Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(6): 1 – 11 (2022)
www.ejabf.journals.ekb.eg

The Wide Spectrum Activity of *Chlorella* and *Spirulina* Extracts on the Viability of Pathogenic and Environmental bacteria in Baghdad, Iraq

Saja H. AL-Abdulameer
Department of Biology, College of Science for Women, University of Baghdad

Corresponding author: saja_hassan@csw.uobaghdad.edu.iq

ARTICLE INFO
Article History:
Received: Sep. 3, 2022
Accepted: Oct. 19, 2022
Online: Nov. 5, 2022

Keywords:
Antimicrobial agents,
***Chlorella vulgaris*,**
***E. coli*,**
Extract,
Pathogens

ABSTRACT

In recent years, many studies have been conducted on the potential use of some algae as an antimicrobial agent, which received a lot of interest. The current study aimed to investigate the effect of *spirulina* and *chlorella* algal extracts on pathogenic bacterial samples collected from patients with burns and urinary tract infections (UTI), as well as environmental bacteria isolated from soil. Four concentrations of *Cholrella* and *Spirulina* algae extract were used (50, 100, 150, 250 mg ml^{-1}), against environmental bacteria such as; *Aeromonas veronii. Pantoea spp, Pasteurella testudinis*, and the pathogenic *Salmonella typhimurium* and *Streptococcus pyogenes*. In this study, *A. veronii* showed to be sensitive towards *Chlorella* and *Spirulina* extracts, where the inhibition zone was (44 mm- 46 mm) respectively. According to the results, the highest inhibition rate was at the concentration of 250 mg ml^{-1} for *Spirulina*, and 150 mg ml^{-1} for *Chlorella* extract. Interestingly, all isolates showed different patterns of susceptibility towards algal extract, as *P. testudinis* was resistant to *Chlorella* concentrations, and *E.coli* was resistant to *Spirulina* extract.

INTRODUCTION

Increasing the usage of industrial drugs, particularly, antibiotics has many unfavorable effects (Frazzini *et al*. 2022). Excessive use of these antibiotics resulted in the development of disease-causing bacteria with high resistance due to the emergence of mutations and plasmid-mediated resistance genes (Benedetti *et al*. 2018; Afrasiabi *et al*., 2022). As a result, using alternative materials to decrease the negative health effects has become popular (Vahdati *et al*. 2021).

Many pharmaceutical firms have started producing various extracts from medical algae with low therapeutic dosages and no toxicity due to the enormous benefits of employing their extracts (Hadi and Brightwell, 2021). Algal species, development stage, light, temperature, and metabolic makeup all differ. If an organism is developed in a

batch culture, the variation in biochemical composition caused by the development stage is typically connected to culture age and nutrient depletion. When an organism is growing photoautotrophical, light serves as its energy supply (Ali *et al*. 2020), mostly sugars, from carbon dioxide into organic molecules in response to light intensity (Barkallah *et al*. 2020; Al Bayati and Amansoori, 2022).

Human skin serves as the body's first line of defence against the outside world (Sauve *et al*. 2015), and as an immune system protector. Managing fluid levels and temperature to prevent the normal colonization of microorganisms and protection against disease causing germs is also provided by the natural and beneficial impact on human skin infections (Gullberg *et al*. 2011). *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* are the bacteria that are most frequently found in wounds and burns among those that cause infections (Lari *et al*. 1998). The most prevalent aquatic creatures are algae, which are a highly diverse groubs in terms of their morphology, ecology, taxonomy, and biochemical characteristics (Alebachew *et al*. 2011). Microalgae and seaweeds are the two primary divisions of algae (Li *et al*. 2016). Seaweeds, also known as macroalgae, are a diverse collection of pluricellular marine organisms that can adapt to the harsh circumstances of marine settings by creating special natural chemicals (Reusch *et al*. 2014). The bioactive components like polysaccharides, proteins, lipids, and polyphenols (Ghosh *et al*. 2022), are particularly well known for their antibacterial, antiviral, and antifungal activity (Anbuchezhian and Karuppiah 2016).

