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Differential Effects of Blue and Green Light Conditions on the Antimicrobial Metabolite Profile of *Arthrospira platensis*

Aynaz Gharajeh[1], Aryan Sateei[1, 2*], Shadman Shokravi[1], Hamid Reza Pordeli[1, 2], Mazyar Ahmadi Golsefidi[2,3]

1. Department of Biology, Go.C., Islamic Azad University, Gorgan, Iran.
2. Medicinal Plants Research Center, Go.C., Islamic Azad University, Gorgan, Iran.
3. Department of Chemistry, Go.C., Islamic Azad University, Gorgan, Iran.

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ABSTRACT

Background: The escalating global threat of antimicrobial resistance underscores the critical need for novel antimicrobial agents. This study aimed to investigate the effect of different light spectra on the biosynthesis of bioactive compounds and the consequent antimicrobial efficacy of propanolic and butanolic extracts from the cyanobacterium *Arthrospira platensis* (Spirulina).

Materials and Methods: *A. platensis* was cultured for 46 days in Zarrouk›s medium under illumination with specific LED light spectra (white, yellow, red, blue, and green). The resulting algal biomass was subsequently extracted using propanol and butanol. The bioactive metabolites within these extracts were identified by gas chromatography-mass spectrometry. The antimicrobial activity of the extracts was evaluated against two gram-positive (*Bacillus cereus, Staphylococcus aureus*) and two gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacterial pathogens using the disk diffusion assay and broth microdilution to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Results: Our results demonstrate that light quality significantly modulates metabolite production and antimicrobial potency. Green light irradiation markedly enhanced the production of known antimicrobial compounds, including bis(2-ethylhexyl) phthalate (DEHP) and phytol, and induced the most potent activity against *B. cereus*. The butanolic extract from green light-treated cultures exhibited exceptional efficacy against *B. cereus* (inhibition zone of 18.67 mm, MIC=8 µg/mL, MBC=16 µg/mL). Conversely, blue light treatment yielded a more diverse metabolome (137 compounds identified), with its propanolic extract showing only modest or no detectable activity against the tested Gram-negative pathogens (MIC≥1024 µg/mL for *E. coli*).

Conclusion: These findings establish light spectrum manipulation as a powerful, non-invasive strategy to selectively enhance the production of antimicrobial secondary metabolites in A. platensis, highlighting its potential as a tunable source for natural antibiotic discovery.

*** Corresponding Author:**
Aryan Sateei, Assistant Professor.
Address: *Department of Biology, Go.C., Islamic Azad University, Gorgan, Iran.*
Phone: *+98 (910) 1719185*
E-mail: *Aryan.Sateei@iau.ac.ir , saateyi@gmail.com*

Introduction

The escalating crisis of antimicrobial resistance not only complicates the direct treatment of bacterial infections but also profoundly disrupts the intricate balance between the invading pathogen and the host's immune system [1]. Multidrug-resistant pathogens, particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa*, have evolved sophisticated mechanisms to evade both antibiotic action and immune clearance, often triggering dysregulated and hyper-inflammatory host responses that contribute significantly to disease pathogenesis and tissue damage [2, 3]. This dual challenge—resolving the infection while mitigating collateral immune-mediated injury—has intensified the search for therapeutic agents that possess either direct antimicrobial activity, immunomodulatory properties, or ideally, both [4]. Natural products derived from microalgae and cyanobacteria have emerged as promising candidates in this context, not only for their well-documented antimicrobial potential but also for their capacity to modulate innate and adaptive immune pathways [5]. Extensive reviews have highlighted that phytoconstituents, including terpenoids, alkaloids, and polyphenols, exhibit significant immunomodulatory activities by regulating inflammatory mediators and immune cell function, making them valuable candidates for combating infectious diseases complicated by dysregulated immune responses [6, 7]. Plant-derived bioactive compounds, including terpenoids, fatty acid amides, and phthalate derivatives, have been shown to interfere with key inflammatory signaling cascades, such as the nuclear factor kappa-B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, thereby reducing the excessive production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) [8, 9]. Furthermore, certain secondary metabolites can enhance the phagocytic activity of macrophages and modulate the differentiation of T-helper cell subsets, thereby promoting a more balanced and effective immune response against persistent infections [10]. Because the biosynthesis of these immunologically active metabolites is highly sensitive to environmental cues, particularly light quality, manipulating cultivation parameters in photosynthetic organisms offers a non-invasive strategy to steer the production of compounds with dual antimicrobial and immunomodulatory functions [11]. Therefore, understanding how specific light spectra influence not only the antimicrobial but also the immunopharmacological potential of cyanobacterial extracts is crucial for developing next-generation therapeutic strategies against AMR-associated infectious diseases [12].

Arthrospira platensis (commonly known as Spirulina), a filamentous, photosynthetic cyanobacterium, is renowned for its nutritional value and richness in compounds such as phycobiliproteins, carotenoids, and essential fatty acids [13]. Beyond its nutritional applications, numerous studies have revealed its potential for producing antimicrobial agents [14, 15]. According to established theory, abiotic stressors, particularly light quality and intensity, can act as powerful elicitors by regulating key biosynthetic pathways such as the methylerythritol phosphate (MEP) pathway for isoprenoids (e.g. phytol) and fatty acid synthesis (FAS) [16, 17].

