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Investigating Antimicrobial Properties of Nanocloropsis.sp Microalgae Extract

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Abstract

Introduction

Treating infectious diseases, especially diseases caused by resistant pathogens, has become one of the biggest health care challenges worldwide. Marine microorganisms are a rich The increasing prevalence of resistant pathogens, makes treating infectious diseases one of the major health care challenges in the world today. Introduction Marine microorganisms are a repository of unique bioactive secondary metabolites, considerably important in novel pharmacological agent discovery.

Materials and Methods

The sample of this article is alcoholic extract of Nanochloropsis algal by Soxhlet method.The antimicrobial effects of alcoholic extract on Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were evaluated. The extracts were first investigated for antibacterial properties by tubular dilution in liquid medium, determining the minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) at varying concentrations of extracts.MIC and MBC test results revealed that

Conclusion

Staphylococcus aureus and Escherichia coliwere the most sensitive andPseudomonas aeruginosawas the most resistant bacterium to algae extract. Hence, it is proposed that extracts of alcoholic algae have antimicrobial effect on Staphylococcus aureus and Escherichia.

1. Introduction

Over the past few decades, antibiotic resistance to bacteria has been increased leading to adverse side effects and increased mortality, and has posed a major public health concern. Because antibiotics have lost their effectiveness and an alarming rate of antibiotic-resistant microorganisms has been developed [1].

Treatment of infectious diseases, especially diseases caused by resistant pathogens, has become one of the biggest health care challenges worldwide. Resistance against antibiotic has emerged in major bacterial species and created a crisis [2]. It is predicted that the human cost of this crisis reaches 300 million premature deaths by 2050, resulting in $ 100 trillion lose of global economy [3]. To address this problem, major cooperation between the nations of the world and

There is a great diversity of microalgae — somewhere close to 10 million species, depending on how you count them, and the total number of algal species ranges from 40,000 to ever-absorbing-into-the-unfolding-pagans. Microalgae are eukaryotic algae that are photosynthetic and are found primarily in aquatic systems; they account for ~40% of global photosynthesis[5]. Each have distinct morphological, physiological, and genetic characteristics and produce multiple bioactive metabolites [6]. Microalgae are capable of high-biotarget corresponding to a broad spectrum of biological activity such as carotenoids, phycobilins, unsaturated fatty acids, proteins, polysaccharides, vitamins, and sterols, and other chemicals [7]. Due to their many advantages for health, tiny (algal) molecules are widely used in various areas such as nutrition, medicine, and applications. Recently, a number of biological active compounds from marine organisms, including microalgae which are fast growing and rich sources of many bioactive molecules between them [8], have attracted significant attention in the research markets.

The use of microalgae as an alternative source of antibiotics and preservatives has attracted much attention. Cell extracts of various microalgae show antibacterial activity against gram-negative and gram -negative bacteria.[9] Bioactive compounds, whose antimicrobial potential have been shown, include amino acids, polysaccharides, proteins, unsaturated fatty acids (PUFAs), especially EPA and DHA, and antioxidants (polyphenols, flavonoids, and carotenoids) [9][10].

Nanochloropsis microalgae belong to the ostigmatophyse family and eukaryotic photosynthetic single-celled microorganisms that live in both freshwater and saline environments [7]. This microalgae contains antioxidants such as carotenoids, phenolics, flavonoids, unsaturated fatty acids, vitamins, sulfated polysaccharides, sterols, minerals, amino acids, phycobiliproteins as well as some other compounds such as MAAF, coal, MAA, coal Q, and peptides [11].

In 2016, Sayegh et al. introduced the potassium salt of fatty acids in nanochlorpecis as an effective factor in the antimicrobial activity of this alga against gram-positive and gram-negative bacteria [9]. The aim of the present study was to investigate the antimicrobial properties of the Nanocloropsis.sp. microalgae extract.

