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#### Determination of the effect of some biological products of *Synechocystis* pevalekii on some pathogenic bacteria isolated from wounds and urinary tract

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### Abstract

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he sample were collected from different environments, growth and diagnosis of Cyanobacteria Species Synechocystis pevalekii was conducted in AL-Dour district / Salahaldeen governorate rocks having algae taken from the edge of the river, a water sample and mud sample from the river, and a soil sample from a planted land. The medium BG11 was used for its growth and measuring the daily increase of growth and chlorophyll these indicators were compared during their growth in four modified media of BG11 which were BG11+0.5g/L NaNo<sub>3</sub>, BG11+0.006g/L (NH<sub>4</sub>)<sub>5</sub>[Fe(C<sub>6</sub>H<sub>4</sub>O<sub>7</sub>)<sub>2</sub>]2H<sub>2</sub>S and BG11+0.1µ/L MoO<sub>3</sub> and the media BG11 was subjecte to all the mentioned additions, and the last was the medium BG11 was subject to all the mentioned additions the most effective in increasing daily growth and chlorophyll. Its bio- products were also extracted and the inhibitory efficacy of biological products of Synechocystis pevalekii was selected for species of bacteria isolated from wounds and urinary tract E.coli, Klebseilla pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis with the concentrations 10,000, 7000, 5000, 3000, 2000, 1000 µgm/ml. The concentration 10,000 µgm/ml showed a clear inhibitory effect E.coli 12mm, klebseilla pneumonia 5.5mm, Staphylococcus aureus 14 mm, Staphylococcus epidermidis 15.5mm whereas the concentration 1000 µgm/ml did not show any inhibitory effect for the growth of isolated bacteria under study. The other concentrations showed varied effects. The active compound of the bio-product for the species of Synechocystis pevalekii was diagnosed by the device GC-Mass as a Ndi-Alanylglycin. In addition the IR device was used and showed the active groups the alkanes and the amides and alkines and aldehydes and aminos.

#### Introduction

In order to Cyanobacteria to be active in the growth, reproduction and synthesis of protein, chlorophyll, nitrogen fixation and other activities, it needs different proportions of nutrients such as nitrogen, phosphorus, sodium, calcium, magnesium, potassium, iron, molybdenum, cobalt and other elements. Each environment posses limited concentrations of nutrient elements in which its proportions determine according to the change of physical, chemical and biological conditions [1]. In addition Cyanobacteria have the ability to release chemical substances as products of secondary metabolism which are antimicroorgansms, as well as the release of various compounds of biological activity such as toxins and some material inhibition some species of bacteria which are anti-bacteria [2].

Some species of cyanobacteria produce antibiotics and medical drugs of therapeutic effects ,most notably the genus of *Microcystis sp., Sytonema sp., Nostoc sp., Phormidium sp., Oscillatoria sp* [3], where the compound diterpenoids has been isolated from Cyanobacteria species *Nostoc commune* which is considered an active anti-bacteria [4]. Studies and researches referred to that fact the cyanobacteria is

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considered a potential power source for a number of chemicals and medical drugs not yet exploited. There are many isolates of cyanobacteria that secrete toxic substances that can be used as anti-cancer drugs, an aesthetic substances ,and others [5]. Thus , the aim of this study was to know the effect of some nutrient elements on the increase of cyanobacteria growth and quantity of chlorophyll, in addition to determine the effect of the biological extract of the cyanobacteria species Synechocystis pevalekii against some species of isolated bacteria from wounds and urinary tract, and evaluate its efficiency compared to some antibiotics and to detect its active compounds.

Synechocystis pevalekii It is a species of cyanobacteria which is Unicellular. Its Cells are spherical or hemispherical surrounded by a very thin membrane the size of its cells is 2.5-3.5 µ and exists as single or in colonies in freshwater of ten with a light blue green color [6].



Figure (1) : Photomicrographs of Synechocystis pevalekii (40X)

#### Materials and methods

#### **Collection of Samples:**

The samples of the study were collected from different environments with in the areas of AL-Dour district . The samples consisted of rocks having algae taken from the edge of the river, a water sample and mud sample from the river, and a soil sample from a planted land . The samples were transferred in sterilized bottles and bags prepared for this purpose.