The strategic use of light quality to manipulate the metabolic profile and thereby enhance antimicrobial efficacy remains underexplored. Furthermore, comparative studies on the efficacy of different extract types (e.g. propanolic, butanolic) against a panel of both Gram-positive and Gram-negative pathogens, under standardized and comparable conditions, are limited. Other limitations in the existing literature include a frequent lack of reporting precise quantitative data (e.g. MIC values), making cross-study comparisons difficult, and a lack of discussion regarding the implications of identifying compounds with known toxicity concerns [18, 19]. Therefore, the present study was designed to address these research gaps by systematically investigating the hypothesis that light quality is a key determinant of the antimicrobial metabolite profile in *A. platensis*.

Materials and Methods

Microalgal strain and cultivation conditions

The cyanobacterium *A. platensis* was acquired as a pure, axenic culture from the SpiruPlas Company (Gonbad Kavos-Iran). The strain was cultivated in standard Zarrouk's medium [20]. The culture was maintained in 500 mL Erlenmeyer flasks containing 250 mL of medium at 25–35 ˚C. Medium pH was adjusted to pH 8 at the beginning for all treatments.

Experimental light treatments

The cultures were subjected to five different light treatments using LED lamps: White, yellow, red, blue, and green. All light conditions were calibrated to a uniform photon flux density of 50 µmol photons m^{-2} s^{-1} at the surface of the flasks, with a continuous (24:0) light-dark cycle. The cultivation period was 46 days.

Growth monitoring and biomass harvesting

Algal growth was monitored regularly by measuring the optical density at 750 nm (OD_{750}) using a UV-Vis spectrophotometer (Shimadzu, Model UV-1800, Japan). Following the 46-day cultivation period, the entire content of each 250 mL algal suspension was harvested by centrifugation. The pellet was weighed to determine the total fresh biomass yield per culture.

Sequential extraction of bioactive compounds

The harvested fresh biomass was immediately subjected to a sequential extraction protocol to isolate compounds of varying polarities. First, the biomass was extracted with 50 mL of 96% ethanol (polar solvent) for a week at 4°C. The mixture was centrifuged at 4000×g for 15 min, and the supernatant (ethanolic extract) was collected. The remaining pellet was then subjected to a second extraction with 20 mL of propanol (intermediate polarity) for a week at 4 ˚C. After centrifugation under the same conditions, the supernatant (propanolic extract) was collected. Finally, the residual pellet was extracted with 20 mL of butanol (less polar solvent) for a week at 4 ˚C. The supernatant (butanolic extract) was collected after centrifugation. All supernatants were concentrated under reduced pressure using a rotary evaporator. The dried extracts were then redissolved in 5 mL of 10% dimethyl sulfoxide (DMSO).

Antimicrobial activity assays

Microbial strains

The antimicrobial activity of the extracts was evaluated against two Gram-positive bacteria (*Bacillus cereus* PTCC 1154 and *S. aureus* PTCC 1431) and two Gram-negative bacteria (*Escherichia coli* PTCC 1338 and *P. aeruginosa* PTCC 1430).

Disk diffusion assay

The antimicrobial activity was screened using the standard disk diffusion method [21], according to clinical and laboratory standards institute (CLSI) guidelines (CLSI M02). The results were interpreted based on CLSI 2024 interpretive criteria (CLSI M100, 34th edition) to classify the susceptibility of the tested bacterial strains as susceptible (S), moderately susceptible (MS), or resistant (R) [22]. Sterile paper disks (6 mm in diameter) were impregnated with 20 µL of each extract solution. The disks were then allowed to dry under a laminar flow hood for 15-20 minutes to ensure complete solvent evaporation. Subsequently, the disks were aseptically placed on the surface of inoculated Mueller-Hinton agar plates. A negative control disk was prepared by impregnating a disk with 20 µL of 10% DMSO, which confirmed the absence of any inhibitory effect from the solvent alone.

Standard antibiotic disks, including nalidixic acid (30 µg), gentamicin (10 µg), amoxicillin (10 µg), and vancomycin (30 µg), were used as positive controls. The plates were incubated at 37 ˚C for 24 h, after which the diameters of the inhibition zones were measured in millimeters using a digital caliper. All assays were performed in triplicate.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined by the broth microdilution method in accordance with the CLSI M100 (34th edition) guideline [22]. Stock solutions with a uniform concentration of 1024 µg/mL were prepared from the extract solutions in DMSO. Two-fold serial dilutions of the extracts were prepared in Mueller-Hinton broth in a 96-well microtiter plate to achieve a final concentration range of 4 to 1024 µg/mL. Each well was then inoculated with a bacterial suspension to achieve a final concentration of approximately 5×10^5 CFU/mL. The plates were incubated aerobically at 37 ˚C for 18–24 h. The MIC was defined as the lowest concentration that completely inhibited visible growth in all three replicates. To determine the MBC, 100 µL aliquots from wells showing no visible growth were subcultured onto fresh Mueller-Hinton agar plates. The plates were incubated again at 37 ˚C for 18–24 h. The MBC was defined as the lowest extract concentration that resulted in no growth on the subculture in all three replicates, indicating ≥99.9% killing of the initial inoculum. All tests were performed in three independent replicates.