2.Materials and Methods

Nanochloropsis algae powder is prepared from the Caspian Ecology Research Institute. 25 g of algae powder was combined with 250 ml of 95% ethanol alcohol as a solvent and placed in a Soxhlet extractor for 24 hours to separate the effective ingredients of the algae. The obtained extract was filtered with Whatman paper, concentrated using a rotary evaporator at 45°C, and finally dried by placing it at 55 °C for 48 hours. Then, it was placed in a freezer at -20°C for the next investigation. To evaluate the antimicrobial activity of the extracts of standard and freeze-dryer strains, three bacteria of Escherichia coli PTCC 1330, Staphylococcus aureus PTCC 1113, and Pseudomonas aeruginosa PTCC 1557 were prepared from Iran Scientific and Industrial Research Organization and kept in -20°C freezer until the experiments were performed. First, according to the instructions and under sterile conditions, the heads of the vials were broken and the contents were transferred to calf Brain Heart Infusion (BHI) broth medium. The samples were then incubated at 37 °C for 24 hours and the grown colonies were used to prepare a standard microbial suspension. Thus, after the initial post-culture of the colonies in BHI medium, and incubation at 37 ° C for 16 hours and observation of turbidity in the broth medium, the samples were centrifuged at 6000 rpm for 15 minutes. The samples were centrifuged again after washing with sterile phosphate buffer serum. This process was repeated 3 times and finally the turbidity of each bacterium was compared with a standard McFarland tube (0.5 McFarland equals 1.5 $\times 10^8$ cfu/ml). Dilution method was used to determine the minimum inhibitory concentration of MIC and the minimum lethal concentration of MBC.

0.2 ml of the studied bacteria at a concentration of approximately 106 cfu/ml was added separately to the experimental tubes containing 1 ml of BHI broth medium. In the next step, concentrations of 1, 2, 4, 8, and 10 mg / ml of microalgae extract with DMSO were added for 0.2 ml each and then the samples were placed in the incubator for 24 hours at 37 °C. The lowest concentration at which no turbidity was observed in the test tubes was considered as MIC. After determining the MIC, to determine the MBC under sterile conditions, 0.1 ml of the contents of tubes that were still clear after 24 hours of incubation and no turbidity were observed, was cultured at the surface in BHI agar medium.

After 24 hours of incubation at the appropriate temperature, the growth and non-growth of bacteria were examined and the first concentration at which no growth was observed was considered as MBC. To evaluate the growth inhibition zone, 100 µl of each bacterial suspension (106 cfu/ml) was first cultured on the surface of Müller-Hinton agar medium. Then, wells with a diameter of 6 mm were installed in different parts of the culture medium and concentrations of 1, 2, 4, 8, and 10 mg/ml of microalgae extract for 50 µl were added to each well. DMSO and trimethoprim sulfamethoxazole antibiogram disc (30 mg) were used as negative and positive controls, respectively. Bacterial media were incubated for 37 h in a 37 °C incubator and the diameter of non-growth zones around the wells was measured using a caliper and determined in millimeters (AOAC, 2000). Statistical analysis of data was performed using SPSS software version One-way analysis of variance was used to check the presence or absence of significant differences at the 5% level between the values of each index, and if a significant difference was observed, the Tukey test was used for the post hoc test. Statistical calculations were performed using Prism software version 7 at a significant level of P <0.05.

3.Results

The antimicrobial properties of the hydroalcoholic extract of Nanochloropsis algae were studied in five different concentrations on three bacteria and the results were shown in Table 1. The mean diameter of the growth inhibition zone was calculated after three replications.

Results The most sensitive bacterium was Staphylococcus aureusand the most resistant bacterium was Pseudomonas aeruginosawith Escherichia colibeing in the median position concerning the level of sensitivity to algae extract; however, their response to use of extract together differed highly significant (p<0.01). Moreover, the increased concentration of the extract within the range used led to an increase in the inhibition of the studied bacteria. Thus, by increasing the concentration of the extract, the diameter of the growth inhibition zone increases markedly. Concentration 10 was the best inhibitory concentration for growth inhibition for all bacteria and exerted the most powerful inhibition against Staphylococcus aureus, the next PS. aeruginosa and Escherichia coli were less sensitive to this concentration of the extract and differences between their sensitivities were significant (p<0.01).The antimicrobial properties of algae extract in three different concentrations by dilution in the tube are shown in Table 2. The minimum concentration at which the bacteria did not grow was considered equal to the minimum inhibitory concentration (MIC). Staphylococcus aureus and Escherichia coli were the most sensitive bacteria (lowest MIC) and Pseudomonas aeruginosa was the most resistant bacterium (highest MIC) to algae extract.The antimicrobial properties of algae extract in three different concentrations by dilution in the tube are shown in Table 3. The minimum concentration at which the bacterium did not grow was considered equal to the minimum bactericidal concentration (MBC).

Table 1_ Comparison of the antimicrobial effect of hydroalcoholic extract of Nanochloropsis algae on the mean diameter of growth inhibition zone in vitro (each data indicates Mean ± SD of 3 replications. Different uppercase and lowercase letters in each row and column show significant differences between data, respectively).

