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Effect of ultraviolet-B radiation on Biochemical composition and antibacterial activities of *Spirulina platensis*

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Abstract

Biomass production of *S. platensis* has been evaluated under ultraviolet-B (UV-B) radiation stress to maximize the algal growth. It was cultivated in Zarrouk's media with applying some stress was treated under high dose rates of (UV-B) with altered time durations of (15, 30 and40 mins). The highest content of its dry weigh, chlorophyll a, carotenoids, protein and carbohydrates are recorded at UVB light (312 nm) for 15 mins. . As its phenolic content was maximized with acetone extract at control while flavonoids were detected with ethanol extract at UV- 45mins. Antibacterial activity of *S. platensis* was prepared in 70% (acetone, methanol and ethanol) at different (UV-B) radiation and tested against some pathogenic bacterial. Its highest antibacterial activities were reported with 70% acetone extract. The impact of UV-B radiation on *S. platensis* led to a drop in protective mechanisms with associated decline in growth and at high intensity studied Antimicrobial activity of *S. platensis*.

Keywords: *Spirulina platensis*, growth, phytochemical, antibacterial, ultraviolet-B radiation
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1. Introduction

In recent years, significant changes have been observed in the aquatic ecosystem owing to increased solar ultraviolet-B (UV-B) radiation penetrating the earth's surface [1]. High rate of UV-B radiation is known to have negative impact on living organisms, starting at the molecular, cellular and ultimately population level [2]. However, photosynthetic organisms depend on solar energy containing damaging UV radiation for their photosynthetic process. Of the total non-ionizing radiation, only 1.5% is constituted by UV-B radiation (280–320 nm) reaching the earth surface having direct damaging effects on both aquatic and terrestrial biota [3]. Cyanobacteria are a promising renewable source of bioactive secondary metabolites can be biotechnologically and commercially exploited in several ways [4]. That UV radiation may induce biosynthesis of carotenoids, allophycocyanin, phycoerythrin, and scytonemin while phycocyanin degrades in response to longtime UV radiation. Moreover, pigment composition [5]. Cyanobacteria also have antioxidant systems, including the synthesis of SOD, exopolysaccharide, microcystin, and light recovery capacity to defend them from UV-B [6]. *Spirulina* has been consumed since ancient times as a perfect food of the earth and has been frequently studied for many purposes, including as a food supplement, a functional food medicine, for cosmetics, and for many other biomaterials [7].It is contains many functional bioactive ingredients with antioxidant and anti-inflammatory activities, including phenolic phytochemicals [8].*Spirulina platensis* is one of the important micro-alga showing antimicrobial activity against many pathogenic bacteria and fungi[9]. The present study is aimed to focus on the effect of UV-B radiation stress on growth, photosynthetic, phytochemical and antimicrobial activities of *Spirulina platensis.*

2. Materials and methods

Culture and growth conditions

Spirulina platensis was obtained from Hydrobiology Lab, National Institute of Oceanography and Fishers, Egypt. It was cultivated axenically as batch culture using Zarrouk medium described by [10]. at pH10, 30±1°C and 26 $\mu E/m^2/s$.

Different doses of UV-B intensities and durations on *Spirulina platensis*

The *Spirulina platensis* culture was treated at different intensities T-8M ultraviolet-B lamp (8 w, 220v, 312 nm) of UV-B radiation for different durations (15, 30 and 45 mins). The UV-B treated cultures were kept under dark for 15 min then transferred to fresh medium and growth parameters recorded for a period of 30 days at a regular time interval of 3 days.

Determination of growth parameters of *Spirulia platensis*

Growth of *S. platensis* was determined by measuring the optical density of the algal suspension at 750 nm with a UV–vis spectrophotometer [11]. And by determination of cellular

dry weight (CDW). Biomass productivity was calculated according to [12].

Estimation of pigment (chlorophyll a and carotenoids)

A known volume (5ml) of *S. platensis* culture was centrifuged at 6000 rpm for 10 min. The supernatant was decanted, and an equal volume of methanol was added to the pellet, then it was incubated in water bath at 55°C for 15 mins, and centrifuged. Absorbance of the extract (A) was measured against blank of free methanol at 650, 665 and 452 nm. Chlorophyll a and carotenoids were estimated as μg ml^{-1} of culture suspension using the following equation [13]:

Chlorophyll a (μg ml^{-1}) = 10.3 E665 - (0.918 E650). And Carotenoids (μg ml^{-1}) = 4.2 E452 – (0.0246 chl.a).