Gas chromatography-mass spectrometry analysis for metabolite profiling

The chemical composition of the extracts was analyzed using an Agilent 7890B gas chromatograph coupled with an Agilent 5977A mass selective detector (gas chromatography-mass spectrometry [GC-MS] system). Separation was achieved using an HP-5MS capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness). The operating conditions were as follows: Injector temperature, 250 ˚C; carrier gas, helium, at a constant flow rate of 1 mL/min; ionization mode, electron impact at 70

eV. The oven temperature program was set as follows: Initial temperature 60 ˚C (hold for 5 min), increased to 300 ˚C at a rate of 5 ˚C min^{-1}, and finally held at 300 ˚C for 10 min. The total GC runtime was 63 min. The sample injection volume was 1 µL in splitless mode. The constituents were identified by comparing their mass spectra with those stored in the National Institute of Standards and Technology (NIST) library.

Statistical analysis

All experiments, except for GC-MS analysis, were conducted using a completely randomized design with at least three independent biological replicates. Data were initially screened using the coefficient of variation, and Dixon's Q-test was applied to identify potential outliers; none were detected. Following one-way analysis of variance (ANOVA), the equality of variances was assessed, and post-hoc comparisons were performed using either Tukey's honestly significant difference (HSD) or the Games-Howell test, depending on whether the assumption of homogeneity of variances was met. All statistical analyses were performed using Microsoft Excel and SPSS software, version 26, and differences were considered statistically significant at $P<0.05$.

For GC-MS analysis, a composite sample was prepared by combining equal weights of plant material from four biological replicates, from which a single alcoholic (ethanolic, propanolic, or butanolic) extract was obtained and analyzed.

Results

Biomass yield under different light conditions the growth of *A. platensis*, measured as final fresh weight biomass yield after 46 days of cultivation, was significantly influenced by the light spectrum (Figure 1). White light resulted in the highest biomass production. Yellow and red light treatments yielded the next highest levels of biomass, showing no statistically significant difference from each other or from the white light condition. In contrast, blue and green light treatments resulted in the lowest biomass yields, which were not significantly different from each other but were significantly lower than those under white, yellow, and red light.

Values represent the means of white, yellow, red, blue, and green light treatments. Different lowercase letters denote statistically significant differences ($P<0.05$, ANOVA with Tukey's HSD post-hoc test; n=4).

Antimicrobial activity: Initial screening and focused analysis

Preliminary screening of all ethanolic extracts, as well as the propanolic and butanolic extracts from yellow and red light treatments, revealed no significant antimicrobial activity (inhibition zone diameter <6 mm) against any of the tested bacterial strains. Consequently, all subsequent detailed analyses—including the determination of MIC, MBC, and GC-MS profiling—were exclusively performed on the propanolic and butanolic extracts obtained from white, blue, and green light treatments, which exhibited antimicrobial potential. Furthermore, *S. aureus* was not affected by any of the microalgae extracts utilized in the present experiment.

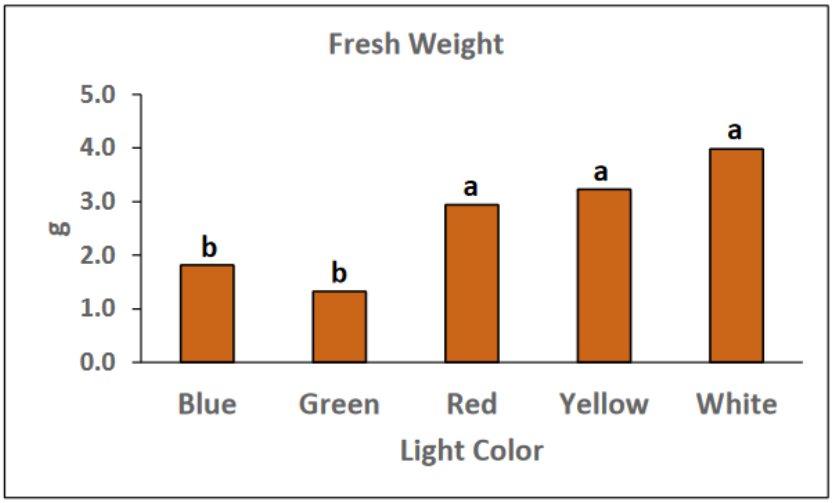

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Figure 1. Total fresh biomass yield (g) of *A. platensis* from individual 250 ml cultures after 46 days of cultivation under different light conditions.

Disk diffusion assay

Table 1 summarizes the antibacterial activity of the effective *A. platensis* extracts, assessed by the disk diffusion method. The susceptibility of the tested bacteria to the extracts and standard antibiotics was interpreted according to CLSI guidelines [21, 22], with isolates classified as S, MS, or R based on the established interpretive criteria. The results demonstrated that the inhibition zone diameters were dependent on three key factors: the light condition during cultivation, the type of extracting solvent, and the bacterial species tested. A consistent observation was that the Gram-positive bacterium (*B. cereus*) was markedly more susceptible to the extracts than the Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Notably, none of the extracts exhibited any inhibitory activity against *S. aureus* (inhibition zone ≤6 mm) across all light treatments and extract types, indicating a remarkable resistance of this Gram-positive pathogen to the microalgal metabolites.

