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Original article

Evaluation of Antibacterial Activity of Some Algae Isolated from Waterfall Derna, Libya

Hanan Abobaker[1]*, Najia Ibrahim[2], Farag Shaieb[3]

[1]*Departmentof Phycology Botany, Faculty of Science, Omar El-Mokhtar University, El -Beida-Libya*
[2]*Department of Phycology Botany, Faculty of Science, Derna University, Derna - Libya*
[3]*Department of Microbiology Botany, Faculty of Science, Omar El-Mokhtar University, El -Beida-Libya*

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Corresponding Email.Hanan.ma.saleh@omu.edu.ly

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ABSTRACT

Aims. The study demonstrated the antibacterial activity of crude freshwater algae extracts of five species (*Chlamydomonas reinhardtii*, *Cladophora sauteri*, *Chlorella vulgaris*, *Ulothrix zonata* and *Nostoc piscine*) isolated from waterfall Derna- Libya by using Agar well diffusion method. ***Methods***. The antibacterial activity was tested *in vitro* against *(Staphylococcus aureus*, *Bacillus* sp., *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Xanthomoas axonopodis and Agrobacterium tumefaciens*). ***Results***. All algae species showed effects against different selected bacteria, *C.vulgaris* crude extracts showed that higher inhibitory effect against *S. enterica*, as for *C. reinhardtii* showed high effect against *K. pneumoniae*, while *U. zonata* showed higher effects against *S. pyogenes*, however *N. piscine* showed high effect against *S. pyogenes*, and *C. sauteri* showed high effect against *S. enterica*, the zone of inhibition was compared with zone of inhibition produced by the standard antibiotic discs in the plate. Our finding suggests the possibility of using algae species in this research as a source of natural antibacterial.

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INTRODUCTION

It has long been believed that pathogenic microorganisms are a primary cause of illness and death in humans. Pharmaceutical companies have developed several novel antibacterial drugs in the past year, but the global spread of multi-drug resistant bacteria is severely impairing the efficacy of existing medications and increasing the likelihood of treatment failure. As a result of the rise in antibiotic resistance, novel antimicrobial agents are desperately needed. Many bioactive chemicals found in the long-studied potential source of novel agent plants may have therapeutic value due to their low toxicity [1].

Since the 1950s, when systematic screening for biologically active ideas began, microalgae have been employed for medical applications. Microalgae, on the other hand, have been the focus of intense research over the last decade, with the goal of

discovering new chemicals that could lead to therapeutically beneficial drugs [2,3]. In the meantime, microalgae have been discovered to manufacture antibiotics: several microalgal extracts and/or extracellular products have been found to be antibacterial, antifungal, antiprotozoal, and antiplasmodial [4,5]. The aim of this study was to evaluate the antibacterial activity of selected algae on some pathogenic bacteria.

METHODS

The five samples of freshwater algae was collected from five different sites of waterfall Derna, (C*hlamydomonas reinhardtii*, *Cladophora sauteri*,*Chlorella vulgaris, Ulothrix zonata* and *Nostoc piscine)*.
The sample's pure culture was cultured in a water medium. Pure cultures were harvested and collected by centrifuging at 10,000 rpm for three minutes before the stationary phase of growth (10 days). For later use, the micro algal pellets that were collected were ground into a coarse powder using a mechanical grinder and dried under shade.

Bacterial strains

The bacterial strains listed below were employed in the current investigation. *Staphylococcus aureus, Bacillus* sp. *Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella enterica,* obtained from department of microbiology Derna hospital, and *Xanthomoas axonopodis, Agrobacterium tumefaciens* obtained from department of plant protective-faculty of agriculture Omer Almuktar University.

Preparation of algae extracts

Algal cultures were centrifuged and pellets were collected, weighed and used for extraction 250 mg of dried algae were dissolved in 10 ml of 95% ethanol, then shacked at 70 cycle/minute for 30-60 minutes using a vibrating incubator 25°C. The dried algae extraction at a speed of 6000 rpm/ minutes was repeated three times to extract most of the algae components. Final alcoholic extract is collected, and evaporated to obtain precipitate, 2ml of distilled water were added to the precipitate, shake well, then add 5ml of methylene chloride and shake well to obtain layers separated by using a separating funnel, the organic part is collected and concentrated at room temperature [6].