Estimation of total soluble proteins

After pigment extraction, residual cells were extracted using 1 N NaOH in a boiling water bath for 2 h, as described by [14]. Protein concentration, as mg ml^{-1}, was determined according to [15] using bovine serum albumin as a standard reference.

Estimation of total carbohydrates

Total carbohydrates were quantitatively determined by phenol sulphuric acid method described by [16] using glucose as a standard reference.

Preparation of *Spirulina platensis* extracts:

About 0.2 gm of alga was soaked in 70% methanol (5 ml) for 24h, and centrifuged. The residues were re-soaked with 70% methanol (5ml) for 24h, and centrifuged. Then other extractions were repeated separately with 70% acetone and 70% ethanol. The filtrates were concentrated in vacuum until drying. It was analyzed for total phenols and flavonoids; the extracts were diluted by 5 ml of the same solvent used for each treatment.

Estimation of total phenolic compounds

Total phenolics were estimated quantitatively according to [17] using pyrogallol as a standard reference with concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% of pyrogallol were prepared from the stock solution of 1.0 g 100ml of pyrogallol in deionized water.

Estimation of total flavonoid compound

Aluminum chloride colorimetric method, as modified by [18] was used to estimate flavonoids content. Different concentrations of quercetin were used to form calibration curve with concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% of quercetin were prepared from the stock solution of 1.0 g 100ml of quercetin in deionized water.

Microorganisms tested

Reference bacteria required for present studies were obtained from Microbial Physiology-Botany and Microbiology department, Faculty of science, Al-Azhar University. They included five bacterial strains, *Pseudomonas aeruginosa* NCIB-9016 and *Escherichia coli* NCTC-10418 (gram-négative bacteria), *Bacillus subtilis* NCTC-10400, *Bacillus diminuta* ATCC-19146 and *Staphylococcus aureus* NCTC-7447 (gram-positive bacteria). In addition, four pathogénies bacterial strains were obtained from faculty of Medicine, Menoufia University, *Acinetobacter sp, Klebsiella pneumonia, Salmonella typhi* (gram-negative bacteria) and *Staphylococcus aureus* (as gram-positive bacteria). All of the cultures were maintained in nutrient agar slants.

Preparation of bacterial suspension:

The bacteria used were obtained from slants which were less than 30 days old**.** Loop full samples were taken from the slants which were grown in sterile 50 ml nutrient broth medium, autoclaved at 121°C under 1.5 atmospheres pressure for 15 min. Then left grow for 16 h at 37°C in a shaking incubator agitating, at 140 rpm **[19].** All bacterial suspensions of tested microorganism were adjusted to 10^{-8} CFU.

Antibacterial activity test

Paper disc diffusion method **[20]** was used for testing antibacterial activities of *S. platensis.* The media was poured into sterile petri- dishes (9.0cm diameter) and allowed to set. The paper disc diffusion method was employed for antibacterial susceptibility. Whatman filter paper discs (No. 1, Diameter 6mm) saturated with about 50 μ, of the different extracts, was placed on culture medium seeded with the test organism. Disc fed with corresponding solvent alone served as the control. These Agar plates were incubated at 37±2°C for bacteria. After 24 h, inhibition zone around the disc was measured in mm. the experiment was carried out three times and the mean values were presented.

Statistical analyses

Results are presented as mean ± SD (standard deviation) for three replicates. All the data were subjected to one – way analysis of variance (ANOVA) using SPSS software, version 21. Test of significance was carried out using TUKY test at the significance level $P \leq 0.05$.

3. Results

1- Effect of UV-B exposure on growth parameters and pigment

Production of *S. platensis*

The experimental organism, *S. platensis* was grown in liquid medium at pH 10.0 and, expose to UVB lamp for 15, 30, and 45 minutes. The growth parameters and content of pigments were determined on day 21 of incubation period. Effect of Exposure on growth as OD750 at 3 days was studied by 15, 30 and 45mins (Distance 15 cm). Figure (1) shows the Effect of UV exposure time on growth of *Spirulina platensis*.