Propanolic extracts: The most potent activity among the propanolic extracts was observed from microalgae cultured under green light. This extract generated an inhibition zone of 20 mm against *B. cereus*, outperforming the standard antibiotics amoxicillin (10 µg; 13 mm zone) and vancomycin (30 µg; 18 mm zone) under the same assay conditions. The propanolic extract from blue light also showed significant but weaker activity, producing zones of 11.00 mm and 8.00 mm against *E. coli* and *P. aeruginosa*, respectively. The extract from white light demonstrated the weakest effect, with a 10.3 mm zone against *B. cereus*.

Butanolic extracts: Mirroring the trend observed with the propanolic extracts, the butanolic extract from green light conditions exhibited the strongest activity, forming an inhibition zone of 18.7 mm against *B. cereus*, which was more effective than the amoxicillin and vancomycin controls. Butanolic extracts from white light produced smaller zones of 9.3 mm against *E. coli* but showed no activity against *P. aeruginosa*.

Table 2 summarizes the results of the MIC and MBC assays. The most potent activity against the Gram-positive bacterium *B. cereus* was observed in extracts from green light-treated cultures. The butanolic extract from this condition exhibited exceptional potency with an MIC of 8 µg/mL and an MBC of 16 µg/mL, followed by the propanolic extract (MIC=32 µg/mL, MBC=64 µg/mL). In contrast, the efficacy of the propanolic extract decreased markedly when the algae were cultivated under blue or white light, both showing an MIC of 256 µg/mL and an MBC of 512 µg/mL against *B. cereus*. The

Table 1. Antibacterial activity of *A. platensis* propanolic and butanolic extracts from different light treatments against pathogenic bacteria, measured by disk diffusion assay

Bacterial Strain	Light Treatment	Extract Type	Mean±SD		Negative Control (DMSO 10%)
			Inhibition Zone (mm)	Positive Control (Inhibition Zone, mm)	
B. cereus (PTCC 1154)	Green	Propanolic	20.00±1.00 (S)	Amoxicillin (10 µg): 13.00±0.50 ® Vancomycin (30 µg): 18.00±0.80 ®	No inhibition (6 mm)
	Green	Butanolic	18.67±0.58 (S)	Amoxicillin (10 µg): 13.00±0.50 ® Vancomycin (30 µg): 18.00±0.80 ®	No inhibition (6 mm)
	Blue	Propanolic	11.00±0.50 (MS)	Amoxicillin (10 µg): 13.00±0.50 ® Vancomycin (30 µg): 18.00±0.80 ®	No inhibition (6 mm)
	White	Propanolic	10.33±0.58 (MS)	Amoxicillin (10 µg): 13.00±0.50 ® Vancomycin (30 µg): 18.00±0.80 ®	No inhibition (6 mm)
	White	Butanolic	(6 mm) ®	-	-
E. coli (PTCC 1338)	Blue	Propanolic	11.00±0.50 (MS)	Gentamicin (10 µg): 19.00 ± 0.60 ® Nalidixic acid (30 µg): 22.00 ± 0.70 ®	No inhibition (6 mm)
	White	Butanolic	9.33±0.58 (MS)	Gentamicin (10 µg): 19.00±0.60 ® Nalidixic acid (30 µg): 22.00±0.70 ®	No inhibition (6 mm)
P. aeruginosa (PTCC 1811)	Blue	Propanolic	8.00±0.50 ®	Gentamicin (10 µg): 18.00±0.50 ®	No inhibition (6 mm)
S. aureus (PTCC 1112)	All treatments	All extracts	(6 mm) ®		

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Data are presented as inhibition zone diameters (Mean±SD, n = 3). Susceptibility categories (S: Susceptible, MS: Moderately Susceptible, R: Resistant) were determined according to CLSI guidelines [21, 22]. Amoxicillin (10 µg), vancomycin (30 µg), gentamicin (10 µg), and nalidixic acid (30 µg) were used as positive controls. N/A indicates no significant activity detected (inhibition zone ≤6 mm, equivalent to the disk diameter). The negative control (10% DMSO) showed no inhibition against any of the tested strains.

Table 2. MIC and MBC of *A. platensis* extracts against tested bacteria

Bacterial Strain	Light Treatment	Extract Type	MIC (µg/mL)	MBC (µg/mL)
B. cereus	Green	Propanolic	32	64
B. cereus	Green	Butanolic	8	16
B. cereus	Blue	Propanolic	256	512
B. cereus	White	Propanolic	256	512
E. coli	Blue	Propanolic	1024<	1024<
P. aeruginosa	Blue	Propanolic	1024<	1024<

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propanolic extract from blue light treatment showed no activity against the Gram-negative pathogens *E. coli* and *P. aeruginosa* at the highest concentration tested (1024 µg/mL).

Identification of bioactive compounds by gas chromatography-mass spectrometry

GC-MS analysis revealed the presence of a wide range of bioactive compounds in the extracts, the quantity and quality of which were strongly influenced by light conditions. The major compounds (with area% ≥1) identified in each extract are listed in Tables 3, 4, 5, 6 and 7. White light: The extract from this condition mainly contained alkanes and silanes (Tables 3 and 4).

