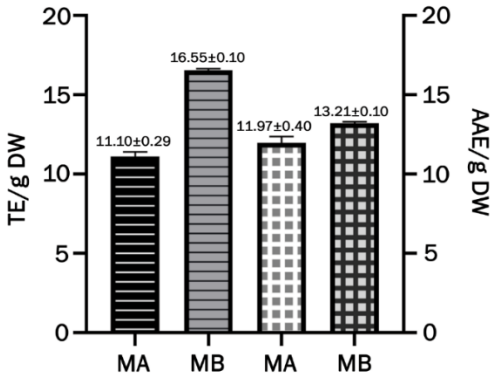

Figure 2. Antioxidant capacity of *Spirulina platensis* extract with FRAP and DPPH method. = FRAP ; = DPPH

The FRAP assay evaluates the ability of antioxidants to reduce a colorless Fe^{3+} to Fe^{2+}, resulting in a blue color change (Benzie and Strain, 1999). Trolox was used as a reference due to its high antioxidant capacity and water solubility (Kusumorini *et al*., 2021). The strong correlation between flavonoid content and FRAP values supports the role of flavonoids as electron donors in neutralizing free radicals (Nur *et al*., 2019). These findings reinforce *S. platensis*'s potential as a natural antioxidant source, highlighting its relevance in pharmaceutical and nutraceutical applications for reducing oxidative damage, a key factor in cancer progression.

Antioxidant activity with DPPH assay

The antioxidant activity test was also conducted using the DPPH method, with ascorbic acid as the standard. Figure 2 shows antioxidant activity measurement results for the 70% ethanolic extract was 11.97 ± 0.40 mg AAE.g^{-1} DW for MA and 13.21 ± 0.10 mg AAE.g^{-1} DW for MB. The inhibition percentage of the antioxidant activity of both extracts was 80.331% and 77.769% for MA and MB, respectively. The results align with the DPPH radical scavenging capacity of *Spirulina* ethanolic extract was 89.55% (El Said *et al*., 2024).

The antioxidant activity of *Spirulina* from two different manufacturers was slightly different. This possibly relates to the different culture condition or handling process. Aouir *et al.* (2017) stated that culture condition, harvesting and drying processes can lead to changes in the bioactive compounds in *S. platensis*. Drying and storage conditions also affect the volatile organic compounds of *Spirulina* (Ughetti et al., 2024). *Spirulina* has been a valuable source of volatile organic compounds for extracting beneficial compounds with potential applications in food, pharmaceutical, and cosmetics industries (Paraskevopoulou *et al.,* 2024).

Anticancer activity on A549 lung cancer cells and HeLa cervix cancer cells

Spirulina platensis extracts demonstrated strong antioxidant activity, effectively mitigating oxidative stress by neutralizing free radicals, a key factor in cancer progression (Misgiati *et al*., 2024). This study evaluated the anticancer potential of *S. platensis* against A549 lung cancer and HeLa cervical cancer cells.

The MTT assay assessed the effects of *S. platensis* extracts across eight concentrations. The highest inhibition was at 100 ppm, with MB (28.31%) outperforming MA (20.6%) (Figure 3). Even at 1 ppm, MB demonstrated greater inhibition (8.28%) than MA (3.2%). Morphological analysis at 100 ppm/48 hours confirmed substantial cell death (Figure 4).

Higher inhibition rates correlated with concentration but plateaued at specific levels, emphasizing the complexity of dose-response relationships (Komariah *et al*., 2022; Chimalakonda *et al*., 2023). The differences in efficacy between MA and MB were linked to active compounds, including phycocyanin, chlorophyll-a, carotenoids, and gamma-linoleic acid (GLA), known for their anticancer properties (Czerwonka *et al.*, 2018).

Doxorubicin, a widely used chemotherapy drug, exhibited 92.07% inhibition at 1 ppm (Figure 5), significantly higher than *S. platensis*. However, doxorubicin also induced substantial cytotoxicity in normal Chang cells, indicating low selectivity. *S. platensis*, in contrast, showed preferential inhibition of A549 cancer cells over normal cells, reinforcing its potential as a safer anticancer agent with minimal side effects (Czerwonka *et al.*, 2018; Baghban *et al*., 2020). Low cytotoxicity in Chang cells (Figure 7) further highlights its selective anticancer potential.

