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Antibacterial properties of the *Chlorella vulgaris* isolated from polluted water in Iraq

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Abstract

This study aims at investigating the effect of the crude bioactive compounds extracted from *Chlorella vulgaris* against some negative and positive gram Pathogenic Bacteria. Antibacterial properties of *Chlorella vulgaris* has been determined in vitro using agar well diffusion methods against some negative and positive gram pathogenic bacteria. The obtained results have shown that the crude bioactive compounds of *Chlorella vulgaris* possess wide ranges of antibacterial properties against gram negative and gram positive bacteria isolated from teeth decay. This investigation exhibits that it can be presumed that the effect of the crude bioactive compounds in *Chlorella vulgaris* has a distinctive effect on various pathogenic life forms in high concentrations. The crude bioactive compounds extracted from *Chlorella vulgaris* have shown effective antibacterial properties against both grams negative and gram positive pathogens except for *Klebsiella* which has been more resistant at different concentrations.

Keywords: Antibacterial; Chlorella vulgaris; Bioactive compounds; Polluted water

INTRODUCTION

Algae a various group belonging to the plant kingdom contains distinctive bioactive substances, the bioactive compounds created by currently developing cells of algae incorporate carbohydrates, enzymes, proteins, free amino acids, fats, lipids, regulators of growth, pigments, toxic materials, antibiotics, phenol, flavonoid, and vitamins. Algae are amazing sources for antimicrobial materials uses in laboratory tests, which inhibited microbial agent that causes diseases to the humans [1]. Photoautotrophic microorganisms collectively termed "Microalgae" that made up micro-eukaryotes and prokaryotic cyanobacteria possess the ability to make a wide range of bioactive substances with various natural activities. The expanded utilization of anti-microbial materials and chemotherapeutics for disease treatment prompts the development of drug-resistant microorganisms and furthermore unfavourably influences the biological system [2]. *Chlorella* is one of the most notable microalgae genera [3]. *Chlorella* is placed below the order Chlorococcales [4], 128 years ago, family Chlorellaceae [5]. Recent molecular genetic studies depending on the polyphyly placed this genus within the Trebouxiophyceae and Chlorophyceae class [6]. *Chlorella* is unicellular, freshwater living and possess bioactive materials such as pigments, vitamins, proteins, sterols and long-chain polyunsaturated fatty acids [7, 8, 9 and 3]. The issue of drug resistance of pathogenic agent to antibiotics is expanding with time. The aimless utilization of antimicrobial materials for the treatment of sicknesses is one of the causes for the improvement of multidrug-resistant microorganisms, up till now resistance of pathogenic microorganisms to almost all antibiotics has been mentioned, moreover, the reactions related with antibiotics has expanded the issue [10 and 11]. For that reason, there is a substantial need to find the new types of antipathogenic materials which have negligible side reactions. In this context, this study aimed to isolate *Chlorella vulgaris* from polluted water and uses an antibacterial agent against Human Pathogenic Bacteria.

MATERIALS AND METHODS

The study area: The samples were collected from the Al-Yahodiyah rivulet in the Hillah city middle of Iraq which was chosen for its intensive residential neighborhoods and green areas and parks within the urban area in the center of the city which is located about 1.8 Km away from the Hillah city in Babil Governorate.

Sample collection: The sample of polluted water collected from the Al-Yahodiyah rivulet in the Hillah city middle of Iraq by using poly polyethylene containers and brought to the laboratory. Necrotic parts were removed and any associated debris.

Isolation of *Chlorella vulgaris*: Microalgae *Chlorella* was isolated by using Streak Plating Technique according to [12].

Identification of *Chlorella*: Microalgae *Chlorella vulgaris* was identified and diagnose according to [13 and 14].

Preparation of solvent: The solvent was prepared by mixing 25ml ethanol, 25ml of acetone, 25ml of Ethel acetate and 25ml of sterile distilled water (1:1:1:1).

Culture and Extraction of Microalgae materials: Microalgae materials were cultured and extracted according to [15]. A quantity of 1gm of bioactive was prepared in 10 ml of sterile deionized water for final concentration 100mg/ml).

