Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
2020, 17(1):34-41 **E-ISSN: 2411-7986**

DOI: http://dx.doi.org/10.21123/bsj.2020.17.1.0034

The Biofilm Inhibitory Potential of Compound Produced from *Chlamydomonas reinhardtii* Against Pathogenic Microorganisms

Ghaidaa, H. A.* ***Neihaya, H.Z.*** ***Nada, Z.Mahdi*** ***Amna, M.A.***

Received 27/1/2019, Accepted 28/8/2019, Published 1/3/2020

Abstract:

Microalgae present much usefulness for antimicrobial research because of its enormous biodiversity and rapid growth rate. From this study results it is reaveled that *Chlamydomonas reinhardtii* were isolated from a pond of water in the province of Diwaniyah. The culture supernatants were obtained when extracted with methanol solvent. Antimicrobial activity of extracts was tested for pathogens, and the best inhibition zone obtained was against *Candida albicans* (32mm), *S.aureus* (15mm), and to *E.coli* (9mm). While it showed no effect against both *S.epidermidis* and *Klebsiella spp.* Biofilm was formed by all tested isolates with differences in its strength formation. *The C. reinhardtii* Algal extract showed higher reduction of the existing *Staphylococcus aureus* biofilm and (10.9 %) was the remaining biofilm, while 55.21% and 54.98% were to *Serratia spp.* and *Escherichia coli* respectively, and it's the lowest reduction. *Klebsiella spp., Candida albicans, Acinetobacter spp., Staphylococcus epidermides,* and *Aspergillus sp.* showed remaining biofilm as (16.7, 18.3, 19.6, 43.6, and 44.1) % respectively. The composition of the volatile compounds of the C. reinhardtii extract was determined by GC/MS. Different groups of compounds were identified such as hydrocarbons, phenols, alcohols and esters, and two bioactive compounds;1-Heptacosanol and Octadecyl chloride which is used in medical and pharmaceutical fields.These results provide an indication of the existence of hopeful antibiofilm compounds in the algal species under study. Further chemical studies are required to illuminate these compounds, structures and activity.

Key words: Antibacterial, Antibiofilm, *Chlamydomonas reinhardtii*, External extract, GCMS analysis.

Introduction:

Biofilm, as assemble together bacterial cells and covered by a large amount of matrix made up of polysaccharides, protein, nucleic acids and lipids called polymeric substance (1, 2). Antibiotic treatment against the inflammatory effects of the human is ineffective or useless because limited diffusion within biofilm reduces the actual dose that reaches the bacteria in other words, Biofilm forming bacteria are 100- 1000 fold resistance to antimicrobial compounds (3). Biofilm adhesion to nonliving and living surfaces, it takes place in a sequence of steps ,which are (i) adhesion to the surface (ii) formation of monolayer and production of the slim (iii) formation of micro colonies (iv) cell form an extracellular matrix (biofilm maturation) (4). Biofilm formation has been linked with their clinical appearance, including cystic fibrosis, urinary tract infection, otitis external, chronic otitis media, endocarditis, chronic lung infections, bacterial keratitis, chronic obstructive pulmonary disease, prostatitis and burn wound infections (5).

The major interest in microalgae of anti-biofilm property, the ability to modulate their metabolism according to environmental circumstance. Furthermore, microalgae are a varied source of bioactive molecules that play physiological roles for themselves and their environment (6, 7). Wide spread of microalgae in different environments for their vast tolerance to change environmental conditions such as temperature, salinity, nutrients, drought and ultraviolet radiation, so they must adapt to such unique conditions, so they are natural sources of effective natural products (7). The ability of microalgae extracts and/or extracellular to produce secondary metabolites (natural bioactive compounds) as well as that product are difficult produced by chemical synthesis due to interest has been extensively documented (8, 9).

Department of Biology, College of Science, AL-Mustansiriyah University, Baghdad, Iraq
*Corresponding author: dr.neihayahz@uomustansiriyah.edu.iq
*ORCID ID:0000-0002-9619-6559

Baghdad Science Journal
P-ISSN: 2078-8665
E-ISSN: 2411-7986

There are numerous reports of compound derives from microalgae with wide range of biological activities like antifungal (10), antiviral (11), antialgal (12), antiprotozoal, anti-inflammatory (13). The chemical structure type includes Phycobiliproteins, carotenoids, fatty acids, t terpens, phenols, volatile, vitamins, and polysaccharides (14,15). Microalgae, makes them as a promising group of organisms for research on drug discovery. Also, it is used in human food, animal and aquaculture feed, cosmetics, bio fertilizer, and fuel (16).

In recent decades, a number of studies have been conducted on finding alternative compounds, low cost and without side effects. Researchers have focused on natural products that can be safe and non-toxic and can be used to treat many bacteria and fungi that infect humans. The aim of this study is to test the efficacy of the methanolic extract of *Chlamydomonas reinhardtii* isolated from the local environment, and to test for inhibiting bacterial pathogenesis biofilm, includes positive and negative gram stain bacteria *(Staphylococcus aureus*, *Escherichia coli, Acinetobacter sp*., *Klebsiella sp.* , *Serratia* sp., *Staphylococcus epidermides* as well as two strains of *Candida albicans* and *Aspergillus sp*. Also to analysis the metabolites by GCMS.