Concentration / **Bacteria**	**1**	**2**	**4**	**8**	**10**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Staphylococcus aureus	R	R	12.50±0.7cA	17.50±0.7bA	25.25±1.06a A
Pseudomonas aeruginosa	R	R	8.00±0.00cB	11.25±1.06b B	16.50±0.7aC
Escherichia coli	R	R	11.50±0.71c A	16.25±0.35b A	21.75±0.35a B

R: Resistant

Results The most sensitive bacterium was Staphylococcus aureusand the most resistant bacterium was Pseudomonas aeruginosawith Escherichia colibeing in the median position concerning the level of sensitivity to algae extract; however, their response to use of extract together differed highly significant (p<0.01). Moreover, the increased concentration of the extract within the range used led to an increase in the inhibition of the studied bacteria. Thus, by increasing the concentration of the extract, the diameter of the growth inhibition zone increases markedly. Concentration 10 was the best inhibitory concentration for growth inhibition for all bacteria and exerted the most powerful inhibition against Staphylococcus aureus, the next PS. aeruginosa and Escherichia coli were less sensitive to this concentration of the extract and differences between their sensitivities were significant (p<0.01).The antimicrobial properties of algae extract in three different concentrations by dilution in the tube are shown in Table 2. The minimum concentration at which the bacteria did not grow was considered equal to the minimum inhibitory concentration (MIC). Staphylococcus aureus and Escherichia coli were the most sensitive bacteria (lowest MIC) and Pseudomonas aeruginosa was the most resistant bacterium (highest MIC) to algae extract.

Table 2_ Comparison of antimicrobial properties of Nanochloropsis algae extract on mean minimum inhibitory concentration (MIC) in vitro

Bacteria	**MIC(mg/ml**
Staphylococcus aureus	8
Pseudomonas aeruginosa	10
Escherichia coli	8

The antimicrobial properties of algae extract in three different concentrations by dilution in the tube are shown in Table 3. The minimum concentration at which the bacterium did not grow was considered equal to the minimum bactericidal concentration (MBC). Pseudomonas aeruginosa is the most susceptible bacterium (lowest MBC) and Escherichia coli and Staphylococcus aureus are the most resistant bacteria (highest MBC) to algae extract.

Table 3_ Comparison of the antimicrobial effect of Nanochloropsis algae extract on the mean minimum bactericidal concentration (MBC) in vitro

Bacteria	**MBC(mg/ml)**
Staphylococcus aureus	**10**
Pseudomonas aeruginosa	-
Escherichia coli	**10**

Discussion

In recent years, the use of new sources with antimicrobial potential, such as natural compounds found in seaweed, has increased due to the reduction of side effects [11]. The first antimicrobial compound, chlorine, was isolated from Chlorella sp. It is a mixture of fatty acids that inhibits the growth of gram-positive and gram-negative bacteria [12]. The results of research by Dvoretsky et al. on the alga Chlorella sp. in 2019 showed that the inhibitory properties of this microalgae are caused by fatty acid compounds [13].In addition, eicosapentaenoic acid (EPA), hexadecaterinic acid, and palmitoleic acid isolated from Phaeodactylum tricornutum have antimicrobial activity against methicillin-resistant gram-positive Staphylococcus aureus [14],[15]. Unsaturated fatty acids extracted from Scenedesmus intermedius, Chaetoceros muelleri, Haematococcus pluvialis, Chlorococcum sp., and Skeletonema costatum also have antimicrobial effects against a wide range of gram-positive and gram-negative bacteria. [16]showed that fatty acids with a chain length of more than ten carbon atoms cause the lysis of bacterial protoplasts. Accordingly,[17] stated that fatty acids primarily affect plasma membranes. [18] reported that unsaturated fatty acids inhibit the synthesis of fatty acids in bacteria.It has been proved in several studies that linoleic acid and oleic acid have antimicrobial activity [19]; [20];[21]. Saturated fatty acids showed little or no antimicrobial activity [16]. In addition, organic extracts obtained from Euglena viridis and S. costatum showed inhibitory activity against Pseudomonas and Listeria monocytogenes[22]; [23]. It was also found that ethanolic extracts of Isochrysis galbana and Dunaliella salina are active against four different bacterial strains with inhibitory concentrations (IC50) of 100 and 80 mg/ml, respectively[24]. Extracts of Coccomyxa onubensis fatty acids against E.coli and P. mirabilis showed minimum inhibitory concentrations (MIC) of 305 and 106 mg/ml, respectively [25]. Another study showed that the ethanolic extract of Rivularia mesenterica has a strong inhibitory effect against various antibiotic-resistant bacteria and fungi and the MIC is between 0.06 and 32.00 mg/ml[26]. Researchers have shown that phytosterols isolated from marine microalgae have interesting biologically active properties such as antimicrobial, anti-inflammatory, anti-cancer, antioxidant, and anti-diabetic[27],[28] ,[29],[30].In 2016, Sayegh et al. The only study on antimicrobial activity of nanochloroplecis refers to potassium salt of fatty acids, which is an effective factor in the antimicrobial activity of this algae so that it can act effectively against gram-positive and gram-negative bacteria [9]. In 2014, Surendhiran et al. Fatty acids from Nannochloropsis oculata were found to be inhibitory to both grampositive and gram-negative bacteria [31]. Nevertheless, highly varying susceptibility was recorded even between members of the gram-negative bacteria [1].Nanochloropsis extract exhibited higher antimicrobial potential against gram-positive Staphylococcus aureus bacteria in the current investigation. These types of bacteria are defined by the cell wall structure in the difference of gram-positive bacteria and gram-negative bacteria. The envelope for gram-negative species is their outer membrane. It is a multilayer, consisting of an outer membrane, a peptid glycan cell wall and a cytoplasmic membrane (inner membrane) [32].The outer membrane is also impermeable to some compounds and removes e.g. antibiotics. The role of the outer cell wall explains the greater sensitivity of gram-positive species to gram-negative bacteria and their greater susceptibility to antibiotics.

