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Antibacterial and Antifungal Activity of Cyanobacteria and Green Microalgae. Evaluation of Medium Components by Plackett-Burman Design for Antimicrobial Activity of *Spirulina platensis*

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Abstract: In this study, 3 cyanobacteria (*Anabaena oryzae, Tolypothrix ceytonic*a and *Spirulina platensis*) and 2 green microalgae (*Chlorella pyrenoidosa* and *Scenedesmus quadricauda*) were tested in compliance with the agar well diffusion method for their antibacterial and antifungal agent production on various organisms that incite diseases of humans and plants (*Escherichia coli, Bacillus subtilis, Staphyllococcus aureus, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus, Penicillium herquei, Fusarium moniliforme, Helminthosporium sp., Alternaria brassicae, Saccharomyces cerevisiae, Candida albicans*). The antimicrobial activity was maintained by using (ethanol, acetone, diethyl ether and methanol). It was found that, *Spirulina platensis* and *Anabaena oryzae* had the highest antibacterial and antifungal activity towards the tested bacteria and fungi. *Spirulina platensis* was evaluated for biological activity against (*A. flavus, F. moniliforme, C. albicans, B. subtilis, P. aeruginosa*) by operating the statistical design of Plackett-Burman for the degree of significance of the eight different trials by using seven independent variables. The results obtained from Plackett-Burman design revealed that highest main effect and t-value were detected with NaCl in case of *A. flavus*. While, they were detected with $MgSO_4$ and micronutrient (a) in case of *F. moniliforme*. Also, they were detected with $FeSO_4$ and micronutrient (a) with *C. albicans*. On the other hand, the results revealed that highest main effect and t-value were detected with micronutrient (b) on *B. subtilis*, while they were detected with NaCl and K_2SO_4 in case of *P. aeruginosa*.

Key words: Cyanobacteria · Green microalgae · Antibacterial activity · Antifungal activity

INTRODUCTION

Algal organisms are rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry [1,2,3].

Cyanobacteria and eukaryotic algae occur in fresh water, marine and terrestrial soil habitats. A number of cyanobacteria and microalgae, produce various, biologically active compounds. These include antibiotics which in laboratory tests inhibited bacteria and fungi that incite diseases of humans [4].

In general, isolation of bioactive compounds from cyanobacteria is done with two objectives. One is to discover new compounds for pharmaceutical, agricultural or biocontrol application. The other is to better understand the interactions of individual organisms within their natural communities. For each of these purposes there is a need to screen new culturable organisms [4,5,6,7,8].

Microalgae, such as *Ochromonas sp., Prymnesium parvum,* and a number of blue green algae produce toxins that may have potential pharmaceutical applications [9,10].

Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity [11]. Nitrogen fixing strains produce compounds with different activity spectra and different molecular weights, but their chemical structures have not been established [12].

The aim of the present work was to study the antimicrobial activity of cell extracts of various

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cyanobacteria (e.g. *Anabaena oryzae; Tolypothrix ceytonica; Spirulina platensis)* and of green microalgae (e.g. *Chorella pyrenoidosa; Scenedesmus quadricauda*) *in vitro* against both Gram-positive, Gram-negative bacteria and some pathogenic fungi.

Also a study has been made to determine which nutrient factors control antimicrobial agent production by the cyanobacterium, *Spirulina platensis* by using the Plackett-Burman experimental design against (*Aspergillus flavus, Fusarium moniliforme, Candida albicans, Bacillus subtilis and Pseudomonas aeruginosa*).

MATERIALS AND METHODS

Culturing and Growth of Algal Organisms: Five axenic algal strains were selected for screening their antimicrobial activity against some species of bacteria and fungi. These are: Cyanobacteria *(Anabaena oryzae; Tolypothrix ceytonica; Spirulina platensis)* and green microalgae (*Chlorella pyrenoidosa; Scenedesmus quadricauda*), these axenic species were gifted by Prof. A.F.Khalafa, Phycological laboratory, Botany Department, Faculty of Science, Alexandria University. *Spirulina platensis* was grown in *Spirulina* medium (Table 1). The other four algal species were grown in MBL medium (Table 2). The test algae were grown for 10 days in their respective growth media before use. The axenic cultures of the five algal strains were grown under controlled laboratory conditions (Temperature at 25±3°C and light at 4000 Lux) and a regime of 16 h light/8 h dark.