Determination of Antibacterial Activity

Agar well diffusion method was followed to determine the antibacterial activity Mueller-Hinton agar (MHA). plates were swabbed (sterile cotton swabs) respective bacteria well 4mm diameter were made in each of these plates using sterile cork borer, about 100µm of organic solvent, added into wells. The plates were incubated at 37°C for 18-24 h. Zone of inhibition were measured using a meter ruler as described by [7].

Antibiotic sensitivity tests

In vitro antimicrobial susceptility to five antibiotics (Clindamycin 2mg/ml, Sulphamethoxazole Trimethoprim 23.75 mg/ml, Clarithromycin 15 mg/ml, Chloramphenicol 30mg/ml, Levofloxacin 5mg/ml). The inoculums were created by introducing isolated microorganism colonies from a nutrient agar plate that was left overnight into 2ml tryptone soya broth (TSB.). The modified suspension was dipped into a sterile cotton swab. To get rid of extra inoculums, the swab was turned several times and firmly pressed against the tube's interior wall above the fluid level. The sterile Mueller Hinton Agar plate was completely covered with swabs. To make sure that the inoculums were distributed evenly, this process was repeated twice more while streaking and rotating the plate. After five minutes of drying, the plates were, and the antimicrobial disks were applied to the surface of inoculated agar plate using an disc dispenser. After that, plates were incubated at 37°C for 18-22 hours. The diameters of the inhibition zones are calculated to the closest mm applying a ruler. Zone diameters were considered to be Sensitive (S) or Resistant (R) according to NCCLS, (2001).

Statistical analysis

One-way ANOVA was used to analyses the obtained data, with acceptable proportion of $P < 0.05$.

RESULTS

The antibacterial activity of algal species was carried out to determine inhibition against some pathogenic bacteria ,the obtained results that the effect of five species algal extracts on nine pathogenic bacteria strains as showed in figure (1) *Cladophora sauteri* extract show the maximum inhibitory activity against *Salmonella enterica* (17mm) were as *Klebsiella pneumoniae* (16mm) inhibition zone using *Chlamydomonas reinhardti*, *Streptococcus pyogenes* (15)mm with *Ulothrix*

zonzta , while *Staphylococcus aureus* recorded highest inhibition zone at 9mm for *Chlorella vulgaris* and the lowest inhibition zone at 5mm for *Klebsiella pneumoniae*, the same effect in case of *Bacillus sp* ,*Echerichia coli* recorded the best inhibition with extract of *Nostoc piscine* (12mm).*Pseudomonas aeruginosa* showed sensitive to all algae extracts except *Chlorella vulgaris* and *Chlamydomonas reinhardti*. The results revealed that the *Xanthomonas axonpodis* was sensitive to the extracts of *Chlorella vulgaris*,*Ulothris zonata* (9mm) whereas resistant to *Cladophora sauteri* and *Chlamydomonas reinhardti* ,however *Agrobacterium tumefaciens* affected by extracts of *Ulothrix zonzta*, *Nostoc piscine* (9,10mm) inhibition zone respectively.