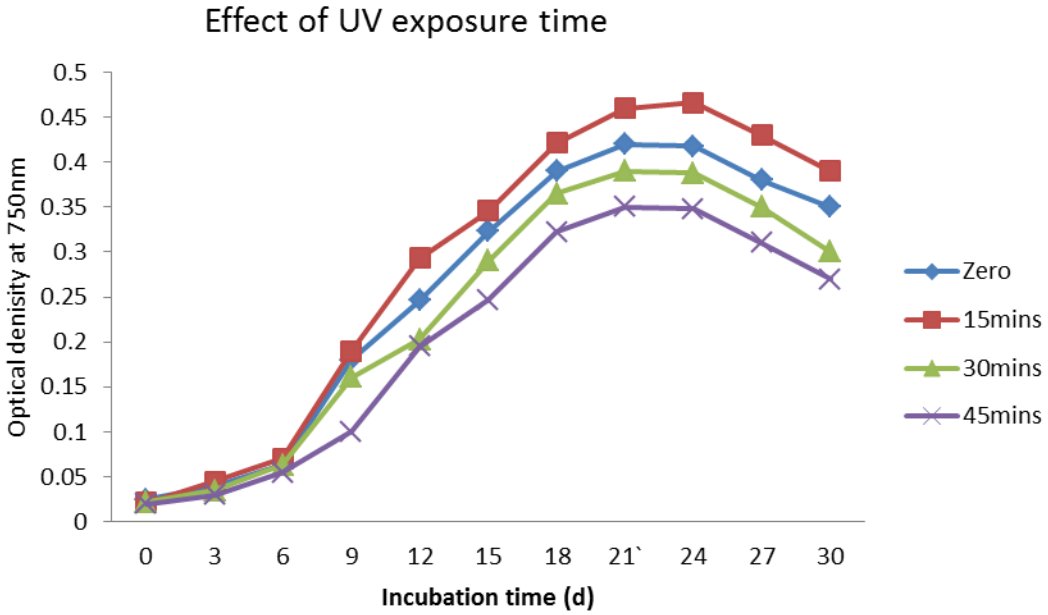

Fig (1): Effect of UV exposure time on growth of *Spirulina platensis* over 30 days of incubation

In general, exposure of the growing culture to UVB light (312 nm) for 15 mins was proven to be the optimal for growth and production of pigment. Table (1) showed that the dry weight recorded the highest value of 0.036g/20 ml. The culture of *S. platensis* showed highest protein content of 466 mg/g and carbohydrate of 48mg/g dry weight which the biomass respectively.

Chlorophyll *a* and carotenoids content of *S. platensis* were shown in Table (1). The optimum UV exposure time of 15 mins resulted in an increase in chlorophyll *a* (2.8 µg/ml) and carotenoids (1.2 µg/ ml). Longer exposure times of the cultures to UV reduced both the growth and formation of pigments.

Total phenolic and flavonoid of their different solvent extracts 70% (acetone, methanol, and ethanol) of *S. platensis* are presented in Tables (2) the highest phenolic content was found in acetone extract (0.52mg/g) at control and flavonoid content found in ethanol extract (9.7 mg/g) at UV- 45mins.

The statistical analysis revealed that the cells exposed to UV irradiation showed highly significant effect on the growth, protein, carbohydrate, pigments (chlorophyll *a* and carotenoids) phenolic and flavonoid content.

Table (1): Effect of UV-B exposure time on growth, proteins, carbohydrates, chlorophyll a and carotenoids contents of *Spirulina platensis*

UV exposure (minutes)	D. Wt. g/20ml	Proteins mg/g d.wt	Carbohydrates mg/g d.wt	Chlorophyll a µg/ml	Carotenoids µg/ml
0.0	0.035 ± 0.0003^{b}	426 ± 2^{b}	39 ± 0.6^{b}	1.7 ± 0.038^{b}	0.78 ± 0.006^{d}
15	0.036 ± 0.0003^{a}	466 ± 4^{a}	48 ± 0.5^{a}	2.8 ± 0.028^{a}	1.24 ± 0.018^{a}
30	0.030 ± 0.0002^{c}	372 ± 4^{c}	35 ± 0.9^{c}	$1.6\pm0.047b^{c}$	1.11 ± 0.009^{b}
45	0.027 ± 0.0004^{d}	363 ± 5^{c}	34 ± 0.7^{c}	1.5 ± 0.010^{c}	0.86 ± 0.010^{c}