Blue light: Although the propanolic extract from blue light was not rich in terms of specific compound quantities, it was notable for its diversity of compounds, with 137 different compounds identified. Among these compounds were phytol (10.13%) (Table 5) and 4-heptanone.

Green light: In butanolic extracts, green light led to the production of compounds such as butanoic acid esters (Table 6). The propanolic extract from this condition contained very high amounts of bis(2-ethylhexyl) phthalate (DEHP) (83.86%) (Table 7 and Figure 2). Other compounds such as phytol (0.88%) and 9-octadecenamide were also identified.

Discussion

The differential accumulation of bioactive compounds observed under various light spectra in our study aligns with recent findings on light-mediated regulation of secondary metabolism in cyanobacteria and microalgae. Recent investigations employing advanced analytical

Table 3. Major compounds identified by GC-MS in the propanolic extract from white light treatment

Compound	RT (min)	Formula	Area (%)	Qual (%)
Heptadecane	28.33	$C_{17}H_{36}$	26.21	92
Propane, 1,1-dipropoxy-	8.02	$C_9H_{20}O_2$	12.3	91
Ethoxy(methoxy)methylsilane	5.09	$C_4H_{12}O_2Si$	11.23	85
9-Octadecenamide, (Z)-	41.27	$C_{18}H_{35}NO$	9.66	94
Propanoic acid, propyl ester	5.03	$C_6H_{12}O_2$	6.98	90
Hexadecanoic acid, ethyl ester	36.11	$C_{18}H_{36}O_2$	2.62	94
Hexadecanoic acid, propyl ester	38.88	$C_{19}H_{38}O_2$	1.23	93
Phytol	39.57	$C_{20}H_{40}O$	1.51	94
Hexadecane	25.37	$C_{16}H_{34}$	1.23	90

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Table 4. Major compounds identified by GC-MS in the butanolic extract from white light treatment

Compound	RT (min)	Formula	Area (%)	Qual (%)
Butane, 1,1-dibutoxy-	15.61	$C_{12}H_{26}O_2$	27.68	93
2,2-Dimethyl-3-hydroxypropionaldehyde	5.25	$C_5H_{10}O_2$	17.34	91
4-Heptanone	7.23	$C_7H_{14}O$	14.35	90
Silane, diethoxydimethyl	7.61	$C_6H_{16}O_2Si$	10.07	86
Butanoic acid, butyl ester	9.03	$C_8H_{16}O_2$	6.6	91
Propanoic acid, 2-methyl-, butyl ester	8.24	$C_8H_{16}O_2$	5.95	93
4-Heptanone, 3-methyl	7.92	$C_8H_{16}O$	3.42	93
1-Butanol, 2-methyl-	6.41	$C_5H_{12}O$	2.91	92
Propanoic acid, 2-methyl-, 2-methylpropyl ester	7.7	$C_8H_{16}O_2$	1.52	94
Propane, 2,2'-(methylenebis(oxy))bis(2-methyl-	13.34	$C_9H_{20}O_2$	1.15	95

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approaches, including principal component analysis and artificial neural network models, have conclusively demonstrated that light spectra exert profound and differential effects on microalgal growth and biochemical composition [23]. Specifically, green light has been shown to significantly modulate protein content and fatty acid profiles, while violet light preferentially enhances the accumulation of polyunsaturated fatty acids [24]. These observations corroborate our findings, where green light treatment resulted in the highest abundance of fatty acid esters and DEHP, suggesting a preferential stimulation of the fatty acid biosynthesis pathway under this spectral condition. Furthermore, studies on the molecular mechanisms of light-regulated metabolism have revealed that

Table 5. Major compounds identified by GC-MS in the propanolic extract from blue light treatment

Compound	RT (min)	Formula	Area (%)	Qual (%)
Ethoxy(methoxy)methylsilane	5.12	$C_4H_{12}O_2Si$	17.07	84
Heptadecane	28.33	$C_{17}H_{36}$	12.66	96
Propane, 1,1-dipropoxy-	8.05	$C_9H_{20}O_2$	10.24	95
Phytol	39.51	$C_{20}H_{40}O$	10.13	94
9-Octadecenamide, (Z)-	41.27	$C_{18}H_{35}NO$	7.17	96
Hexadecanoic acid, ethyl ester	36.12	$C_{18}H_{36}O_2$	4.57	96
1-Butanol	4.15	$C_4H_{10}O$	3.25	89
Hexadecanoic acid, propyl ester	38.88	$C_{19}H_{38}O_2$	2.5	95
trans-(2-Chlorovinyl)dimethylethoxysilane	8.33	$C_6H_{13}ClOSi$	2.51	87
2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undecane, 1-ethyl-	40.14	$C_8H_{17}NO_3Si$	1.75	84
Ethyl 9.cis., 11-trans-octadecadienoate	40.86	$C_{20}H_{36}O_2$	1.17	87
Cycloheptasiloxane, tetradecamethyl-	22.82	$C_{14}H_{42}O_7Si_7$	1.03	88

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Table 6. Major compounds identified by GC-MS in the butanolic extract from green light treatment

