Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com

Research Article

THE EFFECT OF SOME EXTRACTED COMPOUNDS FROM THE ALGAE *OSCILLATORIA TENIUS* AGAINST PATHOGENIC BACTERIA

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DOI: 10.7897/2277-4572.0419

Received on: 10/12/14 Revised on: 01/01/15 Accepted on: 07/01/15

ABSTRACT

In this study, *Oscillatoria tenius* isolated and identified from Al-Mustansiriyah University gardens, BG-11 culture media used for their cultivation in suitable laboratory conditions [(25c°, 200 µE/m^2/sec) for 16:8 h. Light: dark], each culture harvested at the end of two weeks. Organic solvents used for extraction was 95 % (Ethanol, Acetone, Hexane), study aims to test the effectiveness of extracted intracellular (biomass) and Extracellular (filtrate) products and test their effectiveness against 10 strains of bacteria: seven of them gram negative bacteria (*Enterobacter spp., Serratia spp., Acinetobacter spp., Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Salmonella typhi*) and three gram positive bacteria (*Staphylococcus aureus, Streptococcus spp.*, and *Micrococcus luteus)* by agar well diffusion method. Results showed that the intracellular extract was best than extracellular, and sensitivity of all bacteria to intracellular ethanol extract was better than others. It recorded the best inhibition of *K. pneumonia* at a rate of 36 mm, then *Enterobacter spp.* was 30 mm, while intracellular extract by acetone not shown the effectiveness of inhibition against the bacteria were tested. Chemical analysis showed that active compounds were present in intracellular extracts in all samples of studied algae by using Gas-Liquid Chromatography.

Keywords: blue-green algae, algal extracts, anti-microbiological, inhibitory active compounds

INTRODUCTION

The wide spread of blue- green algae, which represent a large and important part of the algae, is making it goal for many studies and researches to identify the benefits and the possibility of its practical use, especially in the medical and pharmaceutical fields like the rest of the ranks of other algae[1]. It has been to focus on micro-algae because it is a source of natural products[2,3]. Many active compounds such as polysaccharides and alginate, vitamins and pigments, antibiotics and halogenated compounds and inhibitors and other phenolic compounds extracted from many algae in fresh and salt water and used in the fields of medicine[4]. It has been testing effective compounds, purification and characterization them to figure out their chemical and biological properties and estimate their effectiveness and value in medicine[5]. Blue-green algae have highly efficient in producing a range of antibiotics with a direct impact on the pathogenic bacteria resistant to them, it produces two types of active compounds, Intracellular products with in cells and extracellular products outside their cells derived active compounds using solvents, which may be individually or mixture or succession of solvents such as acetone, methanol, ethanol, hexane and doubled chloride and petroleum ether and others[6]. The aim of this study was to test the effectiveness of the extract (inside and outside the cells) of *O. tenius* against seven gram negative bacteria (*Enterobacter, Serratia, Acinetobacter, E. coli, Pseudomonas, Klebsiella* and *Salmonella*) and three gram positive bacteria (*Staphylococcus, Streptococcus* and *Micrococcus luteus*) and report the phytochemical composition of the extracts of algae that showed antibacterial activities.

MATERIALS AND METHODS

Prepare Dried Algae

O. tenuis was isolated from AL- Mustansiriya University Gardens by Patterson Method[7]. The algae identified by using an optical microscope (Olympus compound) according to[8]. It cultured in BG-11 medium and the use of stable cultures in laboratory conditions constant (temperature of 25°C and the intensity of illumination 200 Micro Einstein/m^2/ s for a period of 6:18 hours Lighting: darkness)[9], then deposition culture after two weeks by centrifugation at the speed of 3000 r / min for 15 minutes. Then collect the sediment and dry at a temperature of 40°C for 48 hours[10].

Extraction of Extracellular and Intracellular Active Substances

Produced compounds (extracellular and intracellular) was extracted by taking 1 g of dried algae *O. tenuis* in 250 mL of ethanol and then shaken for two hours using the shaking incubator at a temperature 25°C and quickly 70 cycles / minute. Then extract centrifuge at 6000 r / min for 15 minutes, after that the dry filtrate taken by using a rotary evaporator at a temperature of 40 m[10], then the extracts weigh and the process repeated by using the (acetone and hexane) each one separately with three replications.