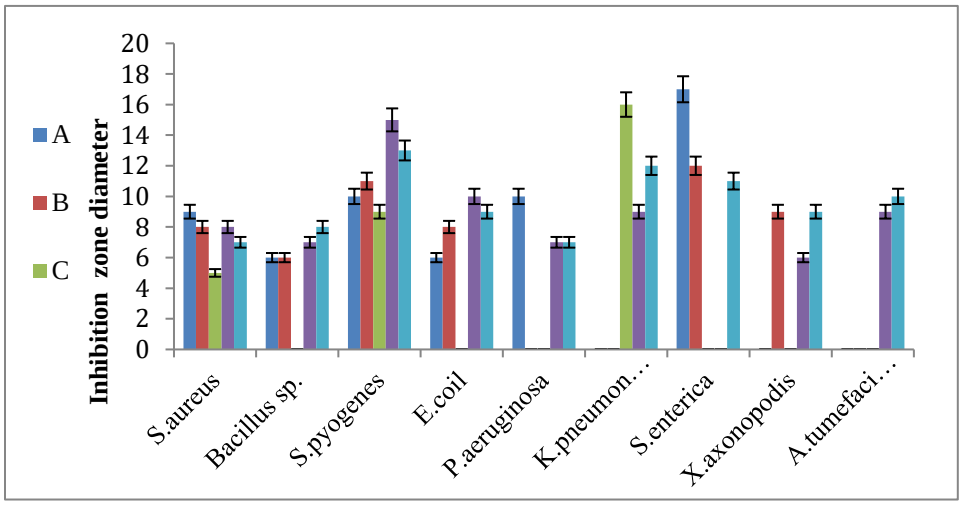

Figure 1. The antibacterial activity of crude algae extracts against Pathogenic bacteria. A: Chlorella vulgaris B: Cladophora sauteri C: Chlamydomonas reinhardtii D: Ulothrix zonata E: Nostoc piscine

Table 1 showed the rates of sensitivity of gram negative and gram-positive bacteria. The results obtained showed that the sensitivity pattern of *S.aureus*, *Bacillus* sp, *S. pyogenes*, *E. coli*, *X.axonopodis*, *K.pneumoniae*, were sensitive to DA, LEV, C, CLR and resistant to SXT. However, *P.aeruginosa* was sensitive to antibiotics SXT,LEV,C, CLR, and resistant to DA.While *S.enterica* was sensitive to antibiotics SXT,LEV,C, DA and resistant to CLR. Also, *A.tumefaciens* was sensitive to C, CLR, and resistant to SXT, DA, LEV.

Table 1. Antibiotic sensitivity test

Pathogenic bacteria	**SXT (23.75 mg/ml)**	**DA (2mg/ml)**	**LEV (5 mg/ml)**	**C (30mg/ml)**	**CLR (15mg/ml)**
S.aureus	R	S	S	S	S
***Bacillus* sp.**	R	S	S	S	S
S. pyogenes	R	S	S	S	S
E. coli	R	S	S	S	S
P.aeruginosa	S	R	S	S	S
K.pneumoniae	R	S	S	S	S
S.enterica	S	S	S	S	R
X. axonopodis	R	S	S	S	S
A.tumefaciens	R	R	R	S	S

DISCUSSION

Antibacterial activities of crude extracts of five freshwater algae (*Chlamydomonas reinhardtii*, *Cladophora sauteri*,*Chlorella vulgaris*, *Ulothrix zonata, and Nostoc piscine*) from the waterfall Derna were studied by agar well diffusion method against seven types of pathogenic bacteria to human and two types of pathogenic bacteria to plant. According to our result demonstrate that the effect of extract *Chlorella vulgaris* on the growth of bacteria was recorded with the highest inhibition

on the *Salmonella enterica, Streptococcus pyogene, Psedomonas aeruginosa, Staphylococcus aureus* and less inhibition on *Bacillus* sp., and resistant to *Klebsilella pneumoniae*, *Xanthomonas axonpodis* and *Agrobacterium tumefaciens.* The results are agreement with the results obtained by [8], who reported that the crude bioactive compounds of *Chlorella vulgaris* possess wide ranges of antibacterial properties against gram negative and gram-positive bacteria.

In the obtained results showed that the effect of crude extract of *Cladophora sauteri* on all selected species of bacteria except some species of gram-negative bacteria *K. pneumoniae, P. aeruginosa A. tumefaciens.* The results are agreement with the results obtained by [9] as source of natural antimicrobial and antioxidant agents for pharmaceutical industries.