Microorganism Strain: All samples used in the current investigation are isolated from people with teeth decay.

Antibacterial Activity Determination: Antibacterial activity was determined according to well diffusion method [16]. The plates of agar were incubated in incubation for 24 hr at 37°C and zones of restraint if any around the well were estimated in millimeter (mm). Each treatment was replicated three times.

Figure 1: *Chlorella vulgaris* isolated from polluted water

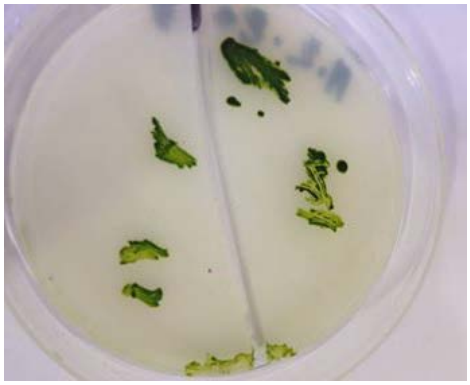

Figure 2: Culture of *Chlorella vulgaris* isolated from polluted water

RESULTS AND DISCUSSION

The antibacterial properties of the crude active compounds extract of *Chlorella vulgaris* against human gram negative pathogenic bacteria are introduced in a (Table: 1).

Table 1: Antibacterial properties of the crude *Chlorella vulgaris* extract against some gram negative bacteria

Bacteria	*Chlorella* extract	
	mg/L	
	10	100
	Zone of Inhibition / mm/ diameter	
Enterobacter	0	25
Proteus	0	24
Escherichia coli	0	15
Klebsiella	0	0
0= Resistant		

Activity was assessed at (10 and 100) mg/ ml. the results uncovered that *Enterobacter*, *Proteus* and *Escherichia coli* bacteria were resistant to crude active compounds extracted from *Chlorella vulgaris* at low concentrations (10 mg/ ml) and sensitive at high (100mg/ml) concentrations (Figure: 3, 4and5). While *Klebsiella* resistant to all active compounds present in *Chlorella vulgaris* at low and high concentrations. The results also revealed that *Enterobacter* was more sensitive to crude active compounds in compare with *Proteus* and *Escherichia coli*.

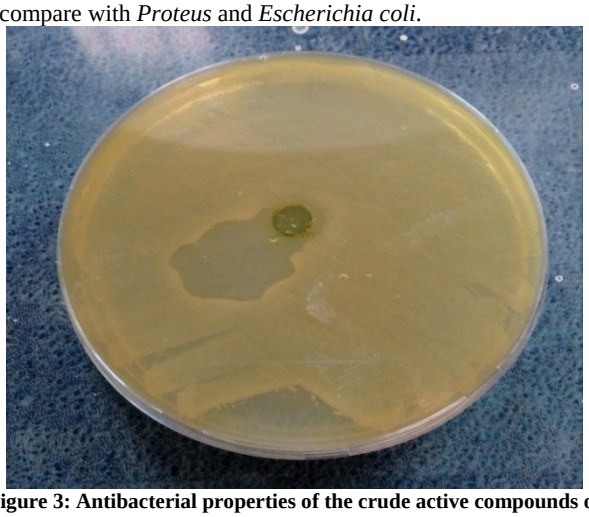

Figure 3: Antibacterial properties of the crude active compounds of the *Chlorella vulgaris* extract against *Enterobacter*

Figure 4: Antibacterial properties of the crude active compounds of the *Chlorella vulgaris* extract against *Proteus*