Conclusion

This indicates that microalgae could be a source of potent antimicrobial agents towards human and plant pathogens, provided that the right solvent and extraction conditions are used. The above bioactive or bactericidal compounds can act individually or in a combination synergistically, although the mechanism of action behind them is yet unclear. So more studies are required to describe the interactions mechanisms of both, microalgae and microalgae so that the antimicrobial compounds be produced commercially or as supplements to diet or be used as biological preservatives.

References

1.Voigt, AM., Ciorba, P., Döhla, M., et. al. (2020) The investigation of antibiotic residues, antibiotic resistance genes and antibiotic-resistant organisms in a drinking water reservoir system in Germany. Int J Hyg Environ Health. Mar;224:113449.

2. Udaondo, Z., Matilla, MA. (2020) Mining for novel antibiotics in the age of antimicrobial resistance. *Microbial biotechnology*. 13(6):1702-1704.

4. Berendonk, T., Manaia, C., Merlin, C. (2015) Tackling antibiotic resistance: the environmental framework. *Nat. Rev. Microbiol*. 13 (5): 310-317.

5.Falkowski PG, Fenchel T, Delong EF. The microbial engines that drive Earth's biogeochemical cycles. Science. 2008 May 23;320(5879):1034-9. doi: 10.1126/science.1153213. PMID: 18497287.

6.Hafsa, MB., Ismail, MB., Garrab, M., Aly, R., Gagnon, J. and Naghmouchi, K. (2017) Antimicrobial, antioxidant, cytotoxic and anticholinesterase activities of water-soluble polysaccharides extracted from microalgae Isochrysis galbana and Nannochloropsis oculata. ***J**. Serb. Chem. Soc*. 82 (5): 509–522.

7.Sanjeewa, K. K. A., Fernando, I. P. S., Samarakoon, K. W., et al. (2016) Anti-Inflammatory and anti-cancer activities of sterol rich fraction of cultured marine microalga Nannochloropsis oculata. *Algae* , 31 (3): 277–287.

8.Jena J., Subudhi E., (2019) Microalgae: an untapped resource for natural antimicrobials. The role of microalgae in wastewater treatment, *Springer*. 99-114.

9. Sayegh, F., Elazzazy, A., Bellou, S., et al. (2016) Production of polyunsaturated single cell oils possessing antimicrobial and anticancer properties. *Ann. Microbiol.* 66 (3): 937–948.

10. Al-Saif, SSAI., Abdel-Raouf, N., El-Wazanani, HA., Aref, IA. (2014) Antibacterial substances from marine algae isolated from Jeddah coast of Red Sea, Saudi Arabia. *Saudi J Biol Sci*. 21:57–64.

11. Ferdous, UT., and Yusof , ZNB., (2021) Medicinal Prospects of Antioxidants From Algal Sources in Cancer Therapy. Front. *Pharmacol*. 12:593116, 1-22.