Test Organisms: The test organisms used in this work were the bacteria (*Escherichia coli, Bacillus subtilis, Staphyllococcus aureus, Pseudomonas aeruginosa*); the fungi (*Aspergillus niger, Aspergillus flavus, Penicillium herquei, Fusarium moniliforme, Helminthosporium sp., Alternaria brassicae)* and the yeast strains (*Saccharomyces cerevisiae, Candida albicans)*, some of them were isolated from the garden soil and were kindly identified by the Regional Center for Mycology and Biotechnology at Al-Azhar University. Others were imported from the institute of Microbiology in Münster, Germany. The bacterial strains were incubated into nutrient broth throughout 24 h, the yeast and fungal strains were incubated into glucose peptone broth throughout 5 days.

Table 1: Composition of the *Spirulina platensis* medium [13]

Macronutrients	Quantity(g)	Macronutrients	Quantity(g)
1-NaCl	1.0	6-K_2HPO_4	0.5
2-$MgSO_4.7H_2O$	0.2	7-$NaNO_3$	2.5
3-$CaCl_2.2H_2O$	0.04	8-K_2SO_4	1.0
4-$FeSO_4.7H_2O$	0.01	9-$NaHCO_3$	16.8
5-Na-EDTA	0.06	10-Distilled H_2O	1000 ml
Solution (a) (mg/l)		Solution (b) (mg/l)	
1- NH_4NO_3	0.0230	1- H_3BO_3	2.820
2- $K_2Cr_2(SO_4).27H_2O$	0.0960	2- $MnCl_2.4H_2O$	1.810
3- $NiSO_4.7H_2O$	0.0440	3- $ZnSO_4.7H_2O$	0.222
4- $Na_2SO_4.7H_2O$	0.0178	4- $CuSO_4.5H_2O$	0.077
5- $Ti(SO_4)_3$	0.0400	5- MoO_3	0.015
6-$Co(NO_3)_2.6H_2O$	0.0440		

Add 1.0 ml from each solutions a and b micronutrients to one litre of the macronutrients. The final pH was adjusted to 9.0-9.5

Table 2: Composition of the Woods Hole MBL medium [14]

Chemical compound	Quantity (g)	Chemical compound	Quantity(g)
1-$CaCl_2.2H_2O$	36.97	1-NaEDTA	4.36
2-$MgSo_4.7H_2O$	36.76	2-$FeCl_3.6H_2O$	3.15
3-$NaHCO_3$	12.6	3-$CuSO_4.5H_2O$	0.01
4-K_2HPO_4	8.71	4-$ZnSO_4.7H_2O$	0.022
5-$NaNO_3$	85.01	5-$CoCl_2.6H_2O$	0.01
6-$NaSiO_3.9H_2O$	28.42	6-$MnCl_2.4H_2O$	0.18
7-Distilled H_2O	1000 ml	7-$NaMoO_4.2H_2O$	0.006
		8-Distilled H_2O	1000 ml

Table 3: Plackett- Burman design for seven variables

	Independent variables						
Trials	1	2	3	4	5	6	7
I	+	+	+	-	+	-	-
II	+	+	-	+	-	-	+
III	+	-	+	-	-	+	+
IV	-	+	-	-	+	+	+
V	+	-	-	+	+	+	-
VI	-	-	+	+	+	-	+
VII	-	+	+	+	-	+	-
VIII	-	-	-	-	-	-	-

Preparation of the Algal Extracts: The algal cultures of 10 days old were centrifuged and the pellets were collected, weighted and used for extraction of antimicrobial agents. 0.5 g of each of the five algal pellets were extracted in 10 ml of either acetone, ethanol, methanol or diethyl ether. All of the extracts were preserved at +4°C [15].

Inhibitory Effect by the Agar-well Diffusion Method: Antibacterial and antifungal activities of cyanobacteria and green microalgae extracts were tested by agar-well diffusion method. Petridishes with 20 ml for each of nutrient agar, then inoculated with 100 µl of a 24 h broth culture of test bacteria and others with 20 ml for each of Sabaroud agar were prepared and inoculated with 100 µl of 5 days glucose peptone broth culture of test fungi and yeast. Indicator microorganisms were spread on agar plates with sterile effusion. Two wells (6 mm) were made and filled with 100 µl extract. The inoculated plates were incubated for 24 h at 37°C for bacteria, and incubated for 3 days at 30°C for (fungi and yeast). After incubation, the diameter of the inhibition zone was measured with calipers and the results were recorded in cm [16].