Determine the Effectiveness of Extracellular and Intracellular Active Substances against Bacteria

Sensitivity of 10 strains of pathogenic bacteria tested, where includes seven types of gram negative bacteria, (*Enterobacter sp., Serratia sp., Acinetobacter, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Salmonella typhi*) and three gram positive bacteria *(Staphylococcus aureus, Streptococcus sp.* and *Micrococcus luteus)*. Anti-bacterial activity identified by using agar well diffusion method, the isolates of bacteria cultured in nutrient broth for 18 hours at temperature of 37°C, then spread the approximate number of bacteria (10^5 cells/ mm) at Mueller Hinton Agar; 100 µL of each algal extract (ethanol, acetone and hexane) each one separately in wells and put same volume of the solvent as a control. Dishes incubated at 4°C for two hours to allow the spreading of the extract in the agar medium after that incubated at 37°C for 18-24 hours and then diameters of inhibition zones measured[10].

Active Compound and Fatty Acids Content

Active compound and Fatty acids in algal extracts were analyzed using Gas-Liquid Chromatography(GC- QP5050A SYSTEM: Model 2010 from SHIMADZU, JAPAN) equipped with a DB-5 ms column

(mm inner diameter 0.25 mm, length 30.0 m, film thickness 0.25 lm) Injector temperature was 280°C; carrier gas was He (20 psi), column flow rate was 1.4 ml/min injection mode-split[11].

RESULTS AND DISCUSSION

The results showed the effectiveness of the intracellular ethanolic extract of the algae *O. tenius* better efficiency against pathogenic bacteria compared to acetonic extract Table 1, all strains of gram positive and negative bacteria used in the study showed sensitivity to ethanolic extract except *Enterobacter spp.*, which did not show any sensitivity towards it. In addition, it found better efficacy to *K. pneumoniae* and *Acinetobacter* spp.which inhibition zone of 36 ml and 30 ml, respectively. At same time, the bacteria did not show any sensitivity to intracellular acetonic extract except *Enterobacter spp.* with inhibition rates of 15 ml. also it was less inhibitory effect to *E. coli* and *P. aeruginosa* with inhibition rate 6 ml.

Table 1: Diameters of inhibition zones exhibited by the bacterial strains toward the intracellular Organic extracts of *Oscillatoria tenuis* algae

Intercellular acetone extract	Intracellular ethanol extract	control	Bacterial strains
15	-	-*	*Enterobacter spp.*
-	30	-	*Acinetobacter spp.*
-	12	-	*Serratia spp.*
-	6	-	*E. coli*
-	15	-	*Micrococcus luteus*
-	6	-	*Pseudomonas aeruginosa*
-	36		*Klebsiella pneumoniae*
-	9	-	*Streptococcus spp.*
-	18	-	*Staphylococcus aureus*
-	9	-	*Salmonella typhi*

(-) =* no inhibition

Table 2: Diameters of inhibition zones exhibited by the bacterial strains toward the extracellular Organic extracts of *Oscillatoria tenuis* algae

Extracellular Hexane extract	Extracellular ethanol extract	control	Bacterial strains
12	30	-*	*Enterobacter spp.*
18	-	-	*Acinetobacter spp.*
-	-	-	*Serratia spp.*
-	18	-	*E. coli*
-	-	-	*Micrococcus luteus.*
15	-	-	*Pseudomonas aeruginosa*
-	-		*Klebsiella pneumoniae*
15	-	-	*Streptococcus spp.*
18	6	-	*Staphylococcus aureus*
-	-	-	*Salmonella typhi*

(-) =* no inhibition

a **b**

Figure 1: Inhibition zones obtained by the ethanol extracts from *Oscillatoria tenius* algae against : a: *K. pneumoniae* b: *Acinetobacter spp.*, c: negative control

a **b**

Figure 2: Inhibition zones obtained by the hexane extracts from *Oscillatoria tenius* algae against: a: *K. pneumoniae*, b: *Streptococcuus*, c: negative control

In this study results exhibited that the extracellular hexanic extract of *O. tenius* algae was highly effective against some gram positive and negative bacteria comparing to ethanol extract Table 2 and inhibition zones limited between 12-18 mm diameters depending on the bacteria tested (Figure 1and 2). It also appeared that the extracellular ethanolic extract of the algae has no effective towards the bacteria except to *Enterobacter spp.* and *E. coli* at a rate of inhibition zones 30 and 18 mm, respectively. The difference in effectiveness against some bacterial isolates to extracellular and intracellular organic extracts of *O. tenius* algae, indicates the presence of more than one active material and could be extracted in more than solvent[11,12]. Organic compounds extracted from algae have a wide effect against bacteria especially when used ethanolic extract and this probably reflects the chemical nature of the active agent, as well as the organic solvents tend to remove hydrophobic compounds from the cell surface[13].