#### Culture medium used for cultivation and growing cvanobacteria:

The selective culture medium BG11 was used which is considered a complete or complex culture medium in nutrient elements, in addition it provides all the nutrient requirements for cyanobacteria [7] which is composed of the following with the concentration indicated for each by (g/L): NaNO<sub>3</sub>-1.5 Na2EDTA-0.001  $(NH_4)_5 [Fe(C_6H_4O_7)_2] 2H_{25} - 0.006$ CH<sub>3</sub>(CH<sub>2</sub>)16CO<sub>2</sub>H-0.0064 K<sub>2</sub>HPO<sub>4</sub> -0.04 ·MgSO<sub>4</sub>.7H<sub>2</sub>O-0.075 · Na<sub>2</sub>CO<sub>3</sub> -0.02 · CaCl<sub>2</sub>.2H<sub>2</sub>O-0.036 and added to it ml/L of Trace metal mix A5 which is composed of the following with the concentrations indicated for each by(g/L): H<sub>3</sub>BO<sub>3</sub> -ZnSO<sub>4</sub>.7H<sub>2</sub>O-0.222, Mncl<sub>2</sub>.4H<sub>2</sub>O-1.81, 2.86, Co(No<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O-0.04, Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O-0.39,

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dissolved in distilled water and the pH adjusted in ranges pH(7.6-7.8) using sodium bicarbonate or diluted hydrochloric acid caliber (0.1), Sterilized with the device Autoclave at temperature 121° C and Pressure 15 pound/inch<sup>2</sup> for 15 minutes and kept in room temperature until use . As for the preparation of the solid medium BG11, agar was added to the liquid medium with a percentage 1%[8].

#### Culture, growth, isolate and diagnosis of **Cyanobacteria:**

Samples were inoculated on petri dishes containing the solid medium BG11 and incubated in a cooled incubator at temperature 25°C with a light intensity 2500 Lux. After two weeks, the colonies of cyanobacteria appeared. Microscopic examination was carried out in the petri dish using an microscope anatomy and marked by a marker pen. Then, each colony was transferred to an independent petri dish of the solid medium BG11. The operation and examination were repeated to ensure purity of the colonies, and pure isolates were transferred to the liquid medium BG11in glass flasks size 250ml in which each flask contains 100ml and placed in the shaker incubator with a speed of 100 rpm[9]. The isolated Synechocystis pevalekii was diagnosed by an optical microscope equipped with a camera, and diagnosis depended on Willey [6].

#### Measuring the daily growth of the selected cvanobacteria:

The daily growth of the cyanobacteria species Synechocystis pevalekii was measured by taking 5 ml from the culture and measured according to optical density on a wave length of 436 nm using the reading of a Spectrophotometer type Apel PD-303 according to Gibson [10].

#### Measuring chlorophyll A :

The method Mackinney[11] was followed in measuring the chlorophyll A for cyanobacteria species Synechocystis pevalekii through taking 5ml from the culture and isolating the cells from the liquid medium using a centrifuge device with a speed of 3500 rpm for five minutes. The residue was taken and then acetone was added with a concentration of 80%. The suspended cells with acetone were broken by an ultrasonic device with a speed of 24,000 freguency /second. The solution resulting from the breaking process was filtrated, and the result which contains chlorophyll A as a solvent was taken and the size was completed to 100ml by acetone with a concentration 80%. The optical density is read by an optical spectrophotometer which consists of a quartz cell with a thickness 1cm on two wave lengths (633,645 nanometer) after resetting the device by blank acetone 80%, and calculating the chlorophyll concentration by applying the formulae:

Chlorophyll (A) = 127\*663A - 269\*645A = mg/ml.

Determine effect of different nutrient in the culture medium BG11 on the growth of the selected cyanobacteria and in the amount of chlorophyll A:

CuSO<sub>4</sub>.5H<sub>2</sub>O-0.079. The medium ingredients were

The species *Synechocystis pevalekii* was cultured in the liquid culture medium BG11 and the modified medium as shown below . The daily readings for growth and chlorophyll A amount were taken for 16 days .

1- BG11Modified(1) + NaNO<sub>3</sub> 0.5 g/L.

2- BG11Modified(2) +  $(NH_4)_5[Fe(C_6H_4O_7)_2]_{2H_{25}} = 0.006$ g/L .