Besides, comparing the antimicrobial activity of cyanobacteria and green microalgae with standard antibiotics (Erythromycin, Tetracycline, Amoxicillin) and fungicides (Itraconazole and Polynoxylin).

Elucidation and Optimization of the Medium Constituents Controlling Antibacterial and Antifungal Activities by the Cyanobacterium *Spirulina platensis*: The most important medium constituents controlling the antimicrobial activity were elucidated by applying the Plackett-Burman experimental design [17,18]. This experiment is a fraction of two level factorial design and allows the investigation of (n-1) variables in (n) experiments. In this work, seven variables were screened in eight combinations to the design shown in (Table 3). The (+) and (-) are symbols used to indicate the presence or absence of variables, respectively. The main effect of each variable was simply calculated as the difference between the average of measurements made at the presence (+) and absence (-) of that factor. For determination of variable significance, statistical t-values for equal unpaired samples were calculated with respect to the observation records. The seven independent variables include: NaCl, $MgSO_4$, $FeSO_4$, Na-EDTA, K_2SO_4, micronutrients a and micronutrients b, at the same time, the other medium constituents ($CaCl_2$, $NaNO_3$, $NaHCO_3$ and K_2HPO_4) remained constant.

RESULTS AND DISCUSSION

The results obtained from the present study concerning the biological activity of the antimicrobial agents produced by some selected Cyanobacteria and green microalgae against different species of bacteria and fungi were recorded in Table 4. It is clear from this table that the diameter of the inhibition zone depends mainly on type of the algal species, type of the solvent used and the tested bacterial and fungal organisms. Concerning the antibacterial effects, the results cleared that acetone extracts of *Spirulina platensis* and ethanol extracts of *Anabaena oryzae* gave the highest biological activities against *Bacillus subtilis* and *Pseudomonas aeruginosa*. The results cleared also that these two extracts had a moderate activities towards *Escherichia coli* and *Staphyllococcus aureus*.

Anent the extracts of the cyanobacterium *Tolypothrix ceytonica*, the results revealed that the antibacterial effect was greater towards *B. subtilis* and *P. aeruginosa* than towards *E. coli* and *S. aureus*. Negative antibacterial effect was recorded towards the latter bacterial species. Concerning, the antibacterial effect of the tested green microalgae, the results recorded in Table 4 cleared that extracts of *Chlorella pyrenoidosa* had the highest

Table 4: Antibacterial and antifungal activities of different extracts obtained from some cyanobacteria and green microalgae

		Diameter of inhibition zone (cm)											
		Bacterial species				Fungal species							
Algal Specie	Organic solvents	*E. coli*	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*A. niger*	*A. flavus*	*P. herquei*	*F. moniliforme*	*Heminthosporium sp.*	*Alt. brassicae*	*Sacch. cerevisiae*	*C. albicans*
Anabaena	Ethanol	2.0	1.2	2.0	4.0	2.0	2.0	3.0	3.0	2.7	3.0	1.5	1.0
oryzae	Acetone	R	R	1.5	2.0	1.5	3.5	2.0	2.0	1.0	1.0	3.0	R
	Diethyl ether	2.0	2.0	R	R	2.0	2.5	2.0	2.5	1.0	2.5	2.0	0.5
	methanol	1.0	2.0	1.5	2.0	1.0	2.0	1.0	2.5	1.0	0.5	2.0	0.7
Tolypothrix	Ethanol	1.0	4.0	1.0	4.5	1.5	3.0	2.5	3.0	1.0	3.0	1.5	0.4
ceytonica	Acetone	1.2	1.5	R	2.0	0.5	2.5	2.0	3.0	1.0	3.5	2.0	0.2
	Diethyl ether	1.0	2.5	R	R	1.3	1.5	1.5	3.5	1.5	2.5	2.0	R
	methanol	1.0	2.0	R	1.5	0.5	2.0	1.5	0.5	1.5	0.5	1.5	0.3
Spirulina	Ethanol	R	2.0	1.5	R	1.5	4.0	2.7	3.0	3.0	3.5	3.0	2.0
platensis	Acetone	1.0	3.3	R	1.5	1.0	4.0	2.0	2.1	2.5	3.0	2.0	2.5
	Diethyl ether	3.0	2.0	0.5	R	3.5	3.0	3.0	3.0	3.0	3.0	R	3.0
	methanol	R	R	1.0	2.5	1.0	2.5	1.5	2.5	3.0	1.5	2.5	1.5
Chlorella	Ethanol	2.5	1.5	R	4.0	1.8	3.0	2.5	4.0	2.5	2.0	3.0	R
pyrenoidosa	Acetone	R	2.5	R	3.0	R	2.5	1.5	3.5	1.0	3.0	2.0	0.2
	Diethyl ether	1.5	2.5	1.0	3.0	0.5	1.0	3.0	2.0	0.5	2.0	2.0	0.5
	methanol	1.0	2.0	1.0	R	1	1.5	2.0	2.0	1.5	2.0	1.5	R
Scenedesmus	Ethanol	R	4.0	1.0	R	2.6	2.6	1.3	4.0	3.0	3.5	2.0	0.3
quadricauda	Acetone	R	3.0	1.5	R	1.0	3.0	3.5	2.0	1.5	1.0	1.5	R
	Diethyl ether	R	2.5	R	R	2.0	2.5	2.5	4.0	3.0	2.5	2.5	0.7
	methanol	1.5	2.0	1.2	R	R	2.0	0.5	2.0	2.5	2.5	4.0	R