Table 3: Active compound in the intra cellular extract of *Oscilatoria tinus*

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	3.26	188885	1.63	40616	1.13	Oxime-, methoxy-phenyl-
2	3.57	24929	0.22	11181	0.31	Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl-
3	6.96	47048	0.41	22046	0.61	1-Dodecene
4	7.14	1725971	14.92	547646	15.20	Caprolactam
5	8.15	19529	0.17	8952	0.25	Octane, 2,3,6,7-tetramethyl-
6	8.66	21832	0.19	9912	0.28	Nonadecane, 4-methyl-
7	8.97	16946	0.15	11259	0.31	2-Butanone, 3,3-dimethyl-1-[5-(1-methylethyl)tetrahydrofuran-2-yl]-
8	9.30	343337	2.97	152064	4.22	1-Tetradecene
9	9.99	20389	0.18	8653	0.24	Trichothec-9-en-8-one, 12,13-epoxy-3,4,7,15-tetrahydroxy-, (3.alpha.,4.beta.,7.alpha.)-
10	10.15	79299	0.69	21996	0.61	1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-en-8-one
11	10.34	566432	4.89	234697	6.51	Phenol, 2,4-bis(1,1-dimethylethyl)-
12	10.46	65294	0.56	19805	0.55	Tetrapentacontane, 1,54-dibromo-
13	10.87	22748	0.20	8068	0.22	Hexadecane, 1-iodo-
14	10.96	19610	0.17	6749	0.19	Diethyl Phthalate
15	11.06	25432	0.22	6425	0.18	Dichloroacetic acid, dodecyl ester
16	11.25	419814	3.63	179581	4.98	n-Nonadecanol-1
17	12.19	130707	1.13	52293	1.45	Eicosane
18	12.28	26834	0.23	9122	0.25	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
19	12.33	79594	0.69	33889	0.94	Benzoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
20	12.38	32445	0.28	13992	0.39	6-Tetradecanesulfonic acid, butyl ester
21	12.57	531058	4.59	161997	4.50	Eicosane
22	12.83	84423	0.73	12554	0.35	Tetradecanoic acid, ethyl ester
23	12.94	303217	2.62	112918	3.13	9-Tricosene, (Z)-
24	14.08	97473	0.84	17570	0.49	Z-11-Pentadecenol
25	14.27	716171	6.19	192907	5.35	l-(+)-Ascorbic acid 2,6-dihexadecanoate
26	14.42	338176	2.92	100174	2.78	Ethyl 9-hexadecenoate
27	14.64	2032651	17.57	718067	19.93	Hexadecanoic acid, ethyl ester
28	14.79	133824	1.16	49392	1.37	n-Tetracosanol-1
29	15.65	175862	1.52	52513	1.46	1-Octadecanol, methyl ether
30	16.06	349357	3.02	84504	2.35	Phytol
31	16.34	464362	4.01	102735	2.85	Methyl 8,11,14-eicosatrienoate
32	16.39	275297	2.38	91317	2.53	Ethyl 5,8,11,14,17-icosapentaenoate
33	16.57	394318	3.41	74040	2.05	7,10-Octadecadienoic acid, methyl ester
34	16.69	363189	3.14	85262	2.37	Ethyl Oleate
35	16.78	74556	0.64	25754	0.71	6-Octadecenoic acid
36	17.07	666727	5.76	169444	4.70	Methyl 17-methyl-octadecanoate
37	17.28	70447	0.61	14653	0.41	1-Octacosanol
38	17.40	228670	1.98	40632	1.13	1,8-Diazacyclotetradecane-2,9-dione
39	23.45	288658	2.49	74888	2.08	1,2-Benzenedicarboxylic acid, diisooctyl ester
40	25.98	106183	0.92	22794	0.63	Octadecanoic acid, 2,3-dihydroxypropyl ester
		11571694	100.00	3603061	100.00	