Materials and Methods:

Algae Isolation and Identification

Microalgae spp. was isolated from a pond of water in the city of Diwaniyah by Sonawale Method (17). The algae identified by using an optical microscope and samples was cultured using Ready TAP (Tris Acetate Phosphate) medium, which cultivation constant laboratory condition (268 µE/m²/s, 25± 2 C˚ and 16:8 lights: a dark period of 10 -14 days). The culture was kept in the above condition for two weeks.

Preparation of Supernatant and Extraction

The culture was harvested after two weeks by centrifugation at 5000 rpm for 15 min. The aqueous supernatant was collected (extracellular) this material mixed with methanol (1:15, w/v) and placed into shaking for 20 min. The culture supernatants and solvent extracts were dried under reduced pressure then product is weighted and stored in -20 °C for further studies (18) and used for antibacterial assay by agar well diffusion method, all experiments have been done in triplicate.

Microorganism Isolates

Six pathogenic bacteria isolate and two species of fungi (*Escherichia coli*, *Acinetobacter sp*., *Klebsiella sp*., *Serratia* sp., Staphylococcus aureus and *Staphylococcus epidermides* as well as two strains of *Candida albicans* and *Aspergillus sp*.) were obtained from the microbiology laboratory in the Department of Biology College of Science in AL-Mustansiriyah University.

Antibacterial and Antifungal Bioassay

Antimicrobial activity of supernatant extract of *C. reinhardtii* was tested by agar well diffusion method. Mueller Hinton agar plates were inoculated with 200 µl of a 24 hours broth culture of the tested microorganisms. Four wells (6 mm) were made and filled with 200 µl of extract. The plates were incubated for 24 hours at 37°C for bacteria. The diameter of the inhibition zone was measured, and the results recorded (19).

Biofilm Formation Assay

Congo Red Method

Bacterial isolates were incubated aerobically at 37 ^{o}C for 24-48 hours., Positive result is indicated by black colonies with a dry crystalline uniformity . The weak slime producers usually stay pink , while an intermittent darkening at the centers of the colonies observed indicates an undefined result,a darkening of the colonies, with the absence of a dry crystalline colonial morphology (20).

Microtitre Plate Method

All microorganisms strains cultured in broth (Hi media /India) and Brain Heart Infusion (BHI) were incubated at 37°C for 18 hours, then 200µl of bacterial culture was used to inoculate 96-well polystyrene Microtiter plates and were incubated at 37°C for 48 hours., All wells were washed after incubation with sterile normal saline to remove unattached cells, and then 200µl of 1% crystal violet was added to each well at room temperature. Wells were rinsed with 200µl sterile saline. 200µl of ethyl alcohol were involved to remove the excessive stain bounded to the biofilm. The absorbance of Microtiter plate was determined at 595 nm using an ELISA reader (Human/Germany). Controls were performed with crystal violet binding to the wells exposed only to the culture medium without bacteria. All the assays were performed in triplicates (21).

Inhibitory Effect of Extracellular *C. reinhardtii* Extract on Bacterial Biofilm:

The modified method of biofilm inhibition spectrophotometric assay was carried out in 96 wall plates, All isolates were cultured in Brain Heart Infusion (BHI) broth (Hi media /India)and incubated at 37°C for 18hours, after that bacterial culture was diluted in BHI broth and adjusted in comparison to MacFarland tube no. 0.5, and 200 µl of this bacterial culture were used to inoculate pre-

sterilized 96- well polystyrene Microtiter plates and later incubated for 48hours at 37C. After incubation, all wells were washed with sterile saline for the judiciary of detached cells. Then, before the staining step, the extracellular extract of algae was added to biofilm containing wells. Subsequently, the tray was incubated for another 24hours after incubation period all wells were washed and stained as the procedure described above (22). The percentage of remaining biofilm was calculated using the equation as follows:
(O.D595 of the extract – O.D595 of the negative control)/ (O.D595 of the positive control – O.D595 of the negative control) × 100% (23).

Thin Layer Chromatography:

To confirm the presence of the metabolite compounds, the TLC plates were exposed to iodine vapor for 5 minutes (Ministry of Science and Technology).

Analysis of GC-MS:

The algal extract was analyzed by GC-MS, using a high-temperature column (Inert cap 1MS; 30 m × 0.25 mm × 0.25 µm film thickness) affiliated to a company (SHIMADZU—Japan). Derivatization of each sample was eliminated. the initial column temperature was set at 100 ℃ while the injector and detector temperatures were set in 280 ℃ . A sample volume was injected about (5µl) in to the column and ran using split (1:10) mode After 1 min, and the temperature of oven was raised to 225 ℃ at a ramp rate of 12.5 ℃/ min (4 min). in addition, the ramp rate of 7.5 ℃/ min (hold time 5 min)when the temperature was raised to 300 ℃ . The compounds were identified by compare of their mass with real standards and NIST library search (24).