Microalgae are known as a sizable category of photosynthetic unicellular eukaryotes (Reusch *et al*. 2014; Decelle *et al*. 2015). They create a wide range of substances, including polysaccharides, lipids, proteins, carotenoids, pigments, vitamins, sterols, enzymes, antibiotics, medicines, and certain fine chemicals, in addition to biofuels (Hassan *et al*. 2022). Microalgal bioactive compounds have neuroprotective, immunostimulating, antibacterial, antiviral, anticancer, antihypertensive, antioxidant, and other health benefits. These substances are highly sought-after in the fields of medicine, cosmetics, the food industry, aquaculture, energy, agriculture, nutrition, and the manufacture of functional foods (Hadi and Brightwell, 2021; Ali *et al*. 2020; Barkallah *et al*. 2020; Al Bayati *et al*. 2022). One of the main health problems that arises as a result of the use of antibiotics is the development of bacterial resistance, which is one of the biggest challenges facing human health. Antibiotic resistance refers to the ability of bacteria to survive in the presence of antibiotics and the reduced effectiveness of antibiotics against bacteria, due to improper use of medication (Sukhikh *et al*. 2022; Bbosa *et al*. 2014). *Chlorella* is very small algae and one of the oldest plants on earth. It is a source of crucial nutrients, and among the numerous vitamins and minerals (Rani *et al*. 2018), it contains iron, calcium, magnesium, zinc, potassium, and sulfur, in addition to proteins. *Chlorella* is considered a source of necessary amino acids for the human body (Kovac *et al*. 2013).

Spirlina is multicellular, filamentous cyanobacteria. *Spirulina* was categorized as algae within the prokaryote kingdom. *Spirulina* includes the genera Arthrospira and Spirulina. The health benefits of using *A. maxima* and *A. platensis*, is mainly for food (Ali and Saleh, 2012). They were also placed in the Spirulina genus, and there has been a significant debate over their proper taxonomic placement. Although they are extremely different from one another scientifically, they were once referred to as "spirulina." (Darienko *et al*. 2010).

The genus is polyphyletic among the groups of Chlorophyceae and Trebouxiophyceae, according to recent genetic studies. *Chlorella* species is single celled, spherical or ovoid, and have a mucilaginous membrane. It has been reported that the identification of *Chlorella* species is difficult because of their extensive evolutionary diversity. Six lineages closely related to *Chlorella vulgaris* were discovered using a polyphasic analysis method which relies on the combination of the internal transcribed spacer (ITS) in ribosomal RNA, and the small subunit (SSU) (Champenois *et al*. 2015). These lineages are typically described by three new species; *C. pituita* sp. nov., *C. coloniales* sp. nov., *C. singularis* (Luo *et al*. 2006). Other three new species without mucilage were also identified and designated (Johansen *et al.* 2013).

The most abundant source of vitamin B12 and chlorophyll is found in plants, and they also include porphyrin, which stimulates cellular metabolism (Li *et al*. 2016). Most of spirulina's health advantages are related to its high antioxidant content, with carotenoids, chlorophyll, and phycocyanin, which are antioxidant pigments (Hoseini *et al*. 2013). Its application in medicine and some of its undiscovered qualities are currently being investigated. *Spirulina* is distinguished by having high protein content between 60% and 80%. Vitamins and minerals are added to the meals of malnourished children in poor nations as a source of protein. Spirulina protein weighs one kilogramme per gramme (Lari *et al*. 1998). Spirulina protein's amino acids are some of the finest found in any veggie in the plant kingdom. *S. platensis* is used to cure several medical disorders as it contains a lot of protein (Al Bayati and Almansoori, 2022). In this study, the ethanolic extract of *Chlorella* and *Spirullina* was used as an alternative antimicrobial agent towards clinically isolated bacteria and environmental bacteria, and to assess the susceptible patterns of each isolate towards both algal extracts.

MATERIALS AND METHODS

2. Materials and Methods

2.1. Bacterial samples collection

Bacterial isolates were collected from patients with wounds and urine, from the city of medicine teaching laboratory, from March 2021 to August 2021(Supplementary file 1, Ethical approval). Swabs were collected from wounds in the early morning before they were cleaned from patients, swabs were previously sterilized with normal saline

solution (0.85%) and directly transported to the laboratory. Environmental bacteria were isolated from different agricultural soil at the University of Baghdad. The soil samples were sterilized under UV light before bacterial isolation.

2.2. Identification of bacterial isolates

All pathogenic and environmental isolates were cultured on nutrient agar (NA) under sterilized conditions for 24 h. The isolates were then subcultured on selective media and incubated for 24 h at 37˚C. A pure culture of each isolate was obtained by re-subcultured of a single colony, and all isolates were identified by VITEK®2 Compact System, according to the manufacturer's instructions**.**

2.3. Algal extracts Preparation:

Algal samples were obtained from the Department of Biology, College of Science for Women, phycology laboratory. Algal species were routinely cultured and prepared for extraction.