Table (2): Effect of UV-B exposure time on phenolic and flavonoid contents of S. platensis

	Phenolic (mg/g d.wt)			Flavonoid (mg/g d.wt)		
UV exposure (minutes)	Acetone	Methanol	Ethanol	Acetone	Methanol	Ethanol
0.0	0.52 ± 0.0004^{a}	0.18 ± 0.0004^{b}	0.17 ± 0.0006^{a}	7.0 ± 0.033^{a}	5.7 ± 0.045^{a}	7.6 ± 0.012^{d}
15	0.24 ± 0.0006^{b}	0.13 ± 0.0003^{d}	0.14 ± 0.0004^{c}	5.0 ± 0.68^{b}	5.2 ± 0.019^{b}	7.7 ± 0.031^{c}
30	0.23 ± 0.0004^{c}	0.26 ± 0.0005^{a}	0.16 ± 0.0003^{b}	5.2 ± 0.032^{b}	5.8 ± 0.031^{a}	9.1 ± 0.038^{b}
45	0.19 ± 0.0004^{d}	0.14 ± 0.0004^{c}	0.13 ± 0.0006^{d}	5.4 ± 0.021^{b}	5.0 ± 0.025^{c}	9.7 ± 0.031^{a}

Each value is the mean of three readings ± standard deviation. Values with the same small letter in the same column showed insignificant difference (at p≤0.05).

2- Effect of UV exposure time on production of antibacterial substances from *S. platensis* extracted with different solvents

The results present in Figures (2, 3, and 4) show that extraction 70% (acetone, methanol, and ethanol) of *S. platensis*, by using paper disc diffusion method. The stronger inhibitory effect on most tested bacteria were induced by normal conditions except *S. typhi* where inhibition zone was 15mm at UVB for 30 mins with 70% acetone extract, *E. coli* NCTC-104189 was 15mm at UVB for 45mins with 70% methanol and *Acinetobacter sp* was 14mm at UV-15 and *Bacillus diminuta* ATCC-19146 was 17mm at UV-45mims with 70% ethanol.

Highly significant variations among the difference extracts of *S. platensis* aganist all bacteria except *Acinetobacter sp* with 70% acetone and 70% methanol extracts**,** while *P. aeruginosa* (NCIB-9016) *and Salmonella typhia* with 70% ethanol finally *Klebsiella pneumonia* with 70% methanol non-significant.