Compound	RT (min)	Formula	Area (%)	Qual (%)
Butane, 1,1-dibutoxy-	15.61	$C_{12}H_{26}O_2$	29.84	92
Propanoic acid, 2-methyl-, butyl ester	8.21	$C_8H_{16}O_2$	15.23	94
1-Butanol, 2-methyl-	6.35	$C_5H_{12}O$	9.59	92
4-Heptanone	7.2	$C_7H_{14}O$	9.02	90
Silane, diethoxydimethyl	7.58	$C_6H_{16}O_2Si$	8.13	87
Butanoic acid, butyl ester	8.96	$C_8H_{16}O_2$	8.6	93
4-Heptanone, 3-methyl-	7.86	$C_8H_{16}O$	6.18	91
1-Butoxy-1-isobutoxy-butane	9.69	$C_{12}H_{26}O_2$	1.48	92
Propane, 2,2'-(methylenebis(oxy))bis(2-methyl-	13.31	$C_9H_{20}O_2$	1.38	94

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different wavelengths affect gene expression patterns in a wavelength-specific manner. For instance, green light has been demonstrated to significantly downregulate the expression of genes associated with central metabolism in cyanobacteria, potentially triggering stress responses that redirect metabolic flux toward secondary metabolite production [25]. The enhanced production of antimicrobial compounds under green light in our study may therefore represent a stress-induced adaptive response, consistent with the reduced biomass yield observed under this condition (Figure 1). Additionally, blue light has been shown to promote the biosynthesis of specific metabolites such as astaxanthin through the upregulation of the MEP pathway, which aligns with the greater chemical diversity (137 compounds) observed in our blue light treatment [26]. These findings collectively support the concept that light quality can serve as a powerful non-invasive elicitor for steering cyanobacterial metabolism toward the production of desired bioactive compounds [27].

Beyond their direct antimicrobial effects, several of the bioactive compounds identified in our *A. platensis* extracts have demonstrated significant immunomodulatory properties that may offer additional therapeutic value in managing infectious diseases [28]. Phytol, a diterpene alcohol that was prominently enhanced in the blue light extract (10.13%, Table 4), has been extensively investigated for its capacity to modulate inflammatory responses. Comprehensive biomedical reviews have documented that phytol exhibits a wide range of pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory effects, largely mediated through its interaction with nuclear receptors and inflammatory signaling pathways [29]. Mechanistically, phytol has been shown to suppress the activation of the NF-κB signaling cascade by inhibiting the phosphorylation and subsequent degradation of nuclear factor kappa-B inhibitor subunit alpha. This primary inhibitory protein sequesters NF-κB in the cytoplasm [29]. This blockade prevents the nuclear translocation of the NF-κB p65

Table 7. Major compounds identified by GC-MS in the propanolic extract from green light treatment

Compound	RT (min)	Formula	Area (%)	Qual (%)
Bis(2-ethylhexyl) phthalate (DEHP)	51.51	$C_{24}H_{38}O_4$	83.86	95
Ethoxy(methoxy)methylsilane	5.15	$C_4H_{12}O_2Si$	4.35	85
Propane, 1,1-dipropoxy-	7.83	$C_9H_{20}O_2$	2.23	97
Propanoic acid, propyl ester	4.33	$C_6H_{12}O_2$	1.78	93
Other minor compounds	-	-	7.78	_

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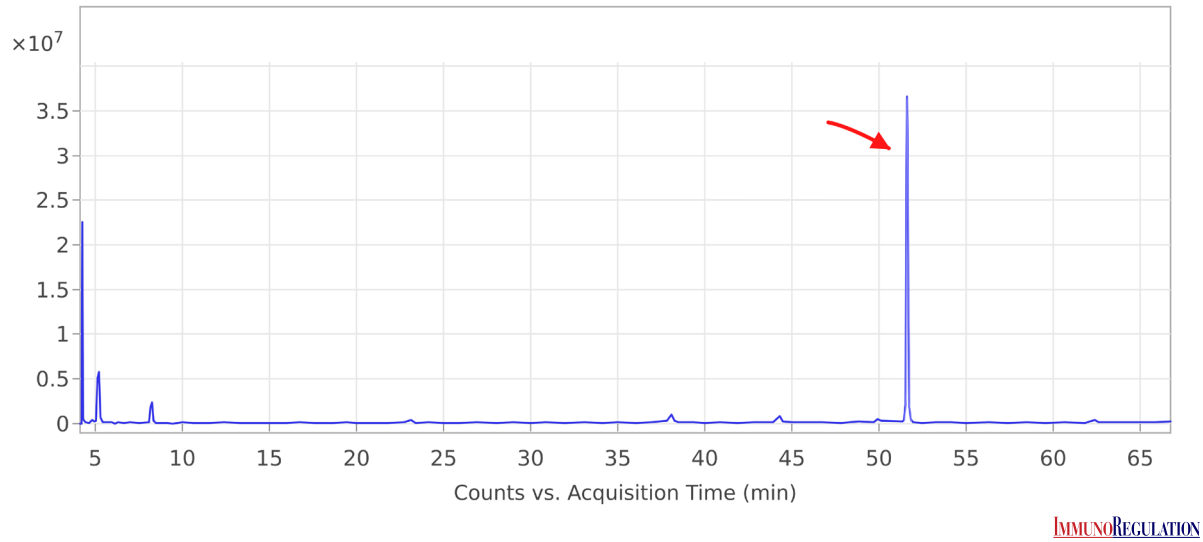