Ethanol extraction (80%) of *Chlorella vulgaris* and *Spirullina* was done according to (Wang *et al.* 2008) with some modifications. Briefly, 1 mg of finely powdered desiccant was dissolved in 10 ml ethanol (80%) in an airtight glass vial. The mixture was heated in a water bath at 80 °C for 4 h. After that, the extract was centrifuged for 15 min at 2000 rpm. The filtrate from the cellular residues was then dried after being evaporated using a rotary evaporator at 50 °C to get the extract in the dry form. The extraction procedure was repeated many times until a good yield was obtained. The resulting pellets were then re-dissolved in methanol (98%). The extract was then centrifuged for 15 minutes at 3000 rpm to remove any remaining material. The filtrate from the cellular residues was then dried after being evaporated using a rotary evaporator at 50 °C to produce a dry extract. The stock solutions of algal extraction were prepared and serial concentrations ranged from 50-250 mg ml^{-1}.

2.4. Antimicrobial assay

Agar well diffusion assay was performed according to (Gonilimali *et al.* 2018). Briefly, an overnight culture of bacterial isolates was inoculated into Mueller Hinton broth (MHB) and incubated aerobically at 37˚C with gentle shaking. The growth was monitored by measuring the OD_{600} until it reaches the mid-exponential phase (OD_{600} of 0.5). A100 µl of each bacterial growth was poured on Mueller Hinton agar (MHA) plates, around 5 mm wells were made using sterile Cork borer, and 200 µl of the algal extract was loaded into the well. All plates were incubated under the above conditions. The antimicrobial activity of algal extracts was determined by measuring the zone of inhibition around each well. The control was assessed using methanol without extract.

2.5. Statistical Analysis

The statistical analysis was done by using GraphPad prism (v.8). Student *t*-test with * $P< 0.05$, ** $P< 0.001$, and *** $P < 0.001$were done.

RESULTS

3.1. Antimicrobial Activity of algal extracts

In order to determine the antimicrobial effect of the algal extract on different environmental and pathogenic bacteria, serial concentrations of *Chlorella* and *Spirulina* extracts were used. The results showed that among all tested species, *Chlorella* extract was only effective against *A. veronii*, *Pantoea* spp. and *S. pneumonia*, as the inhibition zone values were (10, 30, and 31 mm) respectively at a concentration of 50 mg ml^{-1}. The inhibition ability of *Chlorella* extract was increased (44, 35, and 36 mm) respectively, when 250 mg ml^{-1} was used (Table 1). Most notably, *E.coli* was sensitive toward *Chlorella* extract, where the inhibition value was 36 mm at 250 mg ml^{-1}. On the other hand, *S. typhimurium* was slightly inhibited by all concentrations of *Chlorella* extract, whereas, *P. testudinis* was high resistance to all concentrations.

Table 1. Illustrate the inhibition zone (mm) of *Chlorella vulgaris* extract on selected isolates

Bacterial isolates	Conc. Of *Chlorella vulgaris*			
	50 mg ml^{-1}	100 mg ml^{-1}	150mg ml^{-1}	250 mg ml^{-1}
A. veronii	10 mm	25 mm	30 mm	44 mm
***Pantoea* spp.**	30 mm	34 mm	35 mm	35 mm
S. typhimurium	2 mm	4 mm	4 mm	5 mm
P. testudinis	0	0	0	0
S. pneumonia	31 mm	36 mm	35 mm	36 mm
E.coli	0	11mm	22 mm	36 mm

In order to unequivocally determine the killing activity of *Spirulina* extract against selected isolates, and the potential use as an alternative approach to inhibit the growth of pathogenic bacteria, several concentrations of *Spirulina* extract were used (50, 100, 150, 250 mg ml^{-1}). The results revealed that *A. veronii*, *Pantoea spp*., and *S. pneumonia* exert similar patterns of susceptibility toward *Spirulina* extract. The inhibition values of above mentioned isolates were (29 and 30 mm) for *A. veronii* and *S. pneumonia* respectively at 50 mg ml^{-1}, whereas, *Pantoea* spp. was resistance at the same concentration. Interestingly, *Spirullina* extract was more potent against all isolates when a high concentration (250 mg ml^{-1}) was used.