The extract of *Chlamydomonas reinhardtii* displayed a variable degree of antibacterial activities on different bacteria, showed high impact effect against *K.pneumoniae* followed by *S. pyogenes, S. aureus* and the other species of bacteria more resistant to the extract, the results agreed with [10], also in these studied show that the effect of crude *Ulothrix zonata* it was observed that more effect in gram positive bacteria than gram negative bacteria, because the structure of the cell wall of gram negative bacteria is more complex than that of positive gram positive bacteria. Morever, the highest effect of *S. pyogenes*,the results agreed with the results obtained by[11]. Cyanophyta specie in the present study *Nostoc piscine* showed effect on the growth of gram positive bacteria was higher with the gram negative bacteria, recorded with the highest inhibition on the *S. pyogenes.*, and the lowest inhibition on the *P. aeruginosa* and *S.aureus* results are agreement with the results obtained by [12] had shown that a broad spectrum antibacterial by *Nostoc* sp. generally the highest effect of inhibition of algal extracts recorded by using *Cladophora sauteri* on *S.enterica*, these results support that the algae source for active antimicrobial compound, as [13] confirm the algae may be source of potential bioactive compounds of interest in the pharmaceutical industry.

The term antibiotic is usually applied to chemicals that are produced by microorganism and that have the ability to inhibit the growth or to kill bacteria and other microorganisms, many algae were found to release substances which inhibit their growth. Antimicrobial resistance developed by microbes against antibiotics open serious debates in this issue and recognized as a serious problem by global medicinal and research community [14]. *Chlorella vulgaris* extracts was the best effects against gram positive and gram-negative bacteria compared with antibiotic Sulphamethoxazole trimethoprim (SXT) due you *Chlorella vulgaris* contain antibiotic called Chlorellin [15].

CONCLUSION

The freshwater algae in this work were source for biologically active compounds. Further photochemical studies are needed to elucidate these compounds structures and activity for use algae especially micro algae as an alternative natural antibiotic against drugs resistant bacteria.

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تقييم النشاط المضاد للبكتيريا لبعض الطحالب المعزولة من شلال درنة ، ليبيا

حنان ابوبكر[1] *, ناجية ابراهيم[2], فرج شعيب[3]

[1]قسم النبات ، كلية العلوم ، جامعة عمر المختار ، البيضاء ، ليبيا.
[2]قسم النبات - كلية العلوم - جامعة درنة - درنة - ليبيا
[3]قسم النبات الميكروبيولوجي - كلية العلوم - جامعة عمر المختار - البيضاء - ليبيا.

المستخلص

الأهداف. أظهرت الدراسة النشاط المضاد للبكتيريا لمستخلصات طحالب المياه العذبة الخام لخمسة أنواع (Chlamydomonas reinhardtii و Cladophora sauteri و Chlorella vulgaris و Ulothrix zonata و Nostoc piscine) المعزولة من شلال Derna- ليبيا باستخدام طريقة Agar Well. طُرق الدراسة. تم اختبار الفعالية المضادة للبكتيريا في المختبر ضد (Streptococcus pyogenes، Staphylococcus aureus، Bacillus sp.، Escherichia coli، Pseudomonas aeruginosa، Klebsiella pneumoniae، Salmonella enterica، Xanthomoas axonopodis and Agrobacterium tumefaciens). النتائج. أظهرت جميع أنواع الطحالب تأثيرات ضد بكتريا مختلفة منتقاة ، أظهرت المستخلصات الخام للفطر C. vulgaris تأثير مثبط أعلى ضد S. enterica ، بينما أظهر C.reinhardtii تأثيرًا عاليًا ضد K. pneumoniae ، بينما أظهر U. zonata تأثيرًا أعلى ضد S. pyogenes. ومع ذلك ، أظهر N. piscine تأثيرًا عاليًا ضد S. pyogenes ، وأظهر C. sauteri تأثيرًا عاليًا ضد S. enterica. تمت مقارنة منطقة التثبيط مع منطقة التثبيط التي تنتجها أقراص المضادات الحيوية القياسية في اللوحة. الخاتمة. تقترح النتائج التي توصلنا إليها إمكانية استخدام أنواع الطحالب كمصدر طبيعي لمضادات الجراثيم.

الكلمات المفتاحية. نشاط مضاد للجراثيم، شلال، مستخلص الطحالب.