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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]*Department of 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.* ***Conclusion****. Our finding suggests the possibility of using algae species as a source of natural antibacterial.*

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INTRODUCTION

Pathogenic bacteria have been always considered as a major cause of morbidity and mortality in human. Even though pharmaceutical companies have produced a number of new antibacterial drugs in the last year, the global emergence of multi-drug resistant bacteria is increasingly limiting the effectiveness of current drug and significantly causing treatment failure; Due to the increase of resistance of antibiotics, there is a pressing need to develop new and innovative antimicrobial agent. The potential source of new agent plants that have long been investigated contain many bioactive compounds that can be of interest in therapeutic because of 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 were collected from five different sites of waterfall Derna (C*hlamydomonas reinhardtii*, *Cladophora sauteri*, *Chlorella vulgaris*, *Ulothrix zonata* and *Nostoc piscine)*. The pure culture of the sample was incubated in water medium. Pure cultures prior to the stationary phase of growth (10 days) were harvested and collected by centrifuging at 10,000 rpm for 3 min. The Collected micro algal pellets were dried under shade and made into a coarse powder with mechanical grinder for further use.

Bacterial strains

The following bacterial strains were used for this study; *Staphylococcus aureus, Bacillus sp. Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella enterica*, and were obtained from department of microbiology Derna hospital. While *Xanthomoas axonopodis*, *Agrobacterium tumefaciens* were 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 susceptibility 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 prepared by adding isolated colonies of the microorganism from an overnight nutrient agar plate into 2ml tryptone soya broth (TSB). A sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The swab was streaked over the entire surface of the sterile Mueller Hinton Agar plate. This procedure was repeated by streaking two more times, rotating the plate approximately each time to ensure an even distribution of inoculums. Plates were allowed to dry for 5 minutes and then the antimicrobial disks were dispensed onto the surface of inoculated agar plates using a disc dispenser. Plates were then incubated at 37°C for 18-22 hours. The diameters of the inhibition zones are measured to the nearest mm using a ruler. Zones diameters were interpreted as being susceptible 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

Figure 1 shows the effect of five species algal extracts on nine pathogenic bacteria strains. *Cladophora sauteri* extract show the maximum inhibitory activity against *Salmonella enterica* (17mm), *Klebsiella pneumoniae* (16mm), using *Chlamydomonas reinhardti*, *Streptococcus pyogenes* (15mm) with *Ulothrix zonzta*. While *Staphylococcus aureus* recorded highest inhibition zone at 9mm for *Chlorella vulgaris* and the lowest inhibition zone at 5mm for *Klebsiella pneumonia*. The same effects in case of *Bacillus sp*p., *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.