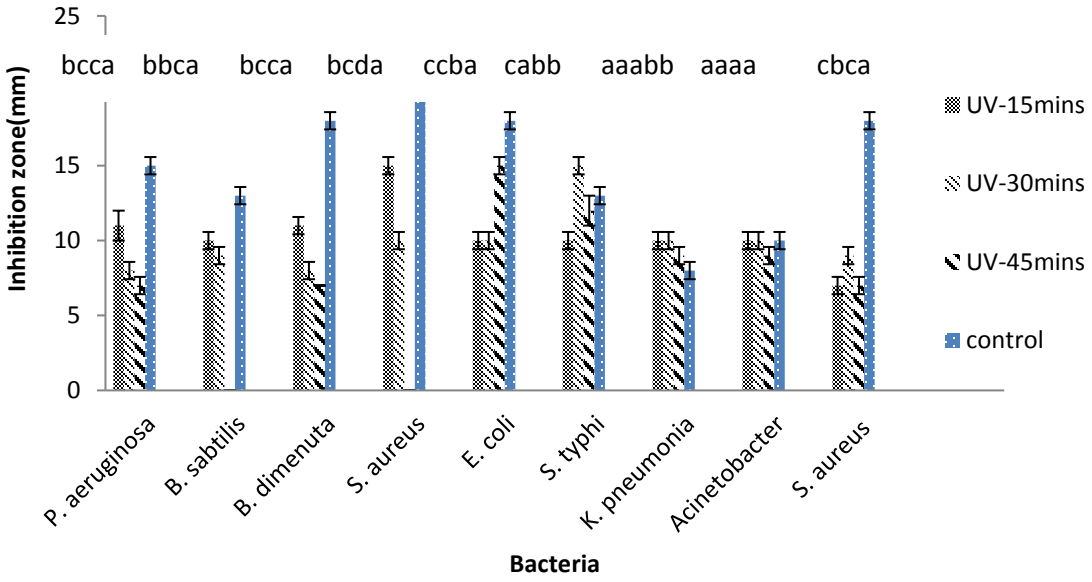

Fig (2): Effect of UV time on antibacterial activities of acetone extract of *Spirulina platensis*

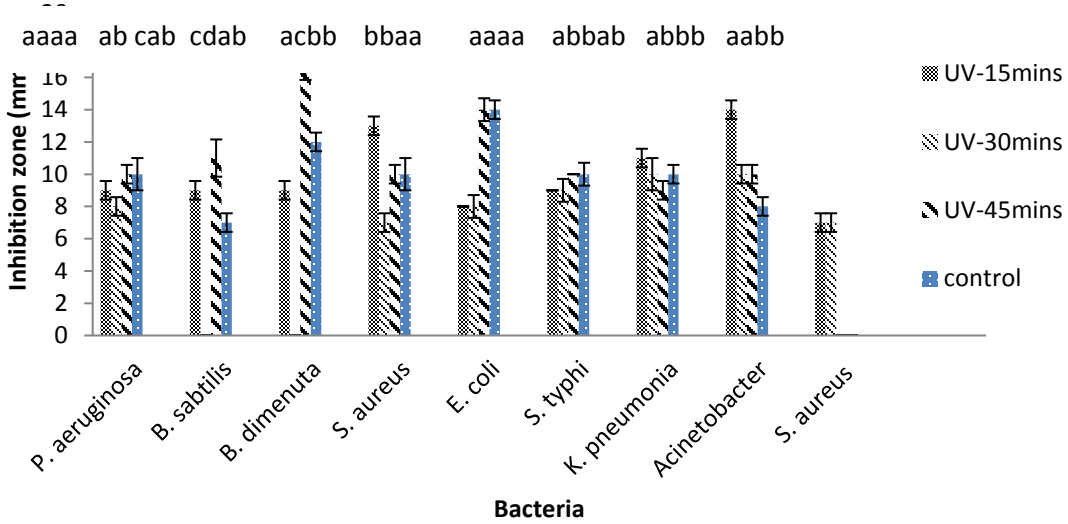

Fig (3): Effect of UV time on antibacterial activities of ethanol extract of *Spirulina platensis*