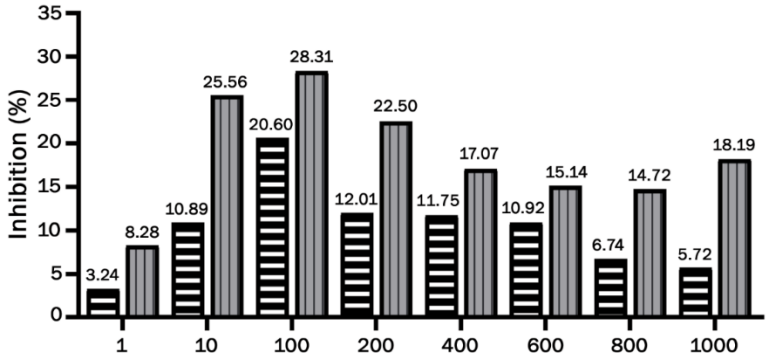

Figure 3. Inhibition of *Spirulina platensis* against A549 lung cancer cells. =MA; =MB

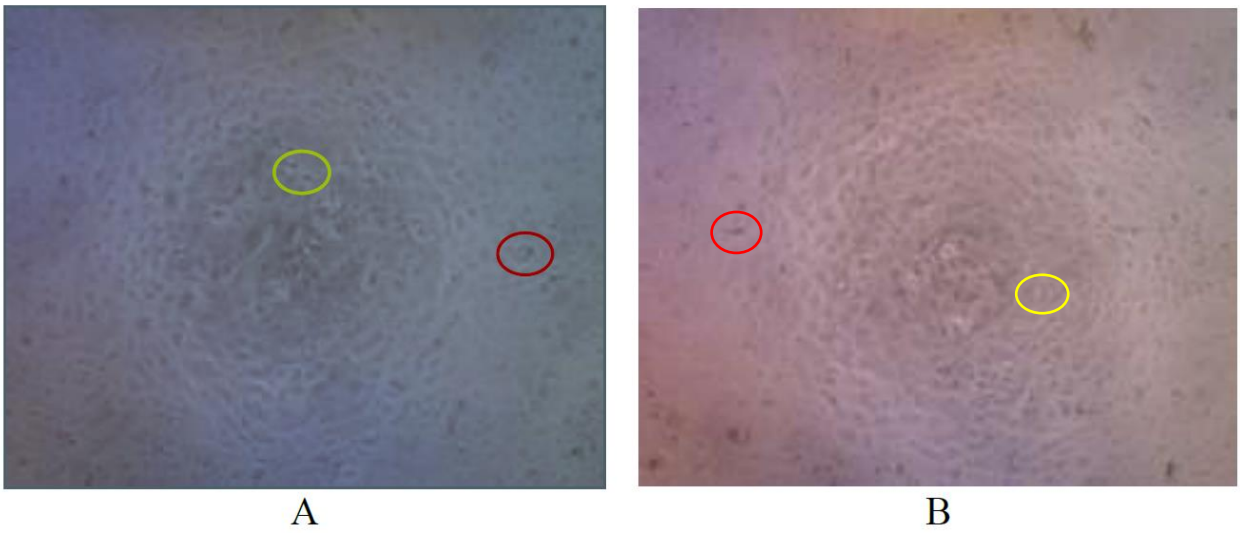

Figure 4. Morphology of A549 lung cancer cells after administration of *Spirulina platensis* (producers A and B) at 100 ppm and 48 hours of incubation (32x magnification). Yellow circle live cells; red circle dead cells.

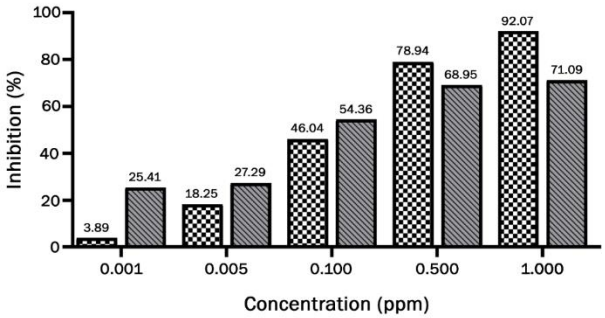

Figure 5. Inhibition of doxorubicin on lung cancer cells and Chang cells .