antibacterial activity against *B. subtilis* and *P. aeruginosa*, moderate activity against *E. coli* and a weak or negligible activity towards *S. aureus*. At the same time extracts of *Scenedesmus quadricauda* had an antibacterial effect towards *B. subtilis* and *S. aureus,* but negative effect towards *P. aeruginosa* and *E. coli.*

These results go in harmony with those obtained by [19], they found that some microalgae had high biological activity against *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus megaterium, Escherichia coli*, *Pseudomonas aeruginosa Candida tropicalis* and *Saccharomyces cerevisiae*. The results proved also that ethanol was the best solvent for extracting the antibacterial and antifungal agents from *Anabaena oryzae*, while diethyl ether and acetone were the best organic solvents for extracting the antibacterial and antifungal agents from *Spirulina platensis*. The same results were also obtained by [3,20, 21]. Also, [22] found that extracts of *Spirulina* obtained by different solvents exhibited antimicrobial activity on both Gram-positive and Gram-negative organisms.

Many investigators mentioned that the methanol extracts of *Nostoc muscorum* (a blue green alga) revealed antibacterial activity on *Sclerotinia sclerotiorum* [23,24]. Also, the methanolic extract of a blue green alga has been investigated by [25] for *in vitro* antimicrobial activity against *Proteus vulgaris*, *Bacillus cereus*, *Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus* and *Rhizopus nigricans* using agar cup-plate method.

In the light of the experimental results concerning the antifungal activity against the tested fungal organisms, the results recorded in Table 4 clearly showed that the antifungal activities of nearly all the extracts of both cyanobacteria and green microalgae taxa towards the tested fungi gave positive results but with varying degrees. Ethanol extract of *Anabaena oryzae* and diethyl ether extract of *Spirulina platensis* gave the largest inhibition zones on the plates of the tested fungi. The fungicidal activity of *Anabaena* strains (a cyanobacterium) against a set of phytopathogenic fungi was examined by [26]. The extracellular filtrates from 4 and 8 weeks old cultures of these *Anabaena* strains were further evaluated in terms of hydrolytic enzymes and proteins.

Table 5: Diameters of inhibition zone (cm) exhibited against test microorganisms of standard antibiotics and fungicides.

		Diameter of inhibition zone (cm)				
		Standard antibiotics			Standard fungicides	
Test organism		Erythromycin	Tetracycline	Amoxicillin	Itraconazole	Polynoxylin
Bacterial species	*E. coli*	4.6	4.1	3.5	ND	ND
	B. subtilis	2.8	2.8	5.0	ND	ND
	S. aureus	R	R	R	ND	ND
	P. aeruginosa	2.7	2.7	2.5	ND	ND
Fungal species	*A. flavous*	ND	ND	ND	R	R
	P. herquei	ND	ND	ND	5.0	0.5
	F. moniliforme	ND	ND	ND	2.0	1.3
	Helminthosporium sp.	ND	ND	ND	4.0	2.7
	Alt. brassicae	ND	ND	ND	2.0	0.5
	Sacch. cerevisiae	ND	ND	ND	R	R
	C. albicans	ND	ND	ND	2.0	1.5

ND: Not detected, R: Resistant

Table 6: Diameter of inhibition zone. Main effect, t-value and degree of significance calculated for each of the seven different factors based on antifungal effect of *Spirulina platensis* extracted with diethyl ether affecting *Aspergillus flavus*.