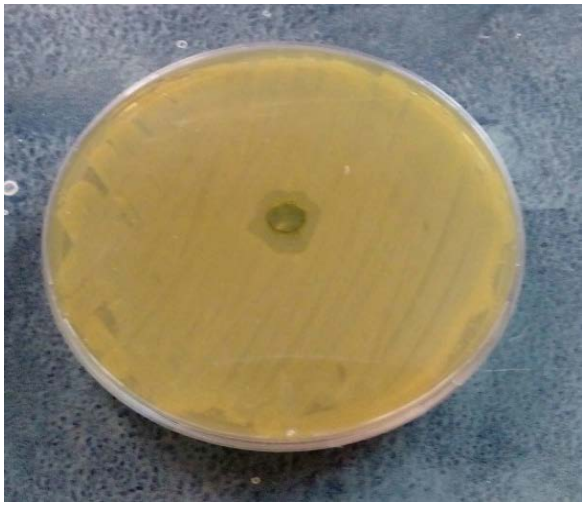

Figure 5: Antibacterial properties of the crude active compounds of the *Chlorella vulgaris* extract against *Escherichia coli*

On the other hand, the antibacterial properties of the crude active compounds extract of *Chlorella vulgaris* against human gram positive pathogenic bacteria are introduced in a (Table: 2).

Table 2: Antibacterial properties of the crude *Chlorella vulgaris* extract against some gram positive bacteria

Bacteria	*Chlorella* extract	
	mg/L	
	10	100
	Zone of Inhibition / mm/ diameter	
Staphylococcus aureus	0	20
Lactobacillus acidophilus	0	18
Streptococcus pyogenes	0	15
0= Resistant		

Activity was assessed at (10 and 100) mg/ ml. the results revealed that *Staphylococcus aureus*, *Lactobacillus acidophilus* and *Streptococcus pyogenes* bacteria were resistant to the crude active compounds extracted from *Chlorella vulgaris* at low concentrations (10 mg/ ml) and sensitive at high (100mg/ml) concentrations (Figure:6, 7and8) . The results also revealed that *Staphylococcus aureus* were more sensitive to the crude active compounds in compare with *Lactobacillus acidophilus* and *Streptococcus pyogenes*.

Figure 6: Antibacterial properties of the crude active compounds of the *Chlorella vulgaris* extract against *Staphylococcus aureus*

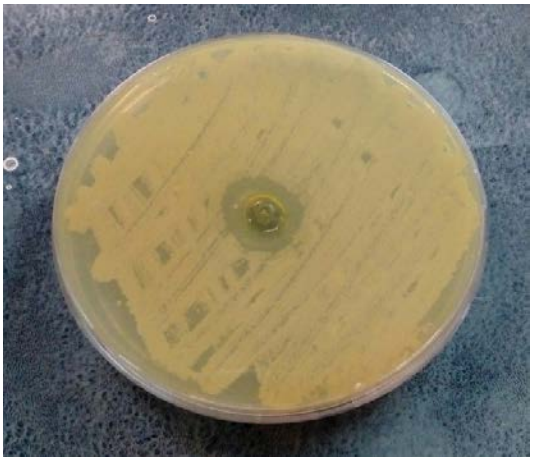

Figure 7: Antibacterial properties of the crude active compounds of the *Chlorella vulgaris* extract against *Lactobacillus acidophilus*

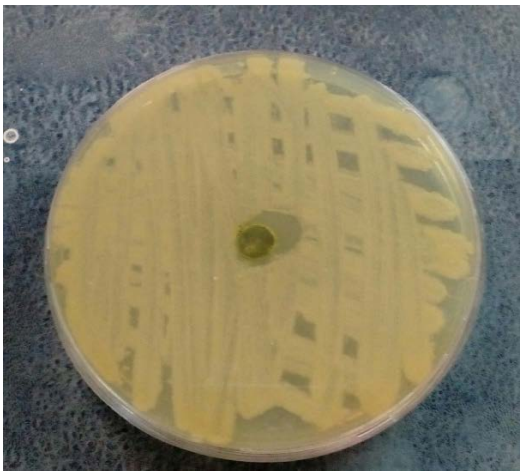

Figure 8: Antibacterial properties of the crude active compounds of the *Chlorella vulgaris* extract against *Streptococcus pyogenes*