Results and Discussion:

Algal Species

Chlamydomonas reinhardtii were isolated from a pond of water in AL- Diwaniyah - Iraq. One of the better microalgae belongs to Chlorophyta (green algae), and the genus, is a single spherical or ellipsoidal cell with two equal flagella located on the interior of the cell, a basal chloroplast surrounding one or more pyrenoids, found in fresh water and marine habitat (25). *C. reinhardtii* is used as a model organism in biology due to short life cycle, grows both in the light and dark, reproduce sexually or asexually and withstand extreme conditions (26).

Extracellular Extract of *Chlamydomonas reinhardtii*

Extract of *C. reinhardtii* was obtained by methanol solvent and dried as mention above. Figure-1showed the shape and quantity of extract.

Figure 1. Extracellular extract of *Chlamydomonas reinhardtii*

results of the present study mentioned first before comparison with previous studies (27) results,they showed that the methanolic extract was more efficient against bacterial strains , where they inhibited S.aureus ,*B. subtilis* and *K. Pneumonia however* , influence on *S. aureus* and with less impact on *B. subtilis* by acetone extract, as well , type of species and the solvent affects the extract activity against the pathogens.

Antimicrobial Activity of Extracellular Extract of *Chlamydomonas reinhardtii*

Extracellular extract of *C. reinhardtii* was tested for antimicrobial activity against five pathogens (2 Gram positive, 2 gram negative and yeast) (Figure-2). The best inhibition zone 32mm was obtained against *Candida albicans* (32mm), then to *S.aureus* (15mm) and to *E.coli* (9mm). While it showed no effect against both *S.epidermidis* and *Klebsiella spp* (Table 1).

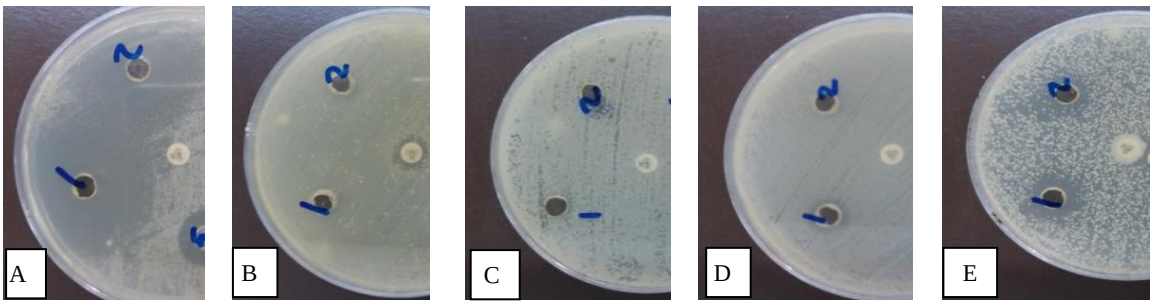

Figure 2. Antimicrobial activity of *Chlamydomonas reinhardtii* on microorganisms. A- *Candida albicans*; B- *S.epidermidis*; C- *Klebsiella spp.*; D-*E.coli*; E. *S.aureus*

Table 1. Antimicrobial Activities of *C. reinhardtii* as presented by inhibition zone diameter(mm)

Microorganisms	Inhibition diameter (mm)
Escherichia coli	9
Acinetobacter spp.	-
Klebsiella sp.	-
Serratia spp.	-
Staphylococcus epidermides	8
Staphylococcus aureus	15
Aspergillus sp.	9
Candida albicans	32

Measuring inhibition zones(MIZ) in agar diffusion assay, grant both qualitative and quantitative results. However, several factors can intervene with the results, like nature of the solution, type of pathogens, and evaporation of organic solvents(28).The variation in the growth inhibition impact on the examined pathogens is concerning the primary structures, different molecular weights, charge characteristics of the polysaccharides and chain modification of different producers (29).

Biofilm Formation Assay

Results found that biofilm formed by all tested isolates with different in biofilm strength formation, colonies of black with a dry crystalline uniformity, indicated very strong biofilm creation (Table 2). *Klebsiella spp.* and *Staphylococcus aureus* isolates were formed the strongest biofilm on Congo red agar.

Congo red Agar (CRA) assay is fast and simple, and Congo red pigment is used directly to characterize the production of exopolysaccharide, which is the fundamental requirement for biofilm formation(30). Bacterial contagion in the clinical exercise are relevant to the biofilm formation by bacteria, and more than 60% of contagion appears to be active in the presence of biofilms (31).

Table 2. Biofilm formation of pathogenic bacteria

Bacteria	Biofilm formation
E. coli	++
Klebsiella spp.	+++
Acinetobacter spp.	++
Staphylococcus aureus	+++
Serratia macescens	++
Staphylococcus epidermis	+
Aspergills niger	++
Candida albicans	++

Antibiofilm Activity

The results of microorganism biofilm reduction assay of treatment of algae extract were shown in Table 3. These results of algal extract showed higher reduction of the existing *Staphylococcus aureus* biofilm to 10.9 % were the remaining biofilm, while the lowest reduction were to *Serratia spp.* and *Escherichia coli* and the remaining biofilm were 55.21% and 54.98% respectively. The other percentage of the remaining biofilm were (16.7, 18.3, 19.6, 43.6, and 44.1) % to *Klebsiella spp., Candida albicans, Acinetobacter spp., Staphylococcus epidermides,* and *Aspergillus sp.* respectively.