	Variables (factors)													
	Diameter of inhibition zone (cm)													
	NaCl		$MgSO_4$		$FeSO_4$		Na-EDTA		K_2SO_4		Micro a		Micro b	
Trials (n=8)	+	-	+	-	+	-	+	-	+	-	+	-	+	-
m_1	4.5		4.5		4.5			4.5	4.5			4.5		4.5
m_2	3.0		3.0			3.0	3.0			3.0		3.0	3.0	
m_3	4.0			4.0	4.0			4.0		4.0	4.0		4.0	
m_4		2.0	2.0			2.0		2.0	2.0		2.0		2.0	
m_5	4.0			4.0		4.0	4.0		4.0		4.0			4.0
m_6		2.5		2.5	2.5		2.5		2.5			2.5	2.5	
m_7		2.5	2.5		2.5		2.5			2.5	2.5			2.5
m_8		2.0		2.0		2.0		2.0		2.0		2.0		2.0
Total	15.5	9.0	12.0	12.5	13.5	11.0	12.0	12.5	13.0	11.5	12.5	12.0	11.5	13.0
Main effect	1.625		-0.125		0.625		-0.125		0.375		0.125		-0.375	
N	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mean	3.875	2.250	3.000	3.125	3.375	2.750	3.000	3.125	3.250	2.875	3.125	3.000	2.875	3250
Sd	0.346		0.747		0.703		0.747		0.732		0.747		0.732	
t-value	4.695		-0.167		0.889		-0.167		0.512		0.167		-0.512	
Degree of significance	95% (+)		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	

As regards the antifungal effects of extracts of the green microalgae *Chlorella pyrenoidosa* and *Scenedesmus quadricauda*, the diameter of the inhibition zone was nearly the same, but less than those obtained for the two test cyanobacteria.

The antimicrobial activity of the test microorganisms against standard antibiotics and fungicides were shown in Table 5. When the effects of extracts obtained from green microalgae and cyanobacteria were compared with standard antibiotics and fungicides used in this study, it was found that the effect of standard antibiotics was more than that of algal extract on *E. coli*. while, the effect of antibacterial agents resulted from algal extracts on *Bacillus, Staphyllococcus* and *Pseudomonas* were higher

Table 7: Diameter of inhibition zone. Main effect, t-value and degree of significance calculated for each of the seven different factors based on antifungal effect of *Spirulina platensis* extracted with diethyl ether affecting *Fusarium moniliforme*

	Variables (factors)													
	Diameter of inhibition zone (cm)													
	NaCl		$MgSO_4$		$FeSO_4$		Na-EDTA		K_2SO_4		Micro a		Micro b	
Trials (n=8)	+	-	+	-	+	-	+	-	+	-	+	-	+	-
m_1	3.5		3.5		3.5			3.5	3.5			3.5		3.5
m_2	1.5		1.5			1.5	1.5			1.5		1.5	1.5	
m_3	2.0			2.0	2.0			2.0		2.0	2.0		2.0	
m_4		0	0			0		0	0		0		0	
m_5	3.2			3.2		3.2	3.2		3.2		3.2			3.2
m_6		3.0		3.0	3.0		3.0		3.0			3.0	3.0	
m_7		0	0		0		0			0	0			0
m_8		4.0		4.0		4.0		4.0		4.0		4.0		4.0
Total	10.2	7.0	5.0	12.2	8.5	8.7	7.7	9.5	9.7	7.5	5.2	12.0	6.5	10.7
Main effect	0.800		-1.800		-0.050		-0.450		0.550		-1.700		-1.050	
N	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mean	2.550	1.750	1.250	3.050	2.125	2.175	1.925	2.375	2.425	1.875	1.300	3.000	1.625	2675
Sd	1.136		0.926		1.182		1.167		1.160		0.957		1.101	
t-value	0.704		-1.945		-0.042		-0.385		0.474		-1.777		-0.953	
Degree of significance	n.s.		90% (-)		n.s.		n.s.		n.s.		90% (-)		n.s.	

than those of standard antibiotics. While the effect of those standard antibiotics on *Pseudomonas* was varied, it was less than that resulted from extracts of *Anabaena* and *Chlorella* but higher than that of *Scenedesmus* extract and nearly equal to the effect of *Spirulina* extract on *Pseudomonas*.