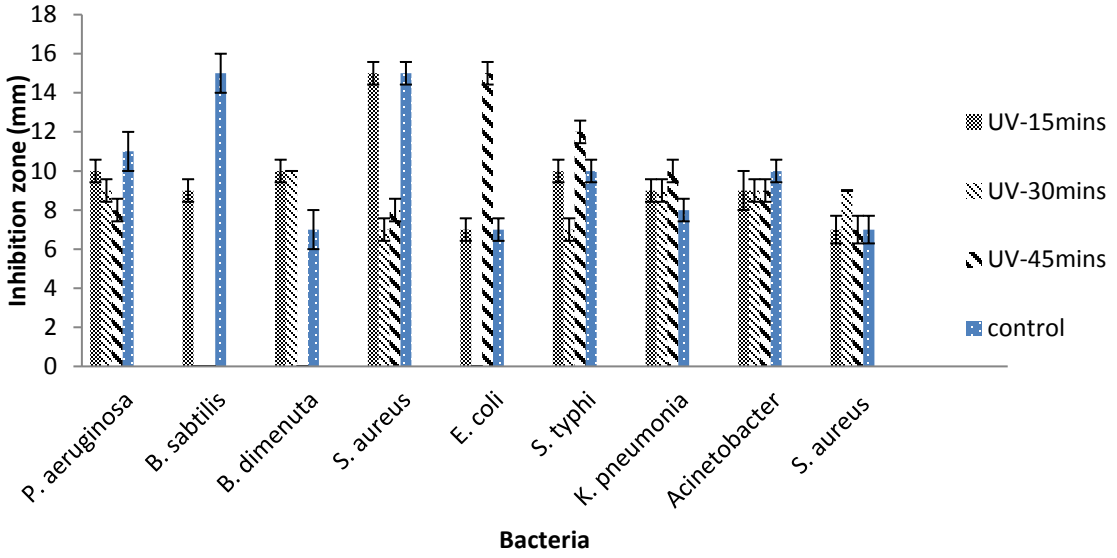

Fig (4): Effect of UV time on antibacterial activities of methanol extract of *Spirulina platensis*

4. Discussion

Spirulina is a photosynthetic, filamentous, multicellular blue-green microalga which grows in wide range fresh, marine and brackish water. It grows well in a highly alkaline environment of pH 10-12 [21]. In the present study, growth of *S. platensis* started to decline after 21days of incubation. In the present study, growth of *S. platensis* started to decline after 21days of incubation. Most of the previous works measured biomass of *S. platensis* after 15 and 25 days and none of them used extra time to follow biomass depletion [22].

In the present study, the exposure of the growing culture to UV light for 15min. lead to increase biomass, chl.*a*, protein, and carbohydrates. The given results were more or less similar with that reported by on this respect [1] experimental alga *Botryococcus braunii* was treated under different doses of artificially enhanced UV-B radiation. The organism was treated under high dose rates of UV-B with altered time durations of 15, 30, 45 and 60 min. It showed large variations in the growth characteristics analyzed. The rate of whole-cell photosynthetic oxygen evolution showed steep drop in high dose compared to low dose-treated cultures.

Effects of ultraviolet-B irradiation on cyanobacteria' *Anabaena variabilis, Oscjlldtoria tenuis Noduland baltica, N. harveyana and Phormidiwn uncinatum* (Baikal and Tubingen strain) indicated that pigmentation and energy transfer to photosynthetic reaction centers were impaired [23]. Stated UV-B irradiation induced an increase in carotenoids of terrestrial Cyahobacterium *Nostoc commune,* especially echinenone and myxoxanthophyll, but did not influence production of chlorophyll *a* [24].

The health benefit of *Spirulina* has been partially attributed to its chemical rich and diverse phenolic compounds, interacting together as potent antioxidants [25]. The present study indicated that total phenolics and flavonoids of *S. platensis*, extracted with different solvent 70% (acetone, methanol and ethanol) realized the highest phenolic content with acetone and flavonoids content with ethanol. That was agreed with [26] who tested the total phenolic and flavonoids with different solvent extracts (acetone, methanol, and ethanol) and showed the highest phenolic contents were found in acetone while flavonoids were reproduced with methanol.

Nowadays, development of alternative pharmaceuticals agents without side effect is more necessary than before. Microalgae-derived compounds, like other natural materials, are biodegradable and environmentally acceptable. *S. platensis* produces intracellular and extracellular metabolites (e.g. antibiotics, functional compounds and functional food ingredients) with antibacterial, antialgal, antiviral, antifungal, immunostimulant, enzyme inhibiting, cytotoxic and anti plasmodial activities [27]. In the present study, three solvents were used 70% (acetone, methanol and ethanol) for extracting of the active substance from *Spirulina platensis*, the results showed that acetone was the strongest solvent for extraction of active materials comparing with the other solvents. Our results agreed with [28] and [29] they stated that the stronger inhibitory effect on the most tested microorganisms was induced by 70% acetone extract followed by 70% methanol and the least was shown by 70% ethanol.

5. Conclusions

Spirulina platensis has been consumed since ancient times as a perfect food and can also be exploited for pharmaceutical applications. The present study important to study the effects of UV-B radiation on commercially important and highly resistant species of microalgae. In present study showed the negative effects of UV-B radiation on *Spirulina platensis*. The *Spirulina platensis* alga was found to retain its growth to a certain level thereafter any increase seemed to be highly lethal for its survival. Hence, it is important to save our earth from the harmful effects of UV-B radiation caused by human activities, which greatly affect the low level of the ecosystem.

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