Diagnosed by device Gas-Liquid Chromatography

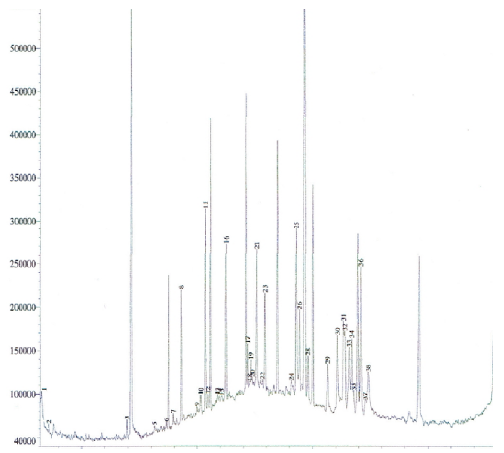

Figure 3: Fatty acids in the intracellular extract of *Oscilatoria tinus* diagnostic by device Gas-Liquid Chromatography

From the data illustrated in (Table 3) it was observed that active compounds (fatty acids, cyclic peptides, alkaloids, flavonoids and others) were present in intracellular extract from all algal samples studied by using Gas-Liquid Chromatography and Results documented in (Figure 3) show that many types of fatty acids were present in all algal samples studied. The results showed a difference in the effectiveness of extracellular and intracellular organic extracts toward bacteria, and reason for this is that the compounds of primary metabolites, such as amino acids, fatty acids and other significance in the building and growth of cells of algae, while the secondary metabolites differ in their behavior from the first one[14]. This study indicates that extracellular and intracellular organic extracts have effectiveness of inhibitory the bacteria, whether it act inside or outside the cell and the reason is because they contain cyclic peptides, alkaloids and polysaccharides[15], also many studies pointed that *Oscillatoria* sp. algae has two kinds of effects on bacterial species: the first type is production of chemicals effective inhibition against bacteria that always appear in the medium, while the second type has shown to be effective when the algae directly in contact with the bacteria[16]. There are many factors that affect on the results that were obtained in tests regarding to the effectiveness of extracts of algae against the activity of bacterial and mold growth, it has attributed this variation to the area and time of collection, methods of keeping the samples used in the test before extraction, the different ingrowth media, environmental factors prevailing, the stage of growth of algae harvested at the farm, type of solvent used in the extraction and extraction method[17,18]. Active antibacterial extracts from different algae have been found to be made up of saturated and unsaturated fatty acids with a predominance of myristic, palmitic, oleic and eicosapentaenoic acids. So, the antibacterial activities of the algae tested could be attributed to the type and amount of free fatty acids which have a role in the overall defense against the studied pathogenic Gram-positive and Gram-negative. Terpenoid content considered to contribute to antibacterial

activity[19,20]. The result of this study suggested that flavonoids could use clinically to treat patients with hypercholesterolemia and hypertension. Previous studies have evolved as promising pharmacological agents in the treatment of cancer. Further results of the present study also indicated remarkable differences in antibacterial activities among the tested bacterial pathogens, which attributed to the exposure of marine algae to the combined effect of light, and oxygen that leads to the formation of free radicals and other strong agents. However, the absence of oxidative damage in structural components of seaweeds and their stability to oxidation during storage suggest that their cells have protective anti-oxidative defense systems[19]. In conclusion, our results revealed that intracellular ethanol extract best than extracellular, and antibacterial effect of it was best than others, so it could be useful to use it against pathogenic bacteria.