12. Pratt, R., (1944) Chlorellin, an antibacterial substance from Chlorella. *Science (Washington).* 351-352.

13.Dvoretsky, D., Dvoretsky, S., Temnov, et.al. (2019) Experimental research into the antibiotic properties of *chlorella vulgaris* algal exometabolites Chem. Eng., 74.

14. Benkendorff, K., Davis, AR., Rogers, CN., Bremner, JB. (2005) Free fatty acids and sterols in the benthic spawn of aquatic molluscs, and their associated antimicrobial properties. *J. Exp. Mar. Biol. Ecol*. 316 (1): 29-44.

15. Smith, VJ., Desbois, AP., Dyrynda, EA. Conventional and unconventional antimicrobials from fish, marine invertebrates and micro-algae. *Mar Drugs*. 8(4):1213-1262.

16.Galbraith, H., Miller, TB. Effect of long chain fatty acids on bacterial respiration and amino acid uptake. *J. Appl Bact*. 36:659–675

17.Wu, JT., Yin-Ru, C., Wen-Ya, H., Wann-Neng, J., (2006) Cytotoxic effects of free fatty acids on phytoplankton algae and cyanobacteria. *Aquat Toxicol*. 80:338–345.

18. Zheng, CJ., Yoo, JS., Lee, TG., et. al. (2005) Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Lett.* 579:5157–5162.

19.Dilika, F., Bremner MMeyer, JJ. (2000) Antibacterial activity of linoleic and oleic acids isolated from Helichrysum pedunculatum: a plant used during circumcision rites. *Fitoterapia*. 71 (4) 450-452.

20.Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V., Hsu, M.J. (2007) Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India *Braz. J. Microbial*. 38 (4):739-742.

21. Kasanah, N., Amelia. W., Mukminin. A., Isnansetyo, AT.(2019) Antibacterial activity of Indonesian red algae *Gracilaria edulis* against bacterial fish pathogens and characterization of active fractions. *Nat. Prod. Res*.33 (22): 3303-3307.

22. Das, UN. (2006) Essential fatty acids—a review. *Current Pharm Biotechnol*. 7:467–482.

23. Terekhova, V., Aizdaicher, NA., Buzoleva, LS. (2009) Influence of extrametabolites of marine microalgae on the reproduction of the bacterium Listeria monocytogenes. *Russ. J. Mar. Biol*. 35 (4): 355-358.

24. Verma. E., (2016) Structural elucidation and molecular docking of a novel antibiotic compound from cyanobacterium Nostoc sp. MGL001. *Front. Microbiol*. 7, p. 1899

25. Navarro, F., Forján, E., Vázquez, M., et. al. (2017) Antimicrobial activity of the acidophilic eukaryotic microalga Coccomyxa onubensis *J. Phycol. Res*. 65 (1): 38-43

26. Skočibušić, M., Lacić, S., Rašić, Z., (2019) Evaluation of antimicrobial potential of the marine Cyanobacterium, Rivularia mesenterica. *J. Adv. Microbiol*. 1-11

27.Yasukawa, K., Akihisa, T., Kanno, H., Kaminaga, T., Izumida, M., Sakoh, T., Tamura, T. & Takido, M. (1996) Inhibitory effects of sterols isolated from *Chlorella vulgaris* on 12-O-tetradecanoylphorbol-13-acetate-induced inflammation and tumor promotion in mouse skin. *Biol Pharm Bull.* 19:573–576.

28. Francavilla, M., Colaianna, M., Zotti, et.al. (2012) Extraction, characterization and *in vivo* neuromodulatory activity of phytosterols from microalga Dunaliella tertiolecta. *Curr Med Chem*. 19:3058–3067.

29. Kim, Y-S., Li, X-F., Kang, K-H., Ryu, B. & Kim, SK. (2014) Stigmasterol isolated from marine microalgae *Navicula incerta* induces apoptosis in human hepatoma HepG2 cells. *BMB Rep*. 47:433–438.

30. Luo, X., Su, P. & Zhang, W. (2015) Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications. *Mar Drugs*. 13:4231–4254.

31. Surendhiran, D., Mani, V., Sirajunnisa Abdul, A., Subramaniyan, T. A. (2014) green synthesis of antimicrobial compounds from marine microalgae Nannochloropsis oculata. *J. Coastal Life Med*. 2 (11): 859-863.

32. Exner, M., Bhattacharya, S., Christiansen, B., et. al. (2017) Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? *GMS Hygiene Infection Control*, 12.