Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 3851 - 3859
Received 20 January 2021; Accepted 08 February 2021.

Antibacterial Effect of *Spirulina platensis* Extracts on the Viability of Bacterial SpeciesIsolated form Acne Patients in Baghdad

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Abstract

Considered as Cyanobacteria are very rich source of secondary metabolites that are biologically active metabolites for anticancer activities, antifungal, antibacterial, antiviral. Current study was to determine the antibacterial Isolated from patients with acne.Efficacy of the Hexane and Methanol crude extracts of cyanobacterium *Spirulina platensis* was determined. Antibacterial activity (In vitro) was estimated by agar disc diffusion assay mod against twelve pathogenic bacteria in which four were Gram-positive and eight of them were Gram-negative bacteria.All bacteria tested with S. platensis extracts found varying degrees of inhibitory activity. The zone of inhibition for different extracts was divers from solvent to solvent. One g of dried *S. platensis* suspended in 100 µl of distill water to prepare the concentration of (25, 50,100)µl was put in the well.*S. platensis* was exhibited with maximum inhibition zones (16 and 13) mm in the hexane extract against *Aerococcus spp*. At 100 and 50 µl respectively, antibacterial activity was found high 13 and 10mm in methanol extract against*Aerococcus spp., Enterococcus* at 25µl under investigation. Also hexane extractshowed no effect at concentration 25 for all bacterial isolates.GC-MS analysis of hexane and methanol extracts of *S. platensis* were showed differentactive compounds like, Hexadecene, Heptadecane, Octadecene, 2-Bromopropionic Acid, Methyl-1-Docosene, Benzenedicarboxylic Acid, and Tetradecanol which they are very important as antibacterial agents .

Key wards:Active Compound, Antibacterial Activity, GC-MS Analysis,*Spirulina platensis*, Solvent Extracts

Introduction

Algae and cyanobacteria have the ability to attract solar energy and convert it into chemical energy that contributes to fixing dioxide gas in the form of an organic compound for the production of food and its metabolites. In addition, they are promising biocatalysts in the field of (green biotechnology) for enhancing production of food, drug, metabolites and green energy source such as biofuel (1).
Spirulina (Arthrospira) is referred to free-floating clusters or fine mats covering substrate with spiral characteristics of its filamentsbelong to class cyanophyta(2). Spirulina is filamentous and multicellular cyanobacteria that have great importance in the health sector, aquacultures, food industry. It has very high content of micro and macronutrients, protein,vitamin, essential amino acids, lipid and anti-oxidants.

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Spirulina is considering safe for human consuming as obvious by its long history of food use and latest scientific findings. In recent years, Spirulina has gathered great attention from research as well as industries as a prosperous source of pharmaceuticals and nutraceutical. (3)

Spirulina platensis its extract display therapeutic properties, like the reduction blood cholesterol level, decrease toxic metals and nephrotoxicity of pharmaceuticals, ability to curb cancers, Protection from harmful radiation(4,5)

The Spirulina, has been known for its antimicrobial property and nutritionalvalue. cyanobacterial exudates many Antimicrobial compounds such as amino acids, polyphenols, glycolipids, fatty acids,terpenoids,alkaloids, antioxidant pigments,vitamins,minerals. Secondary metabolites that produced from cyanobacteria are related with toxic, antimicrobial effects, antineoplastic effects and hormonal (6, 7).In general, compounds produced naturally by cyanobacteria are considered environmentally friendly because they can be easily biodegradable (8).The aim of the study is to: Detectivethe effect of various *Spirulina platensis* extracts*in vitro* against different bacterial isolates from Acne patients and using GC-MS analysis for characterize the structure of active compound.

Material and Methods

Collection of specimens and bacterial identification:

The Acne swabs (20)obtained from Al- Habeebia out patients Hospital, andthe age of patients ranged from 15 to 25 years.collected from patients suffering from acne infections by Sterile swabs,Then transfer the samples immediately to the laboratory .*Bacterial isolates were* isolated from acne Specimens on Nutrient agar, Bacterial isolates were isolated from acne Specimens on Nutrient agar, Mannitol salt agar,MacConkey agar and Blood agar.According to the standard methods which recommended by, Isolates were identified by microscopic, biochemical tests and cultural (9).