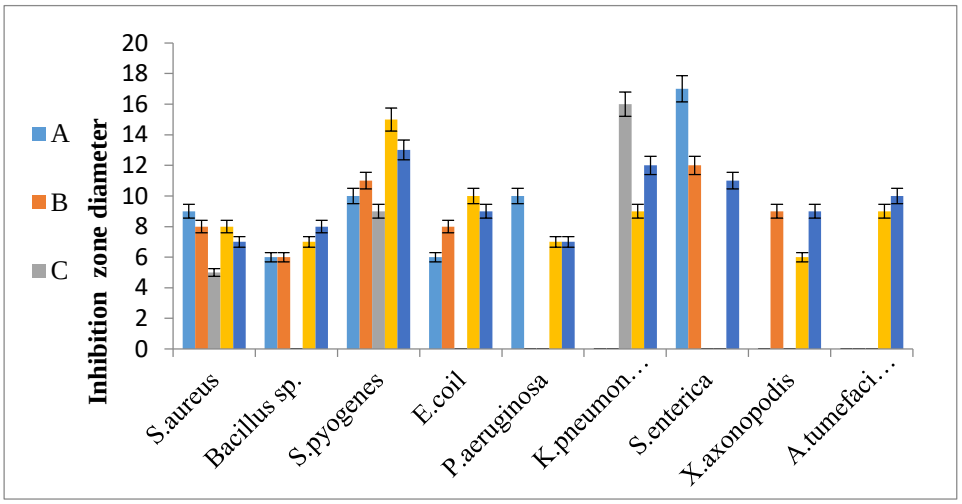

Figure 1. The antibacterial activity of crude algae extracts against Pathogenic bacteria. A: Chlorella vulgaris B: Cladophora sauteri C: Chlamydomonas reinhardtii D: Ulothrix zonata E: Nostoc piscine

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 results, 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.

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 were 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 was 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.

REFERENCES

1. Heel R, Brogden R, Speight T, Avery G. Buprenorphine: a review of its pharmacological properties and therapeutic efficacy. Drugs. 1979;17:81-110.
2. Mendes RL, Nobre BP, Cardoso MT, Pereira AP, Palavra AF. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorganica Chimica Acta. 2003;356:328-34.
3. Cardozo KH, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, et al. Metabolites from algae with economical impact. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2007;146(1-2):60-78.
4. Kellam SJ, Walker JM. Antibacterial activity from marine microalgae in laboratory culture. British Phycological Journal. 1989;24(2):191-5.
5. Ghasemi Y, Yazdi MT, Shafiee A, Amini M, Shokravi S, Zarrini G. Parsiguine, a novel antimicrobial substance from Fischerella ambigua. Pharmaceutical biology. 2004;42(4-5):318-22.
6. Naviner M, Bergé J-P, Durand P, Le Bris H. Antibacterial activity of the marine diatom Skeletonema costatum against aquacultural pathogens. Aquaculture. 1999;174(1-2):15-24.
7. Drago L, Mombelli B, Ciardo G, Vecchi ED, Gismondo M. Effects of three different fish Oil7 formulations on Helicobacter pylori growth and viability: in vitro study. Journal of chemotherapy. 1999;11(3):207-10.
8. Hussein HJ, Naji SS, Al-Khafaji NMS. Antibacterial properties of the Chlorella vulgaris isolated from polluted water in Iraq. Journal of Pharmaceutical Sciences and Research. 2018;10(10):2457-60.
9. Saadatmand S, Khavarinejad R, Nejadsattari T, Soltani S. Antioxidant and antibacterial activities of Cladophora glomerata (L.) Kütz. in Caspian Sea coast, Iran. African Journal of Biotechnology. 2011;10(39):7684-9.
10. Renukadevi K, Saravana P, Angayarkanni J. Antimicrobial and antioxidant activity of Chlamydomonas reinhardtii sp. International journal of Pharmaceutical Sciences and Research. 2011;2(6):1467.
11. Munir N, Rafique M, Altaf I, Sharif N, Naz S. Antioxidant and antimicrobial activities of extracts from selected algal species. Bangladesh Journal of Botany. 2018;47(1):53-61.

12. Kamenarska ZG, Dimitrova-Konaklieva SD, Nikolova C, Kujumgiev AI, Stefanov KL, Popov SS. Volatile components of the freshwater algae Spirogyra and Mougeotia. Zeitschrift für Naturforschung C. 2000;55(7-8):495-9.
13. Ely R, Supriya T, Naik C. Antimicrobial activity of marine organisms collected off the coast of South East India. Journal of experimental marine biology and ecology. 2004;309(1):121-7.
14. Finch RG. Antibiotic resistance: a view from the prescriber. Nature Reviews Microbiology. 2004;2(12):989-94.
15. Hoek C, Mann D, Jahns HM, Jahns M. Algae: an introduction to phycology: Cambridge university press; 1995.

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(Staphylococcus aureus ، Bacillus sp.، Salmonella ،Klebsiella pneumoniae ،Pseudomonas aeruginosa ،Escherichia coli ،Streptococcus pyogenes 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.