Figure 2. GC-MS chromatogram of the propanolic extract of spirulina (*A. platensis*) grown under green light

The prominent peak at a retention time of 51.51 min (indicated by the red arrow), with an area percentage of 83.86%, corresponds to di(2-ethylhexyl) phthalate (DEHP).

subunit, thereby attenuating the transcription of various pro-inflammatory cytokine genes, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) [8]. Additionally, phytol interferes with the MAPK pathway, specifically reducing the phosphorylation of extracellular signal-regulated kinase and p38 MAPK, which are critical upstream mediators of inflammatory responses in immune cells such as macrophages and dendritic cells [9]. In vivo studies have further demonstrated that phytol possesses potent antimicrobial activity against various bacterial pathogens while simultaneously exhibiting immunostimulatory properties, enhancing the host's immune defense mechanisms and promoting recovery from bacterial infections [30]. These immunomodulatory actions are particularly relevant in the context of Gram-positive infections, where excessive production of inflammatory cytokines contributes to septic shock and tissue injury [31]. By simultaneously exerting direct antibacterial activity and dampening hyperinflammatory host responses, phytol-containing extracts may represent a dual-action strategy to mitigate infection-induced immunopathology [32]. This is especially significant given the increasing recognition that bacterial clearance alone is insufficient to prevent morbidity and mortality in severe infections, and that adjunctive immunomodulation is often required for optimal clinical outcomes [33]. The potential of cyanobacterial metabolites as immunomodulatory agents has been increasingly recognized, with recent comprehensive reviews highlighting their capacity to modulate both innate and adaptive immune responses through various mechanisms, including the regulation of cytokine production and immune cell polarization [28]. Our findings contribute to this growing body of evidence by demonstrating that specific light conditions can enrich extracts with immunologically active compounds such as phytol, thereby enhancing their potential for dual therapeutic applications.

One of the most striking and consistent observations in the present study was the exceptionally high relative abundance of DEHP in the propanolic extract from green light-treated cultures, reaching 83.86% of the total identified compounds (Table 6). This compound has frequently been reported in GC-MS profiles of various microalgae and cyanobacteria, yet its interpretation has long been a subject of debate within the scientific community [34]. While some researchers have considered DEHP a genuine microbial metabolite with potential antimicrobial or ecological signaling roles, others have argued that its presence may originate from exogenous contamination, given its widespread industrial use as a plasticizer and its ubiquity as an environmental pollutant [35, 36]. This contamination-centered interpretation, however, should not be generalized uncritically across all biological systems and experimental contexts. A growing body of evidence from multiple independent studies has demonstrated that phthalate esters, including DEHP, can be endogenously biosynthesized by a wide range of photosynthetic organisms, including cyanobacteria, microalgae, and higher plants, and that their accumulation is subject to dynamic regulation by environmental and hormonal elicitors [38, 39]. Babu and Wu [28] provided direct experimental

evidence that several freshwater algae, including cyanophytes, can synthesize phthalate esters de novo, and that their release into the culture medium may increase under stress conditions, suggesting a potential ecological role in allelopathic interactions or chemical defense.

Our previous investigations have further substantiated this biosynthetic capacity across diverse plant taxa. In *Euphorbia trigona*, treatment with the auxin naphthalene acetic acid elicited a remarkable metabolic reprogramming, increasing the content of bis(2-ethylhexyl) phthalate from 9.59% in control plants to 49.31% of the total extractable compounds, alongside significant increases in other bioactive fatty acids and terpenoids [39, 40]. This approximately five-fold increase provides compelling evidence that phthalate accumulation is a biologically regulated response to exogenous elicitors rather than a passive consequence of environmental uptake, similarly, in Agave americana cv. Marginata, urea fertilizer treatment caused outstanding changes in the content of bis(2-ethylhexyl) phthalate, methyl palmitate, and methyl stearate, further confirming the responsiveness of phthalate metabolism to nutritional modulation [41]. Collectively, these findings across multiple species and treatment regimens confirm that phthalates and related esters can be endogenously produced plant metabolites whose profiles are dynamically altered by specific hormonal and nutritional elicitors, providing compelling evidence against their exclusive characterization as anthropogenic pollutants.

In the context of the present study, the dramatic increase in DEHP abundance specifically under green light (83.86%) compared to other light treatments (where DEHP was either absent or present at much lower concentrations) strongly suggests a light-regulated biosynthetic response rather than random contamination. Green light has been shown to induce stress responses in cyanobacteria, characterized by reduced growth rates and redirected metabolic flux toward secondary metabolite production [25]. This stress-induced metabolic shift is consistent with the observed reduction in biomass yield under green light (Figure 1) and the concurrent accumulation of DEHP and other fatty acid derivatives. Furthermore, the presence of DEHP alongside other known antimicrobial compounds—such as phytol, 9-octadecenamide, and various fatty acid esters—in extracts that exhibited potent antibacterial activity (particularly against *B. cereus*) supports the hypothesis that DEHP may be part of a coordinated defense metabolome induced by light stress [42]. It is noteworthy that the DEHP abundance was substantially higher in the propanolic extract (83.86%) than in the butanolic extract from the same green light treatment, indicating selective extraction of this compound based on solvent polarity, which is consistent with its known physicochemical properties as a moderately lipophilic compound.