3- BG11Modified(3) + MoO<sub>3</sub>  $\mu/L$ 

4- BG11Modified(4) + NaNO<sub>3</sub> 0.5g/L + MoO<sub>3</sub>0.1 $\mu$ /L + (NH)) [E<sub>2</sub>(C, H, O<sub>2</sub>)]2H<sub>2</sub> 0.006 g/L

 $(NH_4)_5[Fe(C_6H_4O_7)_2]2H_{25}\,0.006g/L$  .

## Extraction of biological products of cyanobacteria:

The biological products of Synechocystis pevalekii were extracted in the 16<sup>th</sup> day of the implantation by collecting its cells using a centrifuge with a speed 3000Prm for 5minutes to obtain the supernatant and precipitate from the cultivation. The precipitation was taken and dissolved in ethanol of g/10ml [12], The precipitation was broken using an ultrasonic device with a power of 24,000 frequency/second. The solution is then placed in the centrifuge with a speed of 3000 rpm for 10 minutes . The leachate is taken and the ethanol solvents were evaporated from the supernatant using a Rotary Evaporator device with a temperature less than 40°C to obtain on extract. The extract was dissolved in a small quantity of distilled water and the bioproducts using 70% concentration of Ammonium sulphate. The precipitate was separated from the supernatant by placing the solution in the cooled centrifuge tubes with a speed 3000 rpm for 30 minutes. The Ammonium sulphate salt was removed from the precipitation containing the protein by the process of dialysis, and the contents of the bag were poured in a discater which is empty of air under unstable pressure to obtain the proteins which contain the biological products in its dry from, and then dissolved in to the solution Dimethyl Sulpho Oxide (DMSO) with the concentrations 10,000, 7000, 5000, 3000, 2000, 1000 microgram/ml [8].

## The Effect of *Synechocystis pevalekii* bioproducts concentration on the selected bacteria species for study in comparison to antibiotics:

The bacteria species isolated from wounds and urinary tract infection which are E.coli, Klebseilla pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis were obtained from the laboratory of microbiology in the college of Education for Pure Sciences- Tikrit University, 0.05 ml of the mentioned biological products concentrations were placed in wells of 6 mm diameter made by a cork Borer on the trams culture medium Mueller Hinton Agar which is inoculated 0.1 ml of bacterial suspension. In the other half of the petri dish, the antibiotics tablets were placed and incubated at a temperature of 37°C for 24 hours. The dishes were photographed and the results were recorded by measuring the inhibition diameter zone and comparing it to inhibition diameter zones of antibiotic species. The ready antibiotic tablets carrying the trademark Gokhan Laboratuvar san T.I.C.A.S. were used and which are placed in wellsealed boxes in which its names and concentration are indicated in microgram/tablet. These included tetracycline- $30\mu g$  ampicillin- $20\mu g$ , gentamicin- $10\mu g$ , erythromycin- $15\mu g$ , vancomycin- $10\mu g$ .

#### Analysis of active compound biological products using the technique of gas chromatography device – GC-Mass:

The samples of biological products for *Synechocystis pevalekii* were analyzed using the gas chromatography device GC-Mass type Shimadzu Ultra 2010 using Helium as a gas 99.99% in the laboratory of the College of Applied Sciences – Samarra University.

## Analysis of biological products using the technique of Infrared Spectrum I.R:

The sample of biological products for *Synechocystis pevalekii* were analyzed by grinding the sample with potassium bromide (KBr) by a weight of 1% for potassium bromide. The sample was then placed in a mold and pressed as a tablet by the Infrared Spectrum deuce type Shimadzu I.R-435 in the College of Sciences– Tikrit University.

#### Statistical Analysis:

The results were analyzed statistically using Analysis of variance (ANOVA) and using the Minitab program in the analysis, and the arithmatic means of correlations were compared according to Duncan's Multiple Range test with a significance level (P=0.05) [13].

#### **Results and Discussion**

#### The effect of nutrient elements in the culture medium BG11 on the growth of cyanobacteria which is selected for study:

The results showed that the modification of the culture medium BG11 has a clear effect on the increase of the daily growth of the selected cyanobacteria species Synechocystis pevalekii, since there was an increase in the concentration of NaNO<sub>3</sub> in the culture medium BG11 modified 1 on cyanobacteria species Synechocystis pevalekii as result of increase in the daily growth and this recorded the highest reading of 0.686 optical intensity. Thus, growth is 31.7% higher from the highest reading of the same species of cyanobacteria which is 0.469 optical intensity for the natural composition of the culture medium BG11, were as the concentration increase of Ferric ammonium citrate in the modified culture BG11 medium 2 on the some type was less than that of the culture medium BG11 Modified 1. The highest reading of growth was 0.515 optical intensity and this recorded a growth higher by 9% than the growth of the natural composition of culture medium BG11. The effect of addition MoO<sub>3</sub> with a concentration of  $0.1\mu/L$  to the culture medium BG11 modified 3 recorded the highest reading for growth with 0.536 optical intensity, obtaining a growth percentage higher by 13% than the growth for the same species of cyanobacteria on the natural composition of culture medium BG11. The result of concentration increased