REFERENCES

1. Edward GB and David CS. Freshwater Algae: Identification and Use as Bio-indicators. Great Britain. Ltd Chippenham, Wiley Blackwell; 2010. p. 284.
2. Gene EL. Plankton of inland Waters. Academic Press is an imprint of Elsevier; 2010. p. 99-110.
3. Kaushik MP, Abhishek CM, Garima M and Pankal M. Evaluation of *Nostoc commune* for potential antibacterial activity and UV-HPLC analysis of methanol extract. J. of Microbiology 2008; 5(1): 35-41.
4. Rofiza M, Norafattull S, Rahman S and Fatimah M. Isolation and Identification of Marine Algae (*Oscillatoria* Sp.) from Kuantan Coastal Region Malaysia and Studies on Their Bioactive and Antibacterial Properties. World Applied Sciences Journal 2013; 28(2): 200-204.
5. Zbakh H, Chiheb H, Bouziane H, Motilva VS and Riadi H. Antibacterial activity of benthic marine algae extracts from the mediterranean coast of morocco. Journal of microbiology, biotechnology and food sciences 2012; 2 (1): 219-228.
6. Husseini S, Ahmed M, Muhammad A, Anaam N. Industrial water exchange of bacterial contamination using algae alcohol extract. Ass. Univ. Bull. Environ. Ress 2011; 14(1): 45-60.
7. Stein J. Handbook of philological methods: Culture methods and growth measurements. Cambridge University Press; 1973. p. 448.
8. Desikachary TV. Cyanophyta, Indian council of agricultural research: New Delhi; 1959.
9. Rippka RJ, Deruelles J, Waterbury HM and Anier R. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. of Gen. Microbiol 1979; 111: 1-61. http://dx.doi.org/10.1099/00221287-111-1-1
10. Sirikul T, Kun S and Siriporn S. Antibacterial activity of crude extracts of cyanobacteria *Phormidium* and *Microcoleus* species. African Journal of Microbiology Research 2012; 6(10): 2574-2579.
11. Al Saif AS, Abdel Raouf N, El Wazanani H, Aref I. Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. Saudi Journal of Biological Sciences 2014; 21: 57-64. http://dx.doi.org/10.1016/j.sjbs.2013.06.001
12. Rania MA and Halla MT. Antibacterial and antifungal activity of cyanobacteria and green microalgae evolution of medium component by Placket Barman Design for Antimicrobial Activity of *Spirulina platensis*. Global Journal of Biotechnology and Biochemistry 2008; 3: 22-31.
13. Ghasemi YM, Tabatabaei A, Shafiee A, Amini M, Shokravi Sh and Zarrini G. Parsiguine, a novel antimicrobial substance from *Fischerella ambiua*. Pharm. Biol 2004; 2: 318-322. http://dx.doi.org/10.1080/13880200490511918
14. Rosàrio FM, Miguel FR, Lars H, Jose AS, Kaja S and Vitor MV. Antimicrobial and cytotoxic assessment of marine cyanobacteria -*Synechocystis* and *Synechococcus*. Mar. Drugs 2008; 6(1): 1-11. http://dx.doi.org/10.3390/md6010001
15. Mathivanan K, Ramamuthy V and Rajaram R. Antimicrobial activity of *Oscillatoria princeps* and *Lyngbya majuscule* against pathogenic microbes. International Journal of Current Research 2010; 5: 097-101.
16. Chiheb I, Hassane R, José M, Francisco D, Antonio G, Hassan B and Mohamed K. Screening of antibacterial activity in marine green and brown macro algae from the coast of Morocco. African Journal of Biotechnology 2009; 8 (7): 1258-1262.
17. Osman MEH, Abushady AM and Elshobary ME. *In vitro* screening of antimicrobial activity of extracts of some macro algae collected from Abu Qir bay Alexandria, Egypt. African Journal of Biotechnology 2010; 9(12): 7203-7208.
18. Hikmet K, Yavuz B, Belma A, Zehra Y and Tahir A. Screening for Antimicrobial agent production of some microalgae in freshwater. J. of Microb 2006; 2(2): 574-645.
19. Harith Q, Thaer I and Ahmed K. The effectiveness of the extract daitom *Nitzschia palea* (Kuetz.) W. Sm. Anti-bacterial. Iraqi Journal of biotechnology 2009; 8(2): 563-566.
20. Chu W and Goh S. Studies on production of useful chemicals, especially fatty acids in the marine diatom *Nitzschia conspicua* Grunow. Hydrobiol 1994; 285: 33-40. http://dx.doi.org/10.1007/BF00005651

Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
Ghaidaa H. Abd, Neihaya H. Zaki and Merthad A. S. The effect of some extracted compounds from the algae *Oscillatoria tenius* against pathogenic bacteria. J Pharm Sci Innov. 2015;4(1):36-39 http://dx.doi.org/10.7897/2277-4572.0419