Nevertheless, it is crucial to maintain a cautious interpretive framework. Even if DEHP is endogenously produced, its well-documented toxicological profile—including classification as a probable human carcinogen (group 2B) by the International Agency for Research on Cancer and its established role as an endocrine-disrupting chemical—precludes its direct therapeutic application [35, 43]. Therefore, the potent antimicrobial activity observed in the green light propanolic extract should not be attributed solely to DEHP, nor should this extract be considered a candidate for crude therapeutic use. Instead, the co-occurrence of DEHP with potent bioactivity serves as a valuable biological indicator that green light exposure drastically reprograms the metabolome of A. platensis. Future investigations should employ bioassay-guided fractionation coupled with high-resolution mass spectrometry to isolate and identify the specific non-toxic metabolites responsible for the observed antimicrobial effects, while rigorously excluding plastic-derived artifacts [44]. Additionally, transcriptomic and metabolomic analyses under controlled conditions with strict avoidance of plastic consumables would help unequivocally establish the biosynthetic origin of DEHP and other phthalate derivatives in this cyanobacterium (Figure 3) [45].

Conclusion

The findings of this study clearly demonstrate that light conditions are a key factor in regulating the production of secondary metabolites with antimicrobial properties in *A. platensis*. Among the treatments investigated, green light was identified as the most effective condition for enhancing compound production and, consequently, strengthening the antimicrobial activity of propanolic and butanolic extracts, showing potent efficacy against the Gram-positive bacterium *B. cereus*. Blue light, although it induced less antimicrobial activity, led to the production of a wider spectrum of compounds.

While the green light-induced extract showed remarkable antimicrobial activity, it is crucial to acknowledge the safety concerns surrounding certain identified metabolites. Therefore, the practical application of this research lies not in the direct use of the crude extract, but in utilizing green light as a pre-harvest elicitor to enhance the production of target compounds, exploring the use of the functionalized biomass, and deciphering the light

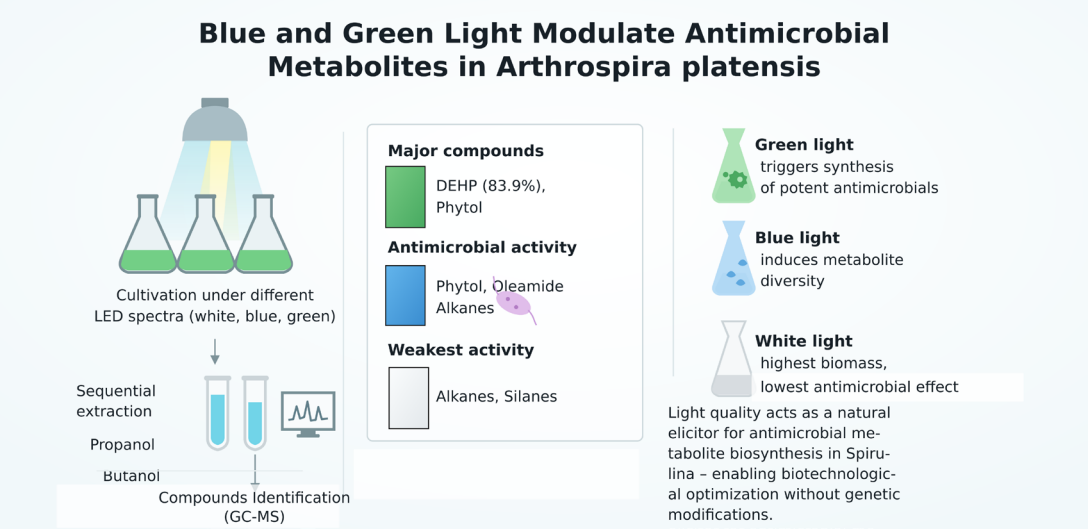

Figure 3. Cultivation of *A. platensis* (spirulina) under green light significantly enhanced the production of antimicrobial compounds, such as DEHP and phytol, in propanolic and butanolic extracts. These green light-induced extracts exhibited potent bactericidal activity primarily against the gram-positive bacterium (*B. cereus*). In contrast, blue light promoted a more diverse metabolome and showed moderate activity against gram-negative bacteria (*E. coli*). White light favored biomass yield but resulted in minimal antimicrobial activity. Our results establish light quality as a powerful, non-invasive tool to steer the metabolic output of *A. platensis* for enhanced antimicrobial compound production.

signaling mechanism for future metabolic engineering. These results confirm the high potential of Spirulina as a natural source for developing new antimicrobial agents and provide a practical, non-invasive strategy for optimizing the production of these valuable compounds.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization, supervision, review & editing: Shadman Shokravi and Aryan Sateei; Methodology: Aryan Sateei, Hamid Reza Pordeli, Mazyar Ahmadi Golsefidi; Investigation and Data analysis: Aryan Sateei and Aynaz Gharajeh; Writing the original Aryan Sateei; Data collection Aynaz Gharajeh and Aryan Sateei.

Conflicts of interest

The authors declared no conflict of interest.

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