Also, by comparing the antifungal effect of all tested cyanobacteria and green microalgae, it was more effective on *Sacch. cerevisiae, A. niger*, *A. flavus, F. moniliforme*, *Alt. brassicae* and *C. albicans* than Itraconazole and Polynoxylin. While, it was less effective than that on *P. herquei* and *Helminthosporium* sp. In this study, the antimicrobial activity of microalgae could be explained by the presence of cyclic peptides, alkaloids and lipopolysaccharides. The antimicrobial activities of *B. subtilis. P. aeruginosa, E. coli, S. aureus, Sacch. cerevisiae* and *C. albicans*, against standard antibiotics and fungicides were studied by [10].

As is in the studies reported, it was observed that the extracts obtained from various solvents used in this study had antibacterial and antifungal activities and that these extracts could be much more effective when compared with contemporary antibiotics and fungicides.

The statistical design of Plackett-Burman for the degree of significance of the eight different trials by using seven independent variables of Zarrouk's medium constituents cleared that antibacterial and antifungal effects of acetone and diethyl ether extracts of *Spirulina platensis* cultured for 10 days differently responded. Data recorded in Table 6 revealed that the largest inhibition zone (4.5 cm) was recorded for *Aspergillus flavus* cultures under the effect of diethyl extract of *Spirulina platensis* at trial (m_1). In this trial NaCl, $MgSO_4$, $FeSO_4$ and K_2SO_4 were present. On the contrary, the smallest inhibition zone (2.0 cm) in trial (m_4) where $MgSO_4$, K_2SO_4 and micronutrient (a) and (b) were present, as well as in trial (m_8) where all the seven variables are absent in the culture medium. It is worth to mention that trials (m_3) and (m_5) gave the same size of the inhibition zone (4.0 cm), while at trials (m_6) and (m_7) the diameter of the inhibition zone was (2.5 cm).

Taking into consideration, the results obtained from Plackett-Burman concerning the calculated main effect, t-value revealed that highest main effect and t-value were detected with NaCl. Consequently, these results revealed the degree of significance of this variable (NaCl) was the highest (95%) within the seven independent variables while, the remaining six factors were found to be non significant. So, the effect of NaCl on the antifungal activity of diethyl ether extract of *Spirulina platensis* on growth of *Aspergillus flavus* is the highest and positive.

Table 8: Diameter of inhibition zone. Main effect, t-value and degree of significance calculated for each of the seven different factors based on antifungal effect of *Spirulina platensis* extracted with diethyl ether affecting *Candida albicans*

	Variables (factors)													
	Diameter of inhibition zone (cm)													
	NaCl		$MgSO_4$		$FeSO_4$		Na-EDTA		K_2SO_4		Micro a		Micro b	
Trials (n=8)	+	-	+	-	+	-	+	-	+	-	+	-	+	-
m_1	1.0		1.0		1.0			1.0	1.0			1.0		1.0
m_2	2.0		2.0			2.0	2.0			2.0		2.0	2.0	
m_3	1.8			1.8	1.8			1.8		1.8	1.8		1.8	
m_4		4.0	4.0			4.0		4.0	4.0		4.0		4.0	
m_5	2.5			2.5		2.5	2.5		2.5		*			2.5
m_6		1.2		1.2	1.2		1.2		1.2			1.2	1.2	
m_7		1.3	1.3		1.3		1..3			1.3	1.3			1.3
m_8		1.0		1.0		1.0		1.0		1.0		1.0		1.0
Total	7.3	7.5	8.3	6.5	5.3	9.5	7.0	7.8	8.7	6.1	9.6	5.2	9.0	5.8
Main effect	-0.050		0.450		-1.050		-0.200		0.650		1.100		0.800	
N	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mean	1.825	1.875	2.075	1.625	1.325	2.375	1.750	1.950	2.175	1.525	2.400	1.300	2.250	1450
Sd	0.776		0.755		0.648		0.772		0.730		0.634		0.705	
t-value	-0.064		0.596		-1.621		-0.259		0.890		1.736		1.135	
Degree of significance	n.s.		n.s.		90% (-)		n.s.		n.s.		90% (+)		n.s.	