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in NaNO<sub>3</sub> and Ferric ammonium citrate and addition of MoO<sub>3</sub> in the culture medium BG11 modified 4 to cyanobacteria species *Synechocystis pevalekii* showed an increase in growth which recorded the highest reading of 0.741 optical intensity and this is the highest percentage of growth 36.7% than the growth for the same species of cyanobacteria on the natural composition of culture medium BG11 the Figure 1 shows the effect of BG11 modified 1and 2 and 3 and 4 transplant media on the daily growth of cyanobacteria species *Synechocystis pevalekii* compared the growth in the natural composition BG11 culture medium. The results of the statistical analysis showed lack of significant differences between the increase of growth for cyanobacteria species *Synechocystis pevalekii* on the two media BG11 modified 2 and 3 and the natural composition of medium BG11. There was also significant differences in the increase of growth for the same species of cyanobacteria in the two mediums culture BG11 modified 1 and 4 and the natural composition for the transplant medium BG11 at the significant level P=0.05 as clarified in the Table 1.



Figure 2 : Measuring the growth of *Synechocystis pevalekii* and treatment by standard and varied of NaNO3 and (NH4)5[Fe(C6H4O7)2]2H25 , addition of MoO3 and treatment for al variables

The result of modifying the culture medium BG11 showed a varied effect which supports the amount of chlorophyll A in the species cyanobacteria selected for study with varied concentrations according to the growth medium. The effect of increasing the concentration of NaNO3 in the culture medium BG11 modified 1 on the amount of chlorophyll A resulting from cyanobacteria species Synechocystis pevalekii was increase. The increase in the amount of chlorophyll A continued to settle on the sixteenth day of the cultivation recording 1.733 mg/ml, so that it becomes higher by 30,4% from the amount of chlorophyll resulting from the same type in the BG11 transplant medium of the natural composition which was 1.206 mg/ml . As for the increase of Ferric ammonium citrate concentration in the medium BG11 modified 2, it had an effect on the increase of chlorophyll A amount resulting from cyanobacteria species Synechocystis pevalekii in a gradual form, recording the amount of chlorophyll A as 1.265 mg/ml, and this is higher by 4.7% from the amount of chlorophyll A resulting from the same species in the culture medium BG11 of the natural composition.

The addition of MoO<sub>3</sub> to the medium BG11 modified 3 showed an increase in the amount of chlorophyll A for the cyanobacteria species *Synechocystis pevalekii* recording 1.281 mg/ml which is higher by 5.9% from the amount of chlorophyll resulting from the same

species of cyanobacteria in the culture medium BG11 of the natural composition. The increase of NaNO<sub>3</sub> and Ferric ammonium citrate and addition of MoO<sub>3</sub> to the medium BG11 modified 4 had an effect on the amount of chlorophyll A for the cyanobacteria species Synechocystis pevalekii which was 1.841 mg/ml which is higher by 34.5% from the amount of chlorophyll A resulting from the same type in the culture medium BG11 of the natural composition. Figure 2 illustrates the effect of culture mediums BG11 modified 1 and 2 and 3 and 4 on the the daily increase of the amount of chlorophyll A for cyanobacteria species Synechocystis pevalekii compared to the daily increase of the amount of chlorophyll A for the same species of cyanobacteria in the culture mrdium BG11 of the natural composition. The statistical analysis results showed a lack of significant differences for the daily increase of the amount of chlorophyll A for Synechocystis pevalekii in the culture medium BG11 modified 2 compared to the culture medium BG11 of the natural composition, were as the presence of significant differences between the daily increase of the amount of chlorophyll A for the same species of cyanobacteria in the culture mediums BG11 modified1 and 3 and 4 compared to the culture medium BG11 of the natural composition at the significant level P=0.05 as illustrated in the Table 1.