The MTT assay assessed *S. platensis*'s effects on HeLa cells, with data provided by Primate Research Center, IPB University (Figure 8). MA exhibited the highest inhibition (34.68%) at 200 ppm, while MB peaked at 50 ppm (33.85%). Doxorubicin (1 ppm) remained the most effective, reaching 70.70% inhibition.

Morphological analysis (Figure 9) showed significant differences before and after treatment. Live cells appeared yellow, while dead cells turned red, confirming dose-dependent cytotoxicity in both *S. platensis* samples and doxorubicin. The observed cell death markers, such as rounded, darkened cells, reinforced the anticancer effects (Andrianto *et al.*, 2024).

These findings *S. platensis* as a dual-action therapeutic agent with antioxidant and selective anticancer properties. While its inhibition rates were lower than doxorubicin, its reduced toxicity to normal cells makes it a promising natural alternative in pharmaceutical and nutraceutical applications. Further studies are needed to identify key bioactive compounds responsible for its anticancer activity. In addition, the anticancer potential of *Spirulina* extracts can be improved, such as using *Spirulina* nanoparticles (Drori *et al.,* 2025). Hussein *et al.* (2025) revealed that the preparation of *Spirulina* extract into nanoemulsion increased its delivery, stability and solubility which possibly makes more effective in cancer treatment.

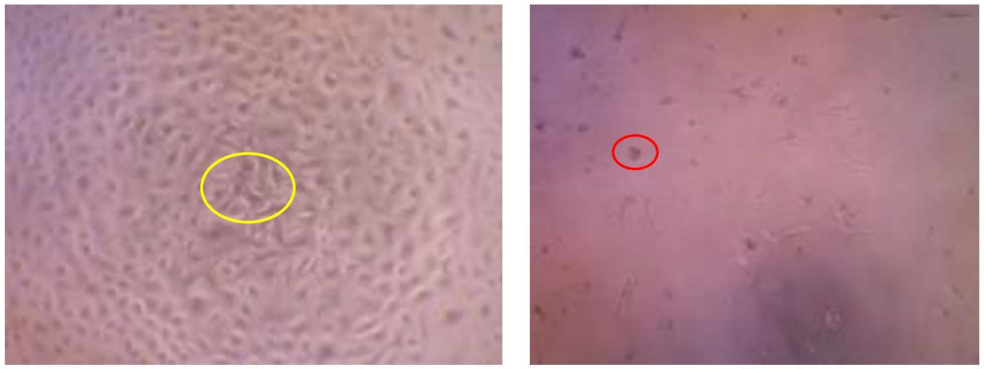

Figure 6. Morphology of A549 lung cancer cells (a) and Chang cells (b) after treatment with 0.005 ppm doxorubicin and 48 hours of incubation (32x magnification). Yellow circle= live cells; red circle= dead cells.

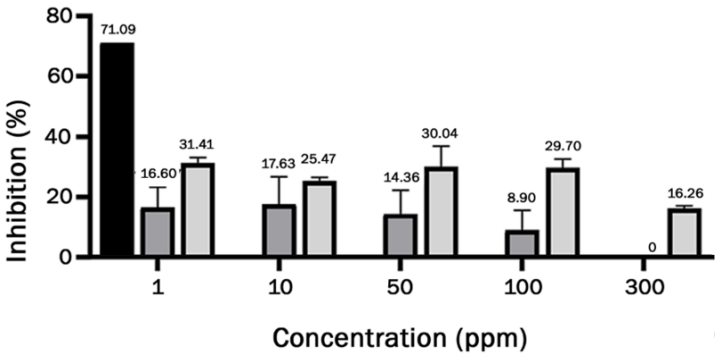

Figure 7 Cell inhibition in Chang cells by doxorubicin , *S. platensis* MA , and MB extracts .