Table 9: Diameter of inhibition zone. Main effect, t-value and degree of significance calculated for each of the seven different factors based on antibacterial effect of *Spirulina platensis* extracted with acetone affecting *Bacillus subtilis*

	Variables (factors)													
	Diameter of inhibition zone (cm)													
	NaCl		$MgSO_4$		$FeSO_4$		Na-EDTA		K_2SO_4		Micro a		Micro b	
Trials (n=8)	+	-	+	-	+	-	+	-	+	-	+	-	+	-
m_1	2.8		2.8		2.8			2.8	2.8			2.8		2.8
m_2	1.9		1.9			1.9	1.9			1.9		1.9	1.9	
m_3	1.2			1.2	1.2			1.2		1.2	1.2		1.2	
m_4		1.3	1.3			1.3		1.3	1.3		1.3		1.3	
m_5	2.0			2.0		2.0	2.0		2.0		2.0			2.0
m_6		0.8		0.8	0.8		0.8		0.8			0.8	0.8	
m_7		3.0	3.0		3.0		3.0			3.0	3.0			3.0
m_8		3.3		3.3		3.3		3.3		3.3		3.3		3.3
Total	7.9	8.4	9.0	7.3	7.8	8.5	7.7	8.6	6.9	9.4	7.5	8.8	5.2	11.1
Main effect	-0.125		0.425		-0.175		-0.225		-0.625		-0.325		-1.475	
N	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mean	1.975	2.100	2.250	1.825	1.950	2.125	1.925	2.150	1.725	2.350	1.875	2.200	1.300	2775
Sd	0.699		0.679		0.697		0.695		0.653		0.688		0.359	
t-value	-0.179		0.626		-0.251		-0.324		-0.957		-0.472		-4.107	
Degree of significance	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		99% (-)	

Table 10: Diameter of inhibition zone. Main effect, t-value and degree of significance calculated for each of the seven different factors based on antibacterial effect of *Spirulina platensis* extracted with acetone affecting *Pseudomonas aeruginosa*.

	Variables (factors)													
	Diameter of inhibition zone (cm)													
	NaCl		$MgSO_4$		$FeSO_4$		Na-EDTA		K_2SO_4		Micro a		Micro b	
Trials (n=8)	+	-	+	-	+	-	+	-	+	-	+	-	+	-
m_1	3.0		3.0		3.0			3.0	3.0			3.0		3.0
m_2	3.0		3.0			3.0	3.0			3.0		3.0	3.0	
m_3	2.1			2.1	2.1			2.1		2.1	2.1		2.1	
m_4		2.0	2.0			2.0		2.0	2.0		2.0		2.0	
m_5	2.0			2.0		2.0	2.0		2.0		2.0			2.0
m_6		1.8		1.8	1.8		1.8		1.8			1.8	1.8	
m_7		1.2	1.2		1.2		1.2			1.2	1.2			1.2
m_8		1.0		1.0		1.0		1.0		1.0		1.0		1.0
Total	10.1	6.0	10.3	6.9	8.1	7.0	7.0	10.1	8.8	6.3	7.3	8.8	8.9	7.2
Main effect	1.025		0.850		0.275		-0.775		0.625		-0.375		0.425	
N	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mean	2.525	1.500	2.575	1.725	2.025	1.750	1.750	2.525	2.200	1.575	1.825	2.200	2.225	1800
Sd	0.364		0.650		0.451		0.667		0.388		0.533		0.527	
t-value	2.818		1.307		0.610		-1.162		1.610		-0.704		0.807	
Degree of significance	95% (+)		n.s.		n.s.		n.s.		90% (+)		n.s.		n.s.	

The treatment of *Fusarium moniliforme* with the diethyl ether extract of *Spirulina platensis* cultured under the eight different trials of Plackett-Burman design (Table 7) revealed that maximum inhibition was detected at trial (m_8), where all the seven variables were absent from the medium. The data also revealed that there is no inhibition zones were detected in trials number (m_4) and (m_7). Based on these observations, the main effect and t-values calculated for each independent variables and cleared that maximum values of these calculations were detected with $MgSO_4$ and micronutrients (a). Consequently, these two variables were the most significant independent variables that affect the production of antimicrobial agents of *Spirulina platensis* on *Fusarium moniliforme*.