Figure 3 : Measuring of the chlorophyll A for *Synechocystis pevalekii* and treatment by standard and varied of NaNO<sub>3</sub> and (NH<sub>4</sub>)<sub>5</sub>[Fe(C<sub>6</sub>H<sub>4</sub>O<sub>7</sub>)<sub>2</sub>]2H<sub>25</sub>, addition of MoO<sub>3</sub> and treatment for al variables

 Table 1 the statistical analysis results for the effect of different some nutrients in the culture medium

 BG11 on the daily increase of growth and amount of chlorophyll A for the species of cyanobacteria

 selected for study

Scietted for Study										
Medium	BG11 Modified	BG11 Modified	BG11 Modified	BG11 Modified	BG11					
type	(1)	(2)	(3)	(4)	Standard					
Effect										
Growth	0.4441±0.1749	0.3558±0.1219	0.3410±0.1493	$0.4679 \pm 0.2096$	0.3078±0.1158					
	А	В	В	А	В					
Chlorophyll	1.1813±0.3885	$0.9099 \pm 0.2584$	1.0414±0.1963	1.2530±0.402	$0.8282 \pm 0.2790$					
	ab	bc	В	а	С					

\* Similar letters indicate lack of significant differences at the level P=0.05

Thus, the culture medium BG11 modified 4 is the best for the increase of daily growth from the best of the culture medium modified and the most efficient for the increase of the a mount of chlorophyll A for the species of cyanobacteria selected for this study. It was noticed that the cyanobacteria cultivation differed in its growth according to the difference of components and if concentrations of culture media, were the cyanobacteria varied in the rapid representation of nutrients and their rapid utilization and consumption. These results were agreed with [14] in that the efficiency of culture media (cultures) vary according to components of elements and nutrients in stimulating growth, time of multiplication and extension of the stability time.

We notice that the nitrates are present in the BG11 culture in the form of NaNO<sub>3</sub> which are very necessary for growth. In addition, the increase in nitrate concentration may be a clear cause of increased growth and rise of chlorophyll amount and especially in the BG11 modified 1 and 4 cultures. This is consistent with what was indicated by Grobbelaar [15] in the variance of concentrations of nutrients from on culture to another and that the growth of cyanobacteria depends in general on the availability of nitrogen, phosphorus and carbon mainly, as well as the rest of elements like potassium, sulphur, iron, manganese, zinc and molybdenum even if in small percentages [16].

Stressed Yang [17] that doubling ferric ammonium citrate has little effect on the increase of growth, where as removing this component from the culture will cause a clear delay in growth and reduction of cell intensity in the culture. This is consistent with what we found during our study of the selected species of cyanobacteria. The molybdenum component is one of the elements needed by cyanobacteria at very low concentrations and is included in the synthesis of enzyme nitrogenase which is composed of two proteins containing elements of iron, sulfur and molybdenum. The first protein is called component- I (Mo-Fe-protein) which is composed of two atoms of molybdenum and  $(30\pm2)$  iron atoms, while the second protein is called component- II (Fe-protein) and consists of four atoms of iron and four atoms of sulfur. The work of the enzyme nitrogenase requires the presence of the two proteins together to perform atoms pheric nitrogen stabilization [18].

#### Sensitivity of some species of bacteria isolated from wounds and urinary tracts towards the antibiotics used:

The inhibitory activity of antibiotic types varied according to their types and concentrations and according to species of bacteria. Table 2 illustrates the sensitivity of each types towards the used antibody.

Table 2 the sensitivity of some species of bacteria isolated from wounds and urinary tracts towards typ	es
antibiotics	

Bacteria		Ampicillin SAM/20		Vancomycin VA/10		Tetracycline T/30		Erythromycin E/15		Gentamicin GM/10	
		I.D	S	I.D	S	I.D	S	I.D	S	I.D	S
			MS		MS		MS		MS		MS
			R		R		R		R		R
Escherichia coli	Wound	8	R	7	R	22	S	/	R	21	S
Klebseilla pneumoniae	Urin	10	R	13	S	10	R	15	R	16	S
Staphylococcus aureus	Urin	14	MS	13	S	15	MS	/	R	19	S
Staphylococcus epidermidis	Wound	19	S	/	R	23	S	/	R	21	S

Concentrations of antibiotics by microgram/disc

I.D= inhibition diameter, S = Susceptible , MS = Moderate Susceptible , R = Resistant