The mechanisms of the reduction of bacterial biofilm by algal extracts can be based on other previous studies, for example algae extracts may contain primary or secondary metabolites which have antibiofilm activity, such as bacteria growth inhibitor, quorum sensing inhibitor (quorum quenching), and disruption of biofilm (32).

Table 3. The percentages of remaining biofilm after treatment with *C. reinhardtii* extract

Microorganisms	% of remaining biofilm
Escherichia coli	54.98
Acinetobacter spp.	19.6
Klebsiella sp.	16.7
Serratia spp.	55.21
Staphylococcus epidermides	43.6
Staphylococcus aureus	10.9
Aspergillus sp.	44.1
Candida albicans	18.3

A clinical study using *Enteromorpha linza* extract in mouth rinse showed that the extract significantly reduced plaque, improved the condition of gingival tissues, and reduced the bleeding after 6 weeks of treatment (33). Another study on furanone, a secondary metabolite derived from red algae, demonstrated that it was able to reduce the biofilm thickness of *Bacillus subtilis* up to 25% (34).(35) showed that the C.reinhardtii has antibacterial activity against bathogenic microbial due to have saturated fatty acids such as myristic acid ,palmitic, acid, stearic and unsaturated fatty acids like, palmitoleic acid,oleic acid, linoleic acid .

Thin Layer Chromatography

The metabolites present in the extract were identified by thin layer chromatography. The results showed the presence of 32 compounds from the extract. Similar work was done with TLC and the presence of compounds in *C. reinhardtii* CC 124 were reported as Gal-acyl$_2$ Gro, acyl$_2$ Gro-Me$_3$Hse,

PtdGro, PtdEtn, SQui-acyl$_2$ Gro, Gal -acyl $_2$ Gro, Ptdlns (36).

Analysis of C. reinhardtii Metabolites by Using GC-MS

The composition of the volatile compounds of the C. reinhardtii extract was determined by GC/MS. Identified Different groups of compounds, like alcohols, hydrocarbons, phenols, and esters are shown in **Table** 4. The compounds which were identified through mass spectrometry were found to exhibit the biological and pharmacological activity **Figure** 3.