The results obtained by applying the diethyl ether extract of the eight different *Spirulina platensis* culture media as antifungal affecting *Candida albicans* (Table 8), revealed that largest diameter of inhibition zone (4.0 cm) was detected at trial (m_4), where $MgSO_4$, K_2SO_4, micronutrient (a) and micronutrient (b) were present. While the minimum diameter of inhibition zone was detected at trials number (m1) and (m_8). Among the seven independent variables Na-EDTA, micronutrient (a) and micronutrient (b) were absent in trial (m_1), while these variables were completely absent at trial (m_8). $FeSO_4$ and micronutrients (a) showed the highest main effect on the antifungal activity (-1.050 and +1.100 respectively) and their calculated t-values were significant at 90% level.

The results and calculations for the Plackett-Burman experiment of the acetone extract of the seven different factors of *Spirulina platensis* affecting *Bacillus subtilis* were presented in Table 9. The maximum inhibition zone (3.3 cm) was detected at trial number (m_8), where all the seven factors were absent from the culture medium, on the other hand, the minimum diameter of inhibition zone was detected at trial number (m_6), where $FeSO_4$, Na-EDTA, K_2SO_4 and micronutrient (b) were present. These results and calculations for this experiment revealed that highest main effect and t-value were detected with micronutrient (b) (-1.475 and -4.107, respectively). Therefore, micronutrient (b) was the most significant factor among all the examined factor of *Spirulina* medium that negatively affecting the antibacterial effect of *Spirulina platensis* extract on *Bacillus subtilis*.

Spirulina platensis cultured under the eight different factors then extracted with acetone and applied to determine the antibacterial effect against *Pseudomonas aeruginosa* were represented in Table 10. These recorded data showed that largest diameter of inhibition zone (3.0 cm) was achieved at trials number (m_1) and (m_2)

where NaCl and $MgSO_4$ were present in both trials and $FeSO_4$ and K_2SO_4 were found at trial (m_1) while Na-EDTA and micronutrient (b) were present at trial (m_2). On the other hand, in the absence of the seven factors, the acetone extract of *Spirulina platensis* exhibited the smallest inhibition zone of *Pseudomonas aeruginosa* (1.0 cm). One can conclude that, NaCl and K_2SO_4 gave the highest main effect (1.025 and 0.625, respectively). Furthermore, these two factors represented the highest and positive value as well as highest degree of significance.

Medium optimization is generally a time consuming and labour-intensive process. The Plackett-Burman experimental design proved to a valuable tool for the rapid evaluation of the effects of the various medium components [27]. Temperature of incubation period, pH of the culture medium, incubation period, medium constituents and light intensity are the important factors influencing antimicrobial agent production [11,28].

NaCl, $MgSO_4$, $FeSO_4$, K_2SO_4, micronutrient (a) and (b) were relatively the important factors that affected the antimicrobial activity of *Spirulina platensis*. Concerning $MgSO_4$, $FeSO_4$, K_2SO_4, probably sulphur was found to be important and necessary for the activity of certain enzymes involved in protein synthesis [29,30].

Optimal concentrations of N, S, P, K^+, Na^+, Ca^{2+} and Mg^{2+}, during the growth and tolytoxin formation of *Sytonema ocellatum* were examined by [8], finding that, in most cases, optimal scytophycin production was associated with maximal vegetative growth. It could be concluded that expected that antibiotic production and activity depend mainly on physiological factors and the tested organism [31].

It is worthily mentioning that the extracts obtained from various solvents used in this study had antibacterial and antifungal activities, and some of these extracts could be more effective than antibiotics and fungicides. However, these microalgae are potential sources of bioactive compounds and should be investigated for natural antibiotics.

It is intended that the present work will contribute to an understanding and determining the medium factors that controlling the bioactive material produced by some algae. So, these bioactive compounds will need further studies to identify the chemical structures of these active compounds and to examine their beneficial effect for inhibition of some pathogenic bacteria and fungi. Because antimicrobial metabolites of algae are of special interest in the development of new environment harmless.

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