Inoculums' preparation

Few colonies from overnight cultures of tested bacterial isolateswere transferred to 1ml of normal saline to prepare the bacterial suspension, and adjusted to 0.5 McFarland turbidity tube that is equal to 1.5×10^7 CFU/ml according to(10).

Sample collection of microalgae and culture characterization

Spirulina platensis isolated from the River Diyala in Nhrawan Baghdad city.The isolates according to (11) were identified using taxonomical approaches and morphological variation.cultivation of S. platensis was uses BG-11 medium.The sample was grown under condition (16h light \ 8h dark) at 25±2°C and 268µE/m²/s light intensity, the cultures were harvested until the end exponential phase of the growth ,then collected biomass and dried in oven for 1 h at 60°C h (12).

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Preparation of various extracts

According to method (13) with some modification,take one gram of *spirulina* powder was extracted with 250 ml of 95% methanol solvent using a Soxhlet extraction apparatus at 60 ºC for 3-4 h hours until the solvent becomes colorless, Then, a rotary evaporator is uses at 40 ºC to get the dried crude extract.

Then the extract was weighted and stored at -20°C in hermetic bottle until antibacterial assay. Repeat the same step using a hexane solvent.

Antibacterial activity of *S. platensis*extracts

The agar well diffusion method was performed to investigate the antibacterial activity of *S. platensis* extracts.The bacterial suspension was inoculated into Nutrient agar plates using a sterile cotton swab, andthen wells (8 mm) has been done on Nutrient agar medium. Hundred micrograms of dried *S. platensis*extracts suspended in 100 µl of distill water to prepare concentration of (25, 50, 100)µland put in the wells. The diameter of inhibition zone around the wells was measured in millimeter. The same procedure has been done to investigate the antimicrobial(14).

Chemical composition of *S. platensis*extracts

The samples from the methanol and hexane extracts of S. platensis were analyzed by GC-MS Ministry of Industry/ Industrial Research and Development Authority/ Ibn al-Bitar Research Center.At 100 ° C the temperature was placed in the initial column and at 280 ° C the temperatures of the detector and injector were set.In the column, inject 5 ml of the sample using the split method (10.1) after one minute, then 225 ° C, raise the oven temperature,at a ramp rate of 7.5 °C/min (hold time 5 min).Through the NIST library and original standards their mass was compared and compounds were identified(15).

Results and discussion

Isolation of acne bacteria

Samples were collected from patients with acne about of 20 swabs.Only twelve samples (60%) were positive for bacterial isolates, including 8 Gram-negative and 4 Gram-positive (Fig 1).

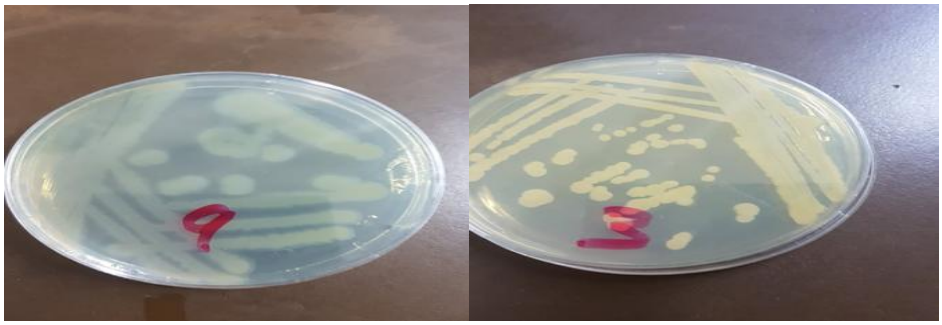

Figure 1: Acne bacteria isolated on Nutrient agar

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Characterization of acne bacteria

Table 1 summarizes numbers and percentage of acne bacteria. *S.aureus* had the highest number of isolates (4), followed by the rest of the species.