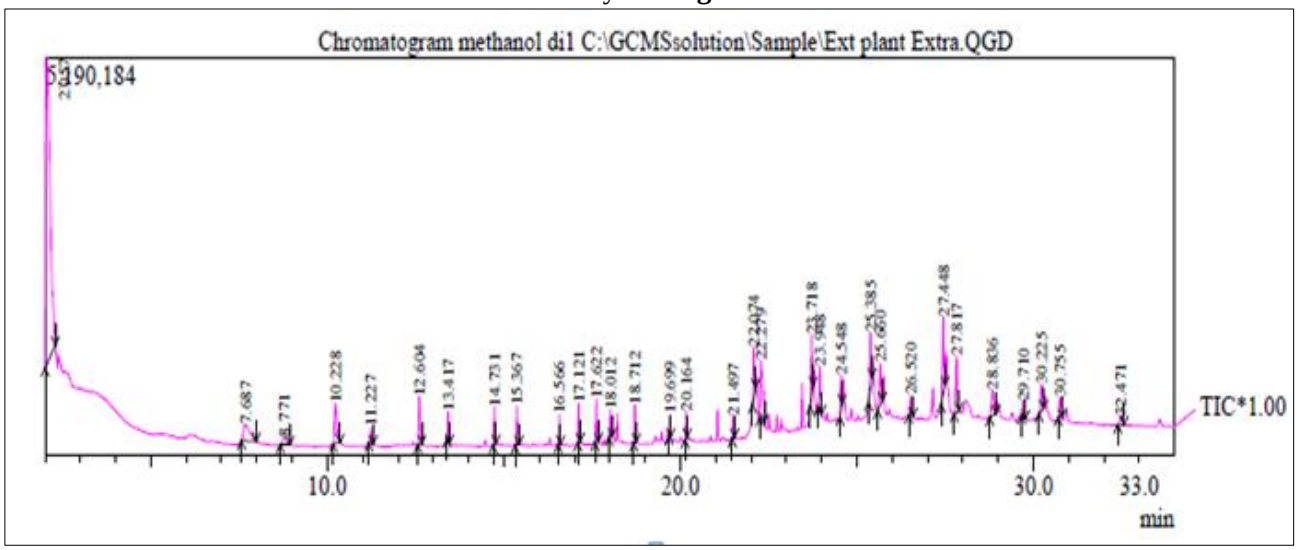

Figure 3. Chromatogram methanol extract of C. Reinhardtii

Table 4. GC-MS profile of C. Reinhardtii extract

Peak	R.Time	Area%	Height%	Compound name
1	2.057	44.96	22.08	Methylene Chloride
2	7.687	3.46	1.19	N-(Trifluoroacetyl)-N,O,O',O"-tetrakis
3	8.771	0.93	0.58	Silane, (2-methoxyethoxy)trimethyl
4	10.228	3.25	2.89	Cyclohexasiloxane, dodecamethyl
5	11.227	0.73	1.16	Benzeneacetic acid, 4-[(trimethylsilyl)oxy]
6	12.604	2.04	3.42	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-tetrasiloxane
7	13.417	0.93	2.14	Silane, [thiobis(methylene)]bis[thiomethyl
8	14.731	1.32	2.71	2-(2,4,4,6,6,8,8-heptamethyltetrasiloxan-2-yloxy)
9	15.367	1.47	2.83	Homogentisic acid, bis(tert-butyldimethylsilyl ester
10	16.566	0.97	2.17	Cyclohexasiloxane, dodecamethyl
11	17.121	1.24	2.82	Heptasiloxane, 1,3,5,7,9,11,13,13-tetradecamethyl
12	17.622	1.69	3.07	Pentadecanoic acid, 14-methyl-, methyl ester
13	18.012	1.03	1.94	n-Hexadecanoic acid
14	18.712	1.24	2.75	beta.-Hydroxypyruvic acid, trimethylsilyl ether
15	19.699	0.62	1.63	Benzenepropanoic acid,alpha, trimethylsilyl ester
16	20.164	0.85	2.00	Mercaptoacetic acid, bis(trimethylsilyl)
17	21.497	0.74	1.65	eptasiloxane,1,1,3,3,5,7,9,11,13tetradecamethyl
18	22.074	2.17	3.71	1-Heneicosanol
19	22.279	4.83	4.21	-1,16-Hexadecanediol
20	23.718	2.21	4.34	Pentafluoropropionic acid, heptadecyl ester
21	23.948	2.14	3.22	-5-Eicosene, (E)
22	24.548	1.16	2.51	3-Dodecanol, 3,7,11-trimethyl
23	25.385	2.41	3.94	1-Octacosanol
24	25.660	2.42	2.99	Octatriacontyl pentafluoropropionate
25	26.520	1.03	1.45	1-Chloroeicosane
26	27.448	4.05	5.49	Docosyl pentafluoropropionate
27	27.817	3.30	4.10	1-Heptacosanol
28	28.836	2.07	1.90	Heptasiloxane, hexadecamethyl
29	29.710	0.93	1.12	Cholest-5-en-3-ol (3.beta.)-, tetradecanoate
30	30.225	1.83	1.92	Octacosyl heptafluorobutyrate
31	30.755	1.21	1.43	n-Octadecyl chloride
32	32.471	0.77	0.62	Octadecane,1-chloro-
		100.00	100.00	

(36) reported that the compounds lick 1-Tetradecene,1-Nonadecene, 1-Octadecene and 1-Heptacosanol were found in both plants and algae antioxidant,anticancer and antimicrobial, and this comparable to our results which showed 1-Heptacosanol (Figure 4), and Octadecyl chloride compared to the library (Figure 5).

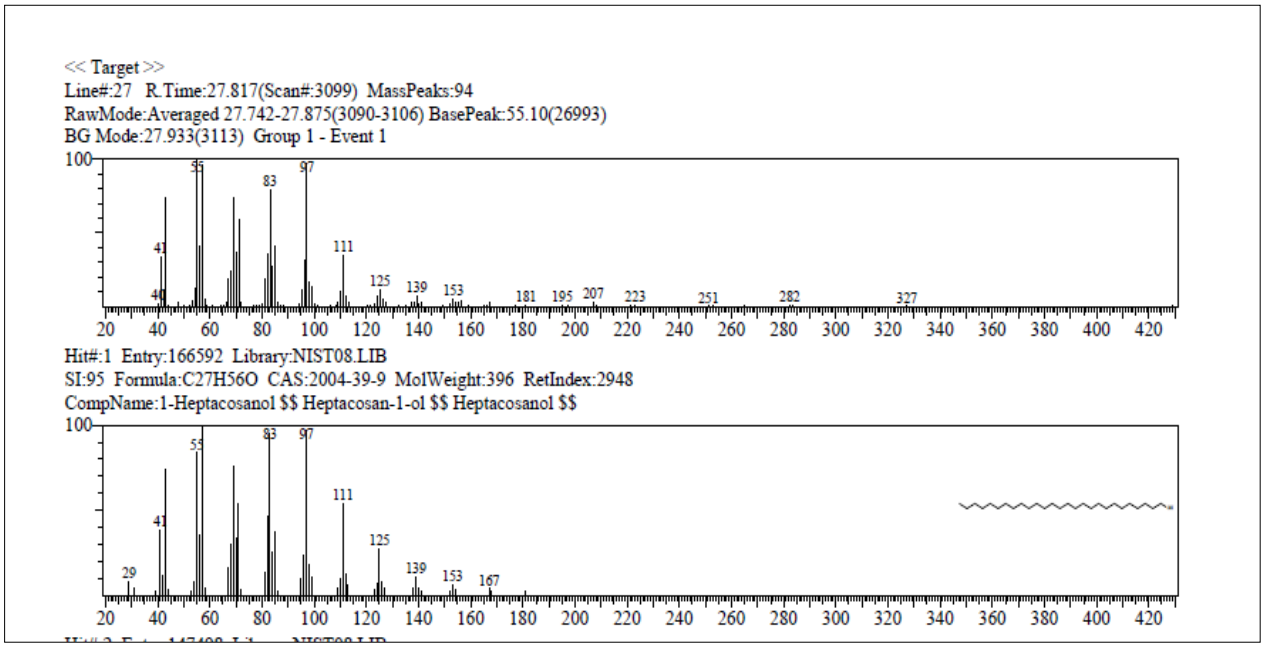

Figure 4. GC-MS result of 1-Heptacosanol compared with library

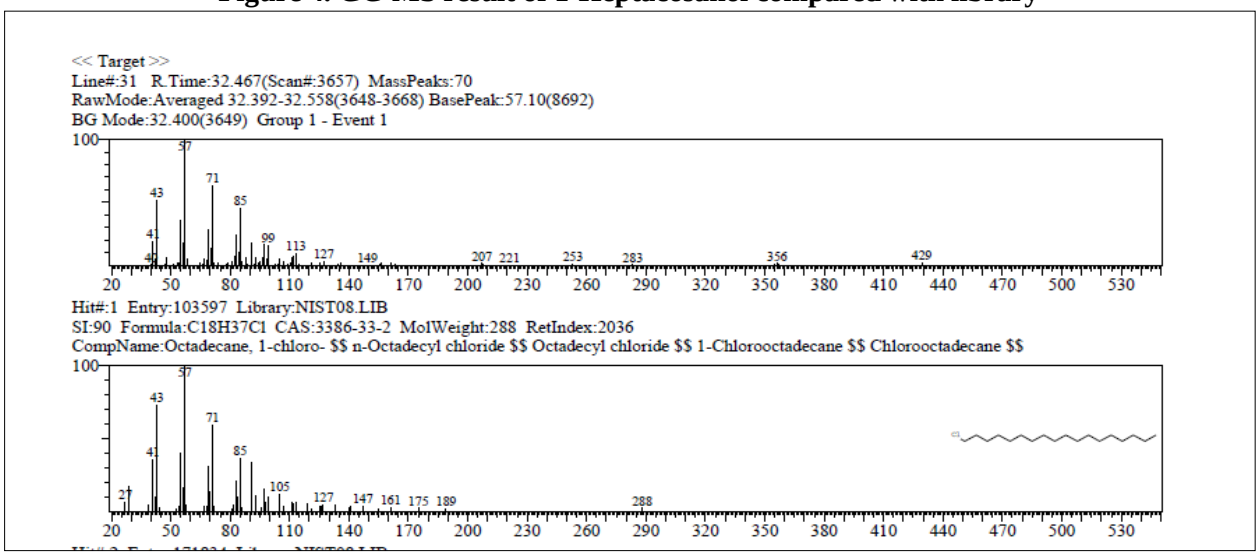

Figure 5. GC-MS result of Octadecyl chloride **compared with library**

Conclusion:

Thin layer chromatography of the methanolic extract of Chlamydomonas reinhardtii isolated from a pond of water in AL- Diwaniyah, Iraq showed the presence of 32 compounds. GC/MS analyses determined different compounds such as hydrocarbons, alcohols, phenols and esters, some of these compounds were bioactive compounds such as 1-Heptacosanol and Octadecyl chloride used in medical and pharmaceutical fields Extracellular extract of C. reinhardtii was tested for antimicrobial activity against several types of pathogens, andresults showed that biofilm formed by all tested isolates with difference in biofilm strength formation. Algal extract showed higher reduction of the biofilm forming by pathogenic bacteria.

Acknowledgment

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq), Baghdad, Iraq for its backing in the current work.

Conflicts of Interest: None.

Reference:

1. Guillaume L, Andrew B, Qiucen Z, David B, Robert A.et al. Physics of biofilms: the initial stages of biofilm formation and dynamics (NJP).2014February ; (16) 045005 .
2. Felix LO,Chari N, Dhamodharan B, Manivel A. Inhibitory Effect of *Spirulina platensis* Extraction Bacteria of Clinical Significance. (PNAS) , 2017 July; 87(2): 537–544.
3. Chari N, Felix L, Selvaraj K, Renganathan K, Dhamodharan B, Manivel A, Naiyf S, Arunachalam C, Sulaiman A , Nooruddin T. Biofilm inhibitory potential of *Chlamydomonas sp*. Extract against *Pseudomonas aeruginosa*. J. Algal Biomass Utln. 2014; 5 (4): 74-81.
4. Sauer K1, Camper AK, Ehrlich GD, Costerton JW, Davies DG. *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. J Bacteriol, 2002 Feb;184(4):1140-54.
5. David L, Ashwini C, Olaya R, Christophe B. From *in vitro* to *in vivo* Models of Bacterial Biofilm-Related Infections. J.Pathogens. 2013 Jun; 2(2): 288–356.
6. Elena T, Sofía G, Javier Á, Azahara R,Antonio A. Virginia M. Bioactive Compounds Isolated from Microalgae in Chronic Inflammation and Cancer. Mar. Drugs. 2015; (13): 6152-6209.
7. Amna A, Shahrazad N, Ghaidaa A. Roaa F. The effect of microalgae extraction on bacterial species isolated from seminal fluid of sexually- active males in Baghdad. J. Genet. Environ. Resour. Conserv. 2016; 4(2):171-177.
8. Minhas, Amritpreet K . "A Review on the Assessment of Stress Conditions for Simultaneous Production of Microalgal Lipids and Carotenoids." Frontiers in microbiology vol. 7 546. 3 May. 2016, doi:10.3389/fmicb.2016.00546
9. Azza MA, Amal AM, Farag AS. *In vitro* antioxidant and antibacterial activities of two fresh water Cyanobacterial species, *Oscillatoria agardhii* and *Anabaena sphaerica*. J APPL pharm.scie..2014 July;4 (7) : 069-075.
10. Sanmukh S, Bruno B, Ramakrishnan U, Khairnar K, Swaminathan S, PaunikarW, Bioactive Compounds Derived from Microalgae Showing Antimicrobial Activities. J Aquac Res Development. 2014; 5(3) 1000224
11- Dina YM, Ahmed SD, Ghaidaa HA. Abdel Latif MJ. Use of *Cladophora glomerata* Extract against Multidrug Resistant Bacterial Pathogens. J. Envir. Sci. Eng. 2013; B2 495-500.
12. Ghaidaa HA, Neihaya HZ, Merthad AS. Effect of some extracted compounds from the algae *Oscillatoria tenius* against pathogenic bacteria. Journal of Pharmaceutical and Scientific Innovation JPSI.2015; 4 (1):36-40.
13. Sayda MA, Mona HH, Waleed MS, Rawheya AS, Gamila HA. Antiviral Activity of Freshwater Algae. J. Appl. Pharm.Sci.2012 January; 2 (2): 21-25.
14. Al-Rubaie HG, Al-hashimi AA, Zaki NH, Rana H. Determination the anti-bacterial activity of Ag nanoparticlas produce biologicaly form different algae spp. Adva. in Environ.l Bio..2016 October; 10(10): 6-12.
15. Ali P, yifeng C. Algal bioactive diversities against pathogenic microbes. Microbial pathogens and strategies for combating them: science, technology and education (A. Méndez-Vilas, Ed.). 2014 January; 796-803
16. Emer S, Nissreen G. Antibacterial Derivatives of Marine Algae: An Overview of Pharmacological Mechanisms and Applications. Mar. Drugs .2016 Apr;14(4): 81.
17. Gurudeo TP. Antioxidant Activity of the Freshwater Microalga *Chlamydomonas* sp. Int. J. Pure App. Biosci. 2016 October ; 4 (5): 55-58.
18. Younes G, Ameneh M, Abdolali M, Shadman S, Mohammad HM. Antifungal and Antibacterial Activity of the Microalgae Collected from Paddy Fields of Iran: Characterization of Antimicrobial Activity of *Chroococcus disperses.* Journal of Biological Sciences. 2007; 7 (6): 904-910.
19. Usharani G, Srinivasan G, Sivasakthi S, Saranraj P. Antimicrobial Activity of Spirulina platensis Solvent Extracts Against Pathogenic Bacteria and Fungi. Adv.in Biol. Res. 2015; 9 (5): 292-298.
20. Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). J. Clin. Diagn. Res.2012 Nov; 6(9): 1478–1482.
21. Yanti RY, Lee KH. Activity of panduratin A isolated from *Kaempferia pandurata* Roxb. against multi-species oral biofilms *in vitro*. J.of Oral Sci..2009 Mar;51(1):87-95.
22. Busetti A, Thompson A, Tegazzini A, Megaw A, Maggs A, Gilmore B. Antibiofilm Activity of the Brown Alga *Halidrys siliquosa* against Clinically Relevant Human Pathogens. Mar. Drugs.2015 June ;13(6): 3581-3605.
23. Yanti, T, Fendy K, Jae-Kwan H.Inhibition of marine algae extracts on *Porphyromonas gingivalis* oral biofilm formation. International J. of Biol. & Pharm. Research.2015 ; 6(5): 370-374.
24. Ahmed Al, Esam AA, Haider YL, Mohammed KS, Mohammed JO, Allaa MA. Algae personification toxicity by GC–MASS and treatment by using material potassium permanganate in exposed basin. EJPE. 2017 September ;26(3): 835-842.
25. Elizabeth HH. Chlamydomonas as a model organism. Annu. Rev. Plant Physiol. Plant Mol. Biol. 2001; 52:363–406.
26. Renukadevi K, P and Pallippatt ,R .Phytochemical Analysis of Chlamydomonas reinhardtii CC-125 and Its Antimicrobial Activity.(IJER) 2015. International J Ext Res. 1:7-13
27. Salem O, Hoballah EM, Ghazi S, Hanna N. Antimicrobial activity of microalgal extracts with special emphasize on *Nostoc* sp. LIFE Sci J. 2014 Journal;4(12):752-758.
28. Qiao J. Antibactrial effect of extracts from two Icelandic algae (*Ascophyllumnodosum* and *Laminariadigitata*). PHD Thesis, Dalian Ocean University 2010.

29. Hristo MN, Liliana GG,Ivan II, Plamen SP, Jaromir L, Iva VT, et al. Antibacterial and antifungal activities of selected microalgae and cyanobacteria. INT J FOOD SCI TECH. 2013 March ;48(7): 1533–1540.
30. Neeli VH, ParvathiT, Krishna PB. Study of Biofilm Production and Anti-microbial susceptibility pattern of bacterial and fungal isolates from urinary catheters. Int.J.Curr.Microbiol.App .Sci.2016 Number; 5(2): 415-424.
31. Balasubramanian A, Singh AR,Alagumuthu G. Isolation and identification of microbes from biofilm of urinary catheters and antimicrobial susceptibility evaluation. ASIA-PAC J of Tropical Biomedicine, 2012;2(3): 1780-1783.
32. Molobela P, Cloete TE, Beukes M. Protease and amylase enzymes for biofilm removal and degradation of extracellular polymeric substances (EPS) produced by *Pseudomonas fluorescens* bacteria. Afr. J. Microbiol. Res. 2010,Vol. 4(14), pp. 1515-1524.
33. Cho HB, Lee HH, Lee OH. Clinical and microbial evaluation of the effects on gingivitis of a mouth rinse containing an *Enteromorpha linza* extract. J med Fod. 2011 Dec;14(12): 1670-1676.
34. Ren D, Sims JJ,Wood TK. Inhibition of biofilm formation and swarming of *Bacillus subtilis* by (5Z) -4-Bromo-5-(bromomethylene) -3-butyl-2(5H)-furanone. Biotechnology & Applied Microbiology. 2002;34: 293-299.
35. Sudalayandi k, kumar A,Sessler R, Sayre R, Falcoa V,Ihemere NJ, et al.Determination of fatty acids and proteins from the fresh water algae *Chlamydomonas reinhardtii* cc2137 and its antagonisms against aquatic bacteria Pak. J.Bot. (2012)44(60): 2139-2144.
36. Renukadevi P,Saravana S,Angayarkanni J. Antimicrobial and antioxidant activity of *Chlamydomonas reinhardtii* sp. (IJPSR) . 2011 April; 2(6): 1467-1472.

القابلية التثبيطيه للمركبات المنتجة *Chlamydomonas reinhardtii* على تثبيط الغشاء الحيوي للاحياء المجهرية المسببة للأمراض

غيداء حسين عبد علي **نهاية حكمت زكي** **ندى زكي مهدي** **امنة محمد**

قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية، بغداد، العراق.

الخلاصة:

اظهرت الطحالب المجهرية و العديد من المزايا للفعالية المضادة للميكروبات بسبب التنوع البيولوجي الضخم ومعدل النمو السريع. من خلال هذه الدراسة تم عزل *Chlamydomonas reinhardtii* من بركة ماء، وتم الحصول على الرائق عند استخلاصه بمذيب الميثانول. تم اختبار الفعالية المضاده للميكروبات لهذا المستخلص عند اختباره ضد الممرضات، وكانت أفضل منطقة تثبيط ضد *Candida albicans* بقطر 32 mm، و*S.aureus* 15mm و 9mm *E.coli* ، في حين لم يظهر أي تأثير ضد كل من *S.epidermidis* و *Klebsiella spp.* تم تكوين البيوفيلم من قبل جميع العزلات المختبرة مع وجود اختلافات في قوة تشكيله. أظهر مستخلص الطحلب *C. reinhardtii* قدرة كبيرة على الحد من تشكيل البيوفيلم للعنقوديات المذهبة ونسبة البيوفيلم المتبقي كانت (10.9٪)، في حين كانت النسب 55.21٪ و 54.98٪ تعود إلى البايوفيلم المتبقي لكل من *Serratia spp* و *Escherichia coli* على التوالي ، وهو أقل انخفاض. اما الانواع *Klebsiella spp.*، *Candida albicans*، *Acinetobacter* spp.، *Staphylococcus epidermides*، *Aspergillus spp* أظهرت نسب للبيوفيلم المتبقي وهي (16.7 و 18.3 و 19.6 و 43.6 و 44.1)٪ على التوالي. تم تحديد تراكيب المركبات المتطايرة لمستخلص *C. reinhardtii* بواسطة جهاز GC / MS. حددت مجاميع مختلفة من المركبات، مثل الهيدروكربونات، الفينولات، الكحولات والأسترات، وتبين ان 1-Heptacosanol ، وكلوريد Octadecyl كانت اكثر المركبات فعالية واستخدمت سابقا في المجالات الطبية والصيدلانية. هذه النتائج الواعده تعطي مؤشرا لوجود مركبات ذات تأثير مضاد للبايوفيلم في الطحلب قيد الدراسة. وهناك حاجة لاجراء دراسات كيميائية إخرى لإلقاء الضوء على هذه المركبات وتركيبها وفعاليتها الحيوية.

الكلمات المفتاحية: مضاد بكتيري ،مستخلص الراشح ،*Chlamydomonas reinhardtii* ، تحليل كروماتوغرافيا الغاز - مطياف الكتلة