The plant kingdom is characterized by possessing a wide range of bioactive compounds with different uses. In contrast, Pathogenic microorganisms are characterized by their ability to resist the antibiotics continuously, for this reason, it is necessary to look within the plant kingdom for more effective natural sources against pathogenic microorganisms. The Results of the current study demonstrated that the crude bioactive compounds extracted from *Chlorella vulgaris* exhibited antibacterial properties against the gram negative bacteria and gram positive bacteria. Syed et al. [15] reported that *Chlorella vulgaris* extracts have positive results for active materials like Flavonoids, Tannin, Terpenoid, Cardiac glycosides, Saponins and Phenolic compounds. Findings in this study an agreement with [17, 18, 19, 20 and 21] Mention that active compounds like Terpenoid, Phenolic and Alkaloid of *Lactuca serriola* L., *Lepidium sativum* L., *Myrtus Communis* L., *Cassia senna* L. and *Ricinus communis* L. extracts showed the inhibitory effect on human gram negative and gram positive pathogenic bacteria. In contrast, the results of this study disagreement with [15] Regarding *Klebsiella* and [22] Regarding *Staphylococcus aureus*, this is due to the use of different solvents that lead to the extraction of different bioactive compounds with different effects. In general, gram positive bacteria are more sensitive to antibiotics due to the simple structure of the cell wall which makes the penetration of antibiotic materials easy in compare with gram negative bacteria [23]. However, in the current study, we found that *Chlorella vulgaris* extract had a wide range of effectiveness against Gram negative bacteria and gram positive bacteria except for *Klebsiella*. This susceptibility of gram-negative bacteria and gram-positive bacteria may be due to the present of Chlorellin "a mixture of fatty acid" in the crude extract was effective against both Gram-negative and Gram-positive bacteria [24]. Alternatively, this may be due to either the presence of bioactive compounds effect on the cell wall, proteins and DNA synthesis or by the hydrophobicity, which impaired the lipids of cell membrane and mitochondria, resulting in leakage and cell death [25]. Based on the results got in this current investigation, could be concluded that the effects of the bioactive substances in *Chlorella vulgaris* have a diverse effect on various pathogenic organisms in various concentrations. The got results may give a help to the utilization of the microalgae in the conventional drug.

CONCLUSION

The crude bioactive compounds extracted from *Chlorella vulgaris* had demonstrated successful antibacterial action against gram negative and gram positive pathogenic bacteria isolated from teeth decay, and *Klebsiella* the most resistant to all bioactive compounds extracted from *Chlorella vulgaris*.

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REFERENCES

1. Kulik MM The potential for using cyanobacteria (blue green algae) and algae in the biological control of plant pathogenic bacteria and fungi. European Journal of Plant Pathology, 1995, 101, 585-599.
2. Pradhan J, Das S and Das B K. Antibacterial activity of freshwater microalgae: A review. African Journal of Pharmacy and Pharmacology, 2014, 8(32), 809-818.
3. Andrade L M, Andrade CJ, Dias M, Nascimento CAO, Mendes M A. *Chlorella* and *Spirulina* Microalgae as Sources of Functional Foods, Nutraceuticals, and Food Supplements; an Overview. MOJ Food Process Technol 2018, 6(1), 00144. DOI: 10.15406.
4. Beijerinck M W. Culturversuche mit Zoochlorella, Lichenen-goniden und anderen niederen Algen. Bot. Ztg. 1890, 45, 726-739, 741-753, 757-767, 781-784.
5. Hoek C, Mann D G and Johns H M. Algae An introduction to Phycology. Cambridge University Press. 1995.
6. Bock C, krienitz L and Pröschold T. Taxonomic reassessment of the genus *Chlorella* (Trebouxiophyceae) using molecular signatures (barcodes), including description of seven new species. Fottea, 2011, 11(2), 293–312.
7. Chisti Y. Biodiesel form microalgae. Biotechnol. Adv. 2007, 25(3), 294-306.
8. Wawrik B and Harriman B H. Rapid, colorimetric quantification of lipid from alga cultures. J. Microbiol Methods, 2010, 80(3), 262-266.
9. Safi C, Liu D Z, Yap B H J, Martin G J O, Vaca Garcia C, et al. A two-stage ultrafiltration process for separating multiple components of *Tetraselmis suecia* after cell disruption. J App. Phcol. 2014, 26(6): 2379-2387.
10. Mendes RL, Nobre BP, Cardoso MT, Pereira AP and Palabra AF. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorg Chim Acta. 2003, 356: 328-334.
11. Mayer AMS and Hamann MT. Marine pharmacology in 2001-2002: marine compounds with antihelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, antiinflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. Comp Biochem Phyco, Part C. 2005, 140, 265-286.
12. Phang S M and Chu W L. Algae culture Collection, Catalogue of Strains. Institute of Post Graduate Studies and Research, University of Malaya, Kuala Lumpur, Malaysia. 1999, 77.
13. Desikachary T V. Cyanophyta. Indian Council of Agricultural Research New Dalhi. 1959, 686.
14. Prescott G W. The algae of the western Great area cranbook Inst.Sci.Michigan. 1973, 977.