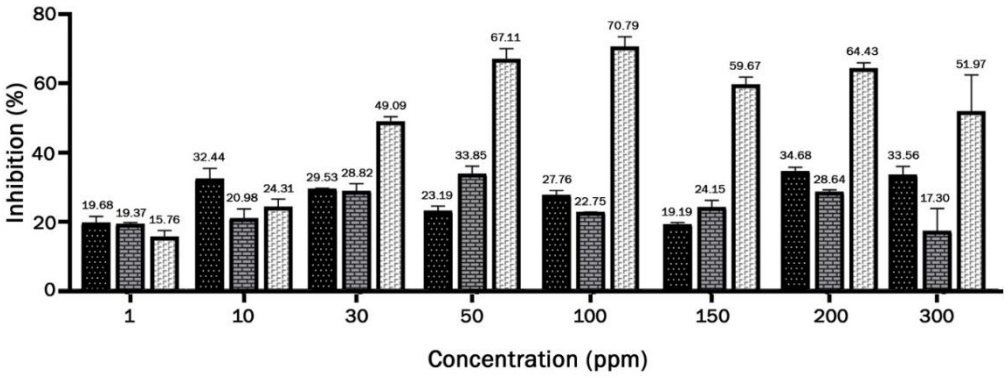

Figure 8. Percentage inhibition of cervical cancer HeLa cells by *Spirulina* extract from MA MB , and doxorubicin

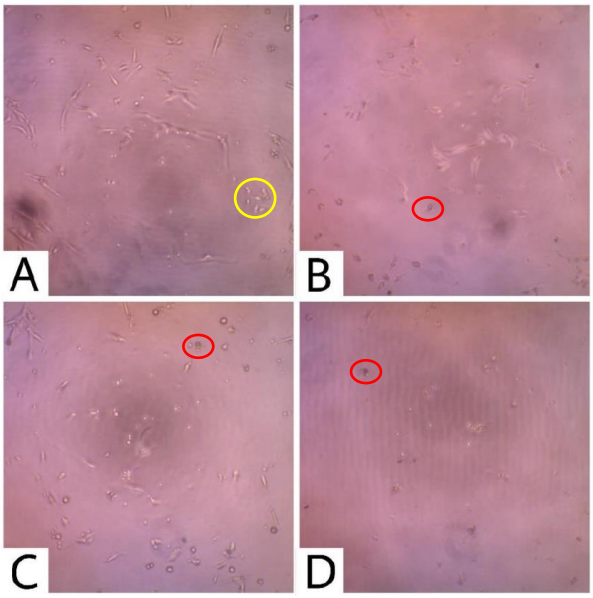

Figure 9. Morphology of cervical cancer HeLa cells: Control cells (A), addition of MA sample at 200 ppm (B), addition of MB sample at 200 ppm (C), and addition of doxorubicin at 1 ppm (D) (32x magnification). Yellow circle= live cells; red circle= dead cells.

Conclusion

This study demonstrated that commercially cultivated *Spirulina platensis* exhibits promising antioxidant and anticancer properties, making it a potential dual-action therapeutic agent. The ethanolic extracts from two manufacturers (MA and MB) varied in their total flavonoid and phenolic content, which directly correlated with their antioxidant capacities (FRAP and DPPH assays) and cytotoxic effects on A549 lung and HeLa cervical cancer cells. MB consistently exhibited higher antioxidant activity and anticancer efficacy compared to MA, likely due to differences in bioactive compound concentrations. Notably, both *S. platensis* extracts displayed selective cytotoxicity, inhibiting cancer cell proliferation while exerting minimal effects on normal Chang cells. This preferential action suggests *S. platensis* may serve as a safer alternative to conventional chemotherapy, which often lacks selectivity and induces severe side effects. While this study highlights the potential pharmaceutical and nutraceutical applications of *S. platensis*, further research is recommended to isolate and identify the bioactive compounds responsible for its cytotoxic effects. Understanding these key compounds will enhance the formulation of targeted anticancer therapies. Large-scale cultivation and processing of *S. platensis* remain costlier than traditional crops, but studies such as this may drive further cultivation efforts by demonstrating its significant therapeutic potential. Continued research into standardized extraction methods, bioactive compound stability, and mechanistic pathways will be crucial for advancing *S. platensis* as a viable natural alternative in cancer treatment.

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