The effect of different concentration of the biological product for cyanobacteria species of *Synechocystis pevalekii* on inhibiting the growth of some species of bacteria isolated from wounds and urinary tracts compared to some antibiotics:

The result of inhibition of *Synechocystis pevalekii* concentrations 10.000, 7000, 5000, 3000, 2000, 1000 microgram/ml showed its effectiveness towards some species of bacteria isolated from wounds and urinary tracts and the most prominent concentration was

10,000 microgram/ml in which the highest inhibiting diameters were in *Staphylococcus aureus* and *Staphylococcus epidermidis* while the lowest inhibiting diameter in *E.coli* and *Klebseilla pneumoniae*. The remaining concentrations gave a varied defect according to species of bacteria compared to the antibiotics illustrated in Table 2. In addition, the concentration 1000 microgram/ml did not show its inhibiting activity for bacteria growth as illustrated in Table 3 and figure 3.

 Table 3 the inhibition diameters biological of Synechocystis pevalekii against some species of bacteria isolated from wounds and urinary tracts infection

	Concentrations of the biological product by microgram/ml						
Bacteria	10,000	7000	5000	3000	2000	1000	
Escherichia coli	12	9	7	3	1	/	
Klebseilla pneumonia	5.5	3.5	3	2	/	/	
Staphylococcus aureus	14	9.5	6	3	2	/	
Staphylococcus epidermidis	15.5	10	8	5	3	/	



figure 3 : the effect of different concentrations of biological product of Synechocystis pevalekii on
 inhibiting the growth of some species of bacteria isolated from wounds and urinary tracts
\* Numbers symbolize the concentration of biological product in thousand microgram /ml .
\*P= Synechocystis pevalekii

\*D=DMSO

The result showed a difference in the effectiveness of inhibition biological products of Synechocystis pevalekii according to different species of bacteria whether positive and negative towards Grams stain and which are isolated from the wounds and urinary tracts cultivated in Mueller Hinton Agar culture compared to some antibiotics. It was noticed that some of these types show its resistance against the antibiotic or the biological product (output) at a particular concentration, while other species of bacterial microbiology show its sensitivity towards the same antibiotic in the same concentration. In addition, some species of bacteria are sensitive towards the antibiotic or biological product at a certain concentration while it shows its resistance at a less concentration and this is consistent by what has been found by [19]. It is be lived that the difference of bacteria sensitivity towards the biological products of cyanobacteria is the chemical genetic makeup of bacteria [20], or it may be due to the nature of the bacteria cell membrane which consists of two layers consisting of high level of peptidoglycan, lipopolysaccharides and phospholipids, which in turn acts as an obstacle to the entry of antimicrobes and reducing its effects on it [21].

## Mass spectrum of active compounds in the biological product of *Synechocystis pevalekii* separated by GC-Mass technology:

Figure 4 shows the mass spectrum of the active compound which is present in *Synechocystis pevalekii* which was separated by GC-Mass chromatography Gas with a time limit of (9.3) minutes. During the matching of the effective compound with the special information base of the device, it was found to be an alkaloid compound N-dl-Alanyglycin of molecular 146 dalton and its chemical formulae is  $C_5H_{10}N_2O_3$  which is the reason for inhibition of bacterial plantation selected for study.

## Diagnosing the biological product of *Synechocystis pevalekii* using I.R device:

Figure 5 illustrates the infrared spectrum with the appearance of the following :

1- The alkanes group C-H with in the spectral absorption site  $2850-2970 \text{ cm}^{-1}$ .

2- The Amides group N-H with in the spectral absorption site 3200-3540 cm<sup>-1</sup>.

3- Appearance of alkines group C=C with in the spectral absorption site  $2100-2270 \text{ cm}^{-1}$ .

4- Appearance of aldehydes group C=O with in the spectral absorption site  $1650-1780 \text{ cm}^{-1}$ .

5- Appearance of Aminos group C-N with in the spectral absorption site  $1180-1360 \text{ cm}^{-1}$ .



Figure 4 : GC-Mass of the biological product for Synechocystis pevalekii



Figure 5 I.R infrared spectrum of the biological product for Synechocystis pevalekii

#### Reference

1- Wilson, J.T.,& Alexander, M., (1979). Effect of soil nutrient status and pH on nitrogen fixing algae in flooded soils. *Soil Sci. Soc. Am. J.* 43:936-939.