Table 1: Numbers and Percentage of acne bacterial isolates

Isolates	No. Isolates	Percentage%
S. aureus	4	33.33
Ps. aeruginosa	1	8.33
Ps. stutzeri	1	8.33
S.epidermides	3	25
Aerococcus spp.	1	8.33
E.coli	1	8.33
Enterococcus	1	8.33
Total	12	100

Ref (16) showed that varide species of anaerobic and aerobic bacteria were contributory in rising acne infection.The results appear that the elevated percent of the isolates belonged to the *Staphylococcus epidermidis*, followed by *Propionibacterium acnes, Micrococcus* and *Staphylococcus aureus*.And these bacteria play an effective role in the pathogenesis of acnecausingapparent skin infection as an important opportunistic pathogen,the optimal state for growth of Propionibacterium acnes,due to the microenvironment Appropriate of the skin,these bacteria have the ability to produce extracellular products which and are responsible for initiating the inflammatory response for example proteases, lipases and chemotactic factors (17).

Antibacterial activity of *S. platensis* extracts

Determined S *platensis* activity extracts against bacteria, were exhibited in the Table - 2.

Table 2: the antibacterial effect of some bacterial isolates treated with *Spirulina platensis*

Bacterial isolate	Inhibition zone (mm)					
	Hexane extract			Methanol extract		
	100%	50%	25%	100%	50%	25%
Aerococcus spp.	**16**	**13**	**-**	**-**	**15**	**13**
Enterococcus	**13**	**12**	**-**	**10**	**-**	**10**
P. aeroginesa	**14**	**10**	**-**	**13**	**12**	**-**
Ps. stuteri	**12**	**--**	**-**	**10**	**-**	**-**
E.coli	**15**	**13**	**-**	**-**	**-**	**-**

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Staph. aureus	**13**	**11**	**-**	**10**	**-**	**-**
Staph. epidermidis	**13**	**12**	**-**	**11**	**-**	**-**

The zone of inhibition of extracts against bacteria was ranged between (16-12mm) at concentration 100µl and ranged between (13-10mm) at 5µl. Also no effect at 25 µl by hexane extract, while The zone of inhibition of test against bacteria by methanol extract was ranged between (13-10mm) at 100µl , *P. aeroginesa* and *Aerococcus spp.* were the best inhibition at 50µl . In addition no effect at 25µl except *Aerococcus spp.* and *Enterococcus*(Fig. 2).

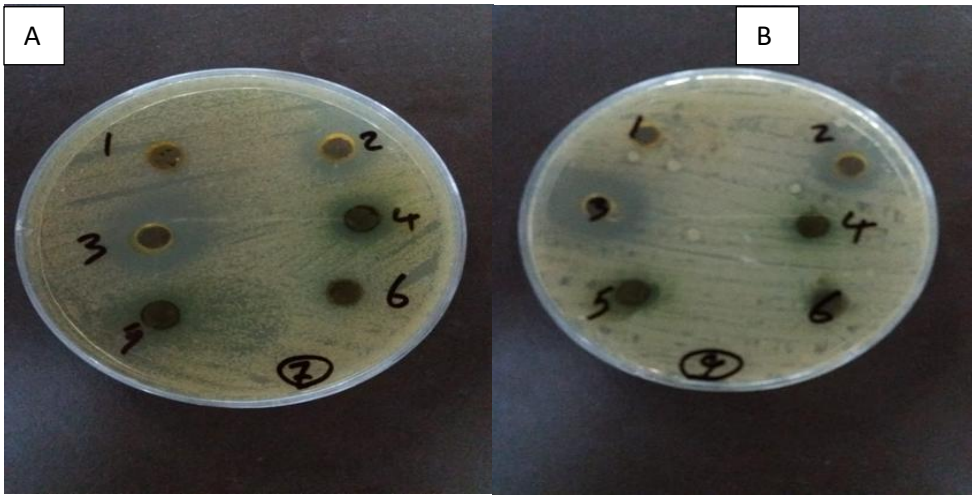

Figure 2: Antibacterial activity of *S. platensis* on acne bacteria.

(A) *Pseudomonas aeruginosa,* (B) *Aerococcus spp*.

Spirulina and its components have been shown to have positive advantage for human health significance from antioxidant properties to malnutrition (18).Algae have been documented to possess and are very rich in antimicrobial compounds in biological systems.In this study, it was found that the active compounds found in the *S. platensis* extracts inhibit the bacteria in a varied and noticeable manner. The inhibitory effectiveness of *S. platensis* extract is different from that ofGram negative bacteriaand Gram positive bacteria, also showed in this studies hexane extract better than methanol extracts.