Table 2: The effect of *Spirulina* extract on the growth of selected isolates presented by inhibition zone (mm).

Bacteria isolates	Conc. of *Spirulina* extract			
	50mg ml $^{-1}$	100 mg ml $^{-1}$	150 mg ml $^{-1}$	250 mg ml $^{-1}$
***A. veronii*.**	29 mm	34 mm	41 mm	46 mm
***Pantoea* spp.**	0	0	21 mm	24 mm
S. typhimurium	3 mm	4 mm	9 mm	15 mm
P. testudinis	0	0	16 mm	21 mm
S. pneumonia	30 mm	34 mm	35 mm	36 mm
E.coli	0	0	0	24 mm

3.2. Susceptibility patterns of isolated bacteria

Based on the above findings, all isolated bacteria exhibit different resistance phenotype towards both extracts as shown in Figure (2). *A. veronii* was almost sensitive to all concentrations for both extracts, and there were significant differences between *Chlorella* extract and *Spirulina* extract as the later was more potent (Figure 2 A). Similarly, *S. pneumponia*; a pathogenic isolate showed to be sensitive to both extracts under different concentrations, however, there is no significant difference between extracts (Figure 2 B). Unlike, *Pantoea* spp. and *E. coli* were more resistant to *Spirulina* extract, except for using high concentrations (150, 250 mg ml^{-1}) (Figure 2 C, and D). In addition, the pathogenic *S. typhimurium* showed a moderate resistance toward both extracts, and the significant differences were assessed (Figure 2 E). On the other hand, *P. testidinis* exhibits a high level of resistance toward both extracts, even at high concentrations, and only a high concentration of *Spirulina* extract was able to inhibit the growth of *E. coli* (Figure F).