2- Biondi, N.; Tredici, M.R.; Taton, A.; Wilmotte, A.; Hodgson, D.A.; Losi, D.; and Marinelli, F. (2007). Cyanobacteria from benthic of antractic lakes as a Source of new bioactivites. J. of Applied Micro. ISSN, 1364-5072.

3- Hikmet, K.; Yavuz, B.; Belma, A.; Zehra, Y.; and Tahir, A. (2006). Screening Antimicrobial agent production of some microalgae in fresh water, Internet J. of Microb. 2(2):574-645.

4- Jaki, B.; Heilmann, J.; and Sticher, O. (2000). New antibacterial metabolites from the cyanobacterium *Nostoc commune*, EAWAG 122b., Nat. prod.63:1283-1285.

5- Liaimer, Anton; Jenson, John B.; Dittmann, Elke. (2016). A Genetic and Chemical Perspective on Symbiotic Recruitment of Cyanobacteria of the Genus *Nostoc* into the Host Plant *Blasia pusilla* L, Frontiers in Microbiology, dio.: 10.3389 / fmicb, Vol.7.:1-16.

6- Willey, J. M.; Sherwood, L.M.; and Woolverton, C.J. (2011). Precscott's Microbiology. 8thed. Mc Graw-Hill companies, Inc.

7- Khamis, H.S.; Sultan, A.M.; Salim, A.A. (2016). Study the effect of Crude Oil in some Physiological in *Oscillatoria chlorin*, Diyala Journal for Pure sciences, vol.12 No4 68-84.

8- Hamoshi, Rawaa M.D. (2012). Effect of some Bioproducts and Genetic Relation of  $N_2$ -Fixing cyanobacteria on some pathogenic bacteria , Ph. D. Thesis ,college of education ,University of Tikrit .

9- Ahmad, Asraa A. M. (2014). Effect of some compound inorganic nitrogen and iron on tow ganera nitrogen fixing cyanobacterium *Nostoc sp. Tu90 & Anabaena sp.Tu91* which isolated from Salahaldeen governorate, master Thesis ,college of education for women ,University of Tikrit.

10-Gibson, C. E. & Fay, R. H., (1983). The photosynthesis and growth efficiency of a planktonic

Blue Green Algae *oscillatoria redeke*. Br. Phycol. J. 18: n 39-45.

11-Mackinney, G., (1941). Absorption of light by chlorophyll Solution. Boil Biochem. Vol 140:315-322.

12-Moncheva, P.; Tishkov, S., Dinmitrova, N., Hipera, V., Antonova- Nikolova S., Bogatzerska. N., (2002). Characteristics of soil Actinomycetes from Antartica. J. Cult. Collect, 3:3-14.

13-Al-Rawi, Qashaa Mahmoud and Abdalazez, Mohammed K. (1982). Design and analysis agricultural experiences, agriculture college – University of Al-Mosul.

14-AL-Aqyli, Thaer M. Ibrahim and Alsalman , Ibrahim M. A. (2016). Stimulating Growth rates and doubling time of green algae scenedesmus quadricauda by using different culture medium, Diyala Journal for Pure sciences, vol:12, No:3,78-93.

15-Grobbelaar, J.U. (2004).Algal niutrition. Mineral nutrition. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*; Richmond, A., Ed.; Blackwell Publishing Ltd.: Oxford, UK, 97–115.

16-Deshmukh, N.S. and. Puranik, P. R. (2016). Influence of Iron on growth and siderophore production by Phormidium sp., Global Journal of Bio-Science and Biotechnology, VOL.5 (3): 355-361 17-Yang, Chih-Chun; Wen, Rex C.; Shen, Claire R.; and Yao, Da-Jeng .(2015). Using a Microfluidic Gradient Generator to Characterize BG-11 Medium for the Growth of Cyanobacteria *Synechococcus elongatus* PCC7942, Micromachines Journal, 6, 1755–1767.

18-Kulasooriya S.A. (2008). *Biological Nitrogen Fixation:Fundamentals and Utilization. Peradeniya Science Publication No: 27*, 143. Science Education Unit, Faculty of Science, University of Peradeniya, Peradeniya.

19-AL-Doury, Nihad Abid Mohammed. (2005). Environmental Study for some species of cyanobacteria isolated from djela river from salahaldeen and testing activity bioproducts effects on some pathogenic bacteria, A thesis Education Collage – University of Tikrit.