The antimicrobial activity of the extract could be due to the presence of different chemicals that may affect growth and metabolism of bacteria include phenolic, triterpenoids,flavonoids. As well as, they could have an inhibitory or activating effect on microbial growth according to their concentration and constitution such as alkaloids, free hydroxyl group, amides (19).

Has been suggest the *S.platensis* extracts higher sensitivity to Gram-positive bacteria were more sensitive than the Gram negative bacteria due to cell physiology, degree of contact, differences in cell wall structure, metabolism (20). The variation structure of the cell wall ,This may be refer to in Gram

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positive bacteria consists of a one layer, while in Gram negative bacterial consists multilayered structure (21,22) lipids and fatty acids cause rupture the cell wall then peptidoclucan penetrate leading to its disintegration, The variation in the results is due to the reason for producing bioactive compounds correlating to time and location ,seasons, , cultivation medium ,type of organic solvents (23,24).

GC-MS analysis

During GC-MS analysis of hexane extract of *S. platensis*, itwas observed that haxane showed 19 compounds1-Hexadecene, Eicosane, 10-Trimethyl, 4-Pentadiene,Hexadecanoic Acid Methyl Ester , Methyl 9-Octadecenoate,2-Bromopropionic Acid, E-15-Heptadecenal,1-Octadecene, Methyl Ester, 13-Octadecadienoic Acid, Methyl, Pentadecyl Ester,Alpha,Phytol, Pentadecyl Ester,Alpha,-D-Glucopyranoside,2-BenzenedicarboxylicAcid, 10-Trimethyl, Heptadecane,Glucopyranoside,14-Ethylene1,Allyl N-Octyl Ether (Fig 3).

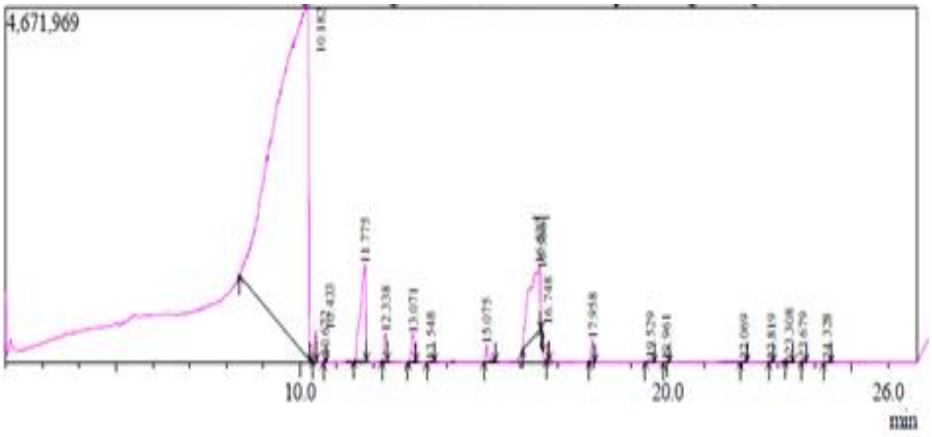

Figure 3: GC-MS Spectrum of hexane extract of S. platensis

While GC-MS analysis of hexane extract of S. platensis, it was observed 7compounds 1-Tridecene, 9-Octadecenoic acid, 2-ethylhexyl isohexyl ester, 1-Pentadecene, Heptadecene, Octane, Sulfurous acid (Fig.4).