15. Syed Sh, Arasu A and Ponnuswamy I. The Uses of *Chlorella Vulgaris* as Antimicrobial Agent and as a Diet: the Presence of Bio-active Compounds which caters the Vitamins, Minerals in General. International Journal of Bio-Science and Bio-Technology, 2015, 7(1), 185-190.
16. Scott AC Laboratory control of antimicrobial therapy. In: Mackie & McCartney Practical medical microbiology (Edited by, Collee JG, Duguid JP, Fraser AG and Marmion B P) 13th Edn. Vol 2, United Kingdom-Edinburgh: Churchill Livingstone, 1989, 161-181.
17. Al-Marzoqi A H, Hussein H J and Al-Khafaji N M S. Antibacterial Activity of the Crude Phenolic, Alkaloid and Terpenoid Compounds Extracts of *Lactuca serriola* L. on Human Pathogenic Bacteria. Chemistry and Materials Research, 2015, (7)1, 8-10.
18. Al-Marzoqi A H, Al-Khafaji, N M S and Hussein, H J. In vitro Antibacterial Activity Assessment of the crude Phenolic, Alkaloid and Terpenoid compounds extracts of *Lepidium sativum* L. on Human Pathogenic Bacteria. International Journal of ChemTech Research, 2016, (9)4, 529-532.
19. Hussein H J, Al-Khafaji, N M S, Al-Mamoori A H, Juaifer W A K, Al-Marzoqi AH and Al-Zobiady R A. Antimicrobial Effect of the Crude Phenolic, Alkaloid and Terpenoid Compounds Extracts of *Myrtus Communis* L. against Human Gram-Negative Pathogenic Bacteria. Journal of Global Pharma Technology, 2017, (9)8, 130-133.
20. Hussein H J, Al-Khafaji, N M S, Al-Mamoori A H and Al-Marzoqi AH. Evaluation of in vitro antibacterial properties of the crude Phenolic, Alkaloid and Terpenoid extracts of *Cassia senna* L. against Human gram-negative Pathogenic Bacteria. Plant archives, 2018, (18)1, 354-356.
21. Hussein H J, Kaizal A F, Al-Khafaji, N M S, Sadiq Z F and Shahad A Sh. Evaluation of antibacterial potential of the crude Phenolic, Alkaloid and Terpenoid extracts of *Ricinus communis* L. against gram-negative Pathogenic Bacteria. Journal of Global Pharma Technology, 2018, (10)03, 171-175.
22. Hend A and Perveen K Antibacterial activity and morphological changes in human pathogenic bacteria caused by *Chlorella vulgaris* extracts. Biomed Res- India, 2017, (28)4, 1610-1614.
23. Beveridge TJ. Structures of gram-negative cell walls and their derived membrane vesicles J. Bacteriol, 1999, 181, 4725-4733.
24. Pratt R, Daniel TC, Eier JB, Gunnison JB, Kumler WD, Oneto JF, Strait L A, Spoehr HA, Hardin G J, Milner HW, Smith H, Strain HH. Chlorellin. An antibacterial substance from chlorella. Science, 1944, 99, 351-352.
25. Rastogi RP and BN Mehrotra: Glossary of Indian Medicinal Plants. National Institute of science communication, New Delhi, India 2002, 20-25.