20-Caccamese S, Azzolina R, Furnari G, Cormaci M, Grasso, S (1981). Antimicrobial and antiviral activities of some marine algae from eastern Sicily. Botanica Mar 1:365-367.

21- Al-Samary I (1999). A study of antibacterial activity of aqueous extract of *Allium sativum* L. (Liliaceae). Eastern Mediterranean Health J 5: 803-810.

# تحديد تأثير بعض النواتج الحيوية لنوع السيانوبكتريا Synechocystis pevalekii على بعض انواع المرضية المرضية المعزولة من اخماج الجروح والمجاري البولية

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#### الملخص

تم عزل وتتمية وتشخيص نوع السيانوبكتريا Synechocystis pevalekii المعزولة من قضاء الدور في محافظة صلاح الدين واشتملت العينات صخور نمت عليها الطحالب مأخوذة من حافة نهر وعينة مائية وعينة طينية من النهر وعينة تربة مأخوذة من ارض مزروعة، حيث استخدم الوسط محفور نمت عليها الطحالب مأخوذة من حافة نهر وعينة مائية وعينة طينية من النهر وعينة تربة مأخوذة من ارض مزروعة، حيث استخدم الوسط محفور نمت عليها الطحالب مأخوذة من حافة نهر وعينة مائية وعينة طينية من النهر وعينة تربة مأخوذة من ارض مزروعة، حيث استخدم الوسط محفور نمت عليها الطحالب مأخوذة من حافة نهر وعينة مائية وعينة طينية من النهر وعينة تربة مأخوذة من ارض مزروعة، حيث استخدم الوسط BG11+0.5g/L للتميتها وقياس الزيادة اليومية للنمو والكلوروفيل فيه ومقارنتها مع اربعة اوساط معدلة على BG11 هي AG11 معيم BG11+0.5g/L ومعنا BG11+0.1p/L MoO3 و NaNO3 و NaNO3 ووسط BG11 اجريت عليه جميع الأضافات التي ذكرت، وكان الوسط الاخير الذي اجريت عليه جميع الاضافات هو الأكفأ في زيادة النمو والكلوروفيل اليومية لهذا النوع، تم استخلاص النواتج الحيوية للما وردي الوسط الاخير الذي اجريت عليه جميع الاضافات هو الأكفأ في زيادة النمو والكلوروفيل اليومية لهذا النوع، تم استخلاص النواتج *الحيوية للسيانوبكتريا Synechocystis pevalekii* على الوع، تم استخلاص النواتج الحيوية للميانوبكتريا المعزولة من والكلوروفيل اليومية لهذا النوع، تم الجروح والمجري البولية التثبيطية النواتج الحيوية للسيانوبكتريا 10,000 على والكلوروفيل اليومية لهذا النوع، تم الجروح والمجري البولي البولية التثبيطية للنواتج الحيوية للسيانوبكتريا 10,000 مايكروغرام/مل فعالية التثبيطية والحمة من الجروح والمجاري البولي البولية تثبيطية واضحة من المهم ركوغرام/مل، واظهر تركيز 10,000 مايكروغرام/مل فعالية تثبيطية واضحة الموسم والمهم من المهم والمهم مايكروغرام/مل فعالية تثبيطية واضحة بينما لم يبري والمجري البولي البولية الموضحة المرمان واظهر تركيز 10,000 مايكروغرام/مل فعالية تثبيطية واضحة بينما لم يبري بينما لم يبري الموسماني الموسماني المائم المائم يبري لي لوليد المهم المهم المهم المهم المهم المهم المهم المهم الموضعة الموضحة الموضم الموضم الموضم المهم المهم المهم مالمهم المهم المهم المهم المهم المهم مركرو فرامم مالم يبني الم يبري مالمهم مالمهم الموضحة الموضم ال

تركيز 1000مايكروغرام/مل اية فعالية تثبيطية لنمو البكتريا المعزولة للدراسة، وابدت التراكيز الباقية فعالية متباينة بينهما، وشخص المركب الفعال للناتج الحيوي لهذا النوع من السيانوبكتريا بجهاز GG-Mass وشخص على انه مركب قلويدي N-dl-Alanylglycin، فضلا عن استخدام جهاز الـIR واظهار المجاميع الفعالة فيه من الالكانات والالكاينات والدهايدات وامينات.

الكلمات المفتاحية : السيانوبكتريا، الكلوروفيل A ، النواتج الحيوية .