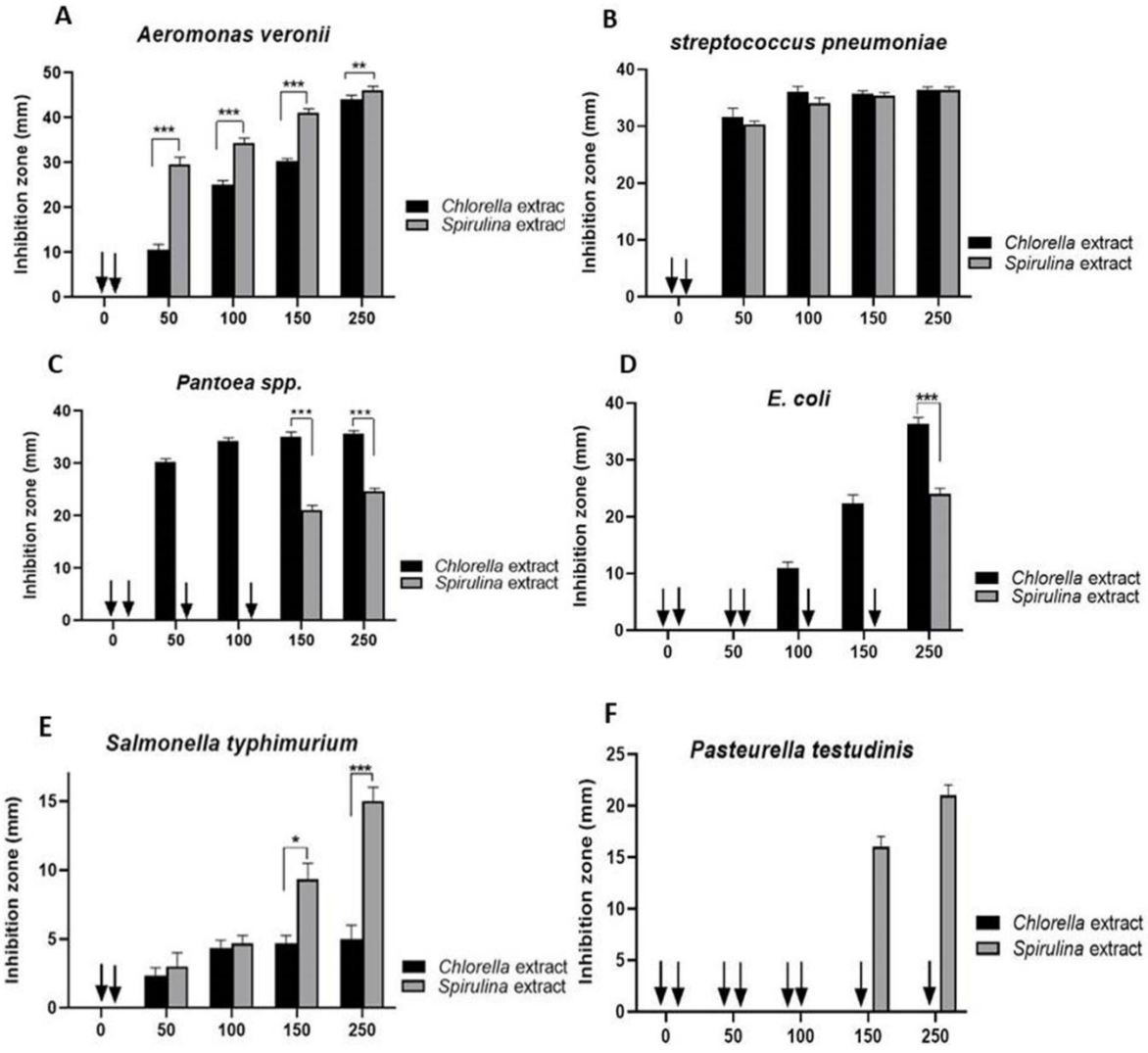

Figure 2: **The antimicrobial activity of *Chlorella* and *Spirulina* extracts against selected isolates.** All isolates bacteria were subjected to well diffusion assays with serial concentrations of both algal extracts. The diameter of the inhibition zone was measured. Each assay was done in three biological replicates. The statistical analysis was done by student *t*-test with * $P< 0.05$, ** $P< 0.001$, and *** $P < 0.001$. the black arrows refer to no inhibition zone . A: *A. veronii*; B: *S. pneumonia*; C: *Pantoea spp*.; D: *E. coli*; E: *S. typhimuurium*; and F: *P. testudinis*.

DISCUSSION

The current study was focused on the potential use of algae as an alternative drug to overcome the persistence and distribution of pathogenic bacteria and environmental bacteria, due to the improper use of antibiotics and the increase in the emergence of multi-drug resistance pathogens. The antimicrobial capacity of *Chlorella* and *Spirullina* extract was examined using the main cause of UTI in Iraqi patients; *E.coli*, and the most virulent *S. typhimurium*. *S. pneumonia* is the most common cause of community-acquired pneumonia (CAP) worldwide. Our findings

indicate that both extracts have antimicrobial activity against most the isolates, particularly at high concentrations. The activity of *Chlorella* and *Spirullina* extract might due to the high phenolic compounds and flavanones present in microalgae, which play an important role as antioxidant and antibacterial agents (El-Chaghaby *et al.* 2019). In addition, many studies reported that many microalgae have bioactive compounds that are used as antimicrobial drugs, including polysaccharides, phycocyanin, neophytadiene, phytol, fatty acid, and diacylglycerol that are produced mainly by Spirulina sp. (de Morais *et al*. 2015). Furthermore, Chlorella vulgaris is the main source of carotenoids, sulfated polysaccharides, sterols, and canthaxanthin, which is used as an antitumor, antibacterial, and anticoagulant activity (Amaro *et al.* 2013, Andrade *et al*. 2017).

The inhibition ability of algal extract toward *A.veronii*, *S. pneumonia*, *Pantoea* spp., and *S. typhimurium*, is attributed to the production of hydrogen peroxide from polyphenols under aerobic conditions and directly associated with hydroxyl groups (Dell'Anno *et al.* 2020). Moreover, *E. coli* was the most resistant isolate toward all concentrations of *Chlorella* extract; whereas, it was slightly affected by the high concentrations of *Spirullina* extract (150, and 250 mg ml^{-1}). The pathogenicity and high resistance ability of *E. coli* toward different pharmaceutical compounds are due to possessing virulence factors and many exotoxins in addition to their biofilm formation ability. It was reported that the polyphenols compound is the active component to target *E. coli* in particular as bacteriostatic and bactericidal effect (Wang *et al.* 2008, Daglia, 2012). On the other hand, Frazzini *et al*. (2022) reported that the extraction method also effect the inhibition efficacy of microalgae, and a high concentration of algal extract gave a high inhibition rate. In this study, using 250 mg ml^{-1} of *Chlorella* extract may not be sufficient to kill *P. testudinis*, thus means that the killing activity is concentration-dependent.

CONCLUSION

The wide spectrum activity of microalgae as an