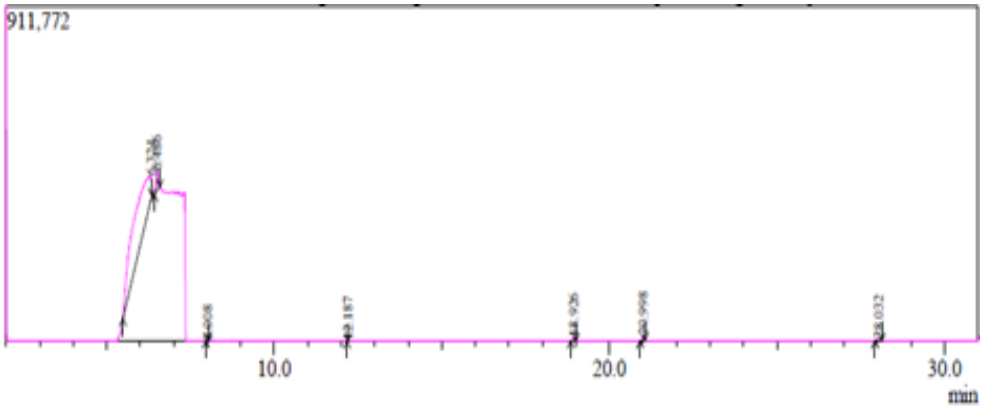

Figure 4: GC-MS Spectrum of methanol extract of S. platensis

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According to the outcome of GC-MS Spectrum datum, it was infer that the compound was n-hexadecanoic acid. The results gained This agree with (9) who tilled main fatty acids extracted from the S. platensis as oleic acid,palmitic acid, linoleic acid and stearic acid etc .(5)Identified of 15 compound for volatile components at *S. platensis* which constituted of the total compounds 96.45%,of the total compounds the major components it is tetradecane and heptadecane.(25) observed that GC-MS analysis, 13 bioactive component were identified of the cyanobacterium *S. platensis* of the partially purified, the major compound was n-Hexadecanoic acid.

Conclusions

From the currently results it can be concluded that the extract of *Spirulina platensis*, contains possibility bioactive compound with an efficient antibacterial activity. This compound can be used for the development of natural antibiotics against resistant bacteria.

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التأثير المضاد للبكتيريا لمستخلصات سبيرولينا بلاتنسيس على حيوية الأنواع البكتيرية المعزولة من مرضى حب الشباب في بغداد

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الخلاصة

تعد الطحالب الخضراء المزرقة (سيانوبكتريا) مصدرًا غنيًا للفعاليات البايولوجية لأحتوائها على مركبات فعالة مضادة للبكتيريا ومضادة للفطريات ومضادة للفيروسات ومضادة للسرطان. الدراسة الحالية تقييم المستخلصات *Spirulina platensis* ضد البكتريا المعزولة من مرضى حب الشباب. تم تحديد فعالية مستخلصات خام الهكسان والميثانول من بكتيريا السيانوباكتيريوم سبيرولينا بلاتنسيس. تم تقييم النشاط المضاد للبكتيريا في المختبر بأعتماد طريقة الأنتشار في وسط الأكار ضد أثنى عشر بكتيريا ممرضة ثمانية منهم سالبة لصبغة كرام وأربعة بكتريا موجبة كرام. أظهرت البكتيريا الزرقاء S. platensis درجات متفاوتة من النشاط المثبط ضد جميع البكتيريا المختبرة. اختلفت منطقة التثبيط لمستخلصين مختلفين من مذيب إلى مذيب. تم وضع غرام واحد من بلاتنسيس المجفف المعلق في 100 ميكرولتر من ماء التقطير لتحضير تركيز (25, 50,100) ميكرولتر لكل حفرة. أذ سجل اعلى فعالية تثبيط ضد *Aerococcus spp* (16 و 13) ملمعند التركيز 100 و 50 ميكرولتر على التوالي في مستخلص الهكسان ،كما وجد أن اعلى فعالية تثبيط 13 و 10 ملم في مستخلص الميثانول ضد *Aerococcus spp*. ،*Enterococcus* عند 25 ميكرولتر. كما لم يظهر تأثير مستخلص الهكسان عند التركيز 25 لجميع العزلات البكتيرية. أظهر تحليل GC-MS لمستخلصات الهكسان والميثانول في S. platensis مركبات نشطة مختلفة مثل Hexadecene و Heptadecane و Octadecene و 2 Bromopropionic Acid-و Methyl-1-Docosene و Benzenedicarboxylic Acidو Tetradecanol وهي مهمة جدًا كعوامل مضادة للجراثيم.

كلمات مفتاحية : المركباتالفعالة، مضاد للبكتيريا ،تحليلكروماتوغرافياالغاز، سبيرولينا بلاتنسيس ، ، مستخلصات المذيب