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. *

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**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 extern, 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).
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, terpenes, 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 epidermidis* 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 epidermidis* 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 °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 48 hours at 37°C. 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 24 hours 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:

\[
\frac{(\text{O.D595 of the extract} - \text{O.D595 of the negative control})}{(\text{O.D595 of the positive control} - \text{O.D595 of the negative control})} \times 100\%
\]

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 °C while the injector and detector temperatures were set in 280 °C. A sample volume was injected about (5μl) into the column and run using split (1:10) mode. After 1 min, the temperature of oven was raised to 225 °C at a ramp rate of 12.5 °C/ min (4 min). In addition, the ramp rate of 7.5 °C/ min (hold time 5 min) when the temperature was raised to 300 °C. 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 1 showed the shape and quantity of extract.

![Figure 1. Extracellular extract of Chlamydomonas reinhardtii](image)

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](image)
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 sp.                 | -                        |
| Staphylococcus epidermidies  | 8                        |
| Staphylococcus aureus        | 15                       |
| Aspergillus sp.              | 9                        |
| Candida albicans             | 32                       |

Table 2. Biofilm formation of pathogenic bacteria

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

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 epidermidies  | 43.6                   |
| Staphylococcus aureus        | 10.9                   |
| Aspergillus sp.              | 44.1                   |
| Candida albicans             | 18.3                   |

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 epidermidies, 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).

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-acyl2 Gro, acyl2 Gro-Me3Hse, Gal-acyl2 Gro, acyl2 Gro, acyl2 Gro-Me2Hse.
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   | Methylen 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    | Benzenacetic 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[thiromethyl]           |
| 8    | 14.731 | 1.32  | 2.71    | 2-(2,4,4,6,6,8,8-heptamethyloxasiloxan-2-yloxy)        |
| 9    | 15.367 | 1.47  | 2.83    | Homogentisic acid, bis(tert-butylidimethylsilyl 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    | Octasilsloxane,1,1,3,3,5,7,9,11,13-tetradecamethyl     |
| 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-Chloroicosane                                        |
| 26   | 27.448 | 4.05  | 5.49    | Docosyl pentafluoropropionate                          |
| 27   | 27.817 | 3.30  | 4.10    | 1-Heptacosan                                           |
| 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 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, and results 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.

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**Conflicts of Interest:** None.
Reference:
1. Guillaume L, Andrew B, Qui cen Z, David B, Robert A et al. Physics of biofilms: the initial stages of biofilm formation and dynamics (NJP). 2014 February; (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, Nayif S, Arunachalam C, Sulaiman A, Nooruddin T. Biofilm inhibitory potential of Chlamydomonas sp. Extract against Pseudomomas aeruginosa. J. Algal Biomass Uln. 2014; 5 (4): 74-81.
4. Sauer K1, Camper AK, Ehrl ich GD, Costerton JW, Davies DG. Pseudomomas 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, Sofia 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 microalgal 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 sp.95. J Appl. Pharm.sci..2014 July;4 (7): 069-075.
10. Samuikh 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. Environ. Sci. Eng. 2013; B2 495-500.
12. Ghaidaa HA, Nehiya 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 nanoparticles produce biologically form different algae spp. Adv. 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, Leo 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 persification 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 Pullippatt 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. Antibacterial effect of extracts from two Icelandic algae (Ascomyllum nodosum and Laminaria digita). PHD Thesis, Dalian Ocean University 2010.
29. Hristo MN, Liliana GG, Ivan II, Plamen SP, Jaromir L, Iva VT, et al. Antimicrobial activities of selected microalgae and cyanobacteria. Int J Food Sci Tech. 2013 March;48(7): 1533-1540.

30. Neeli VH, Parvathi T, 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-buty1-2(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 reinhardtiicc2137 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.

37. 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.

38. Ren D, Sims JJ, Wood TK. Inhibition of biofilm formation and swarming of Bacillus subtilis by 5Z-4- bromo-5-(bromomethylene)-3-buty1-2(5H)-furanone. Biotechnology & Applied Microbiology. 2002;34: 293-299.

39. 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 reinhardtiicc2137 and its antagonisms against aquatic bacteria Pak. J.Bot. (2012)44(60): 2139-2144.

40. Renukadevi P, Saravana S, Angayarkanni J. Antimicrobial and antioxidant activity of Chlamydomonas reinhardtii sp. (IJPSR). 2011 April; 2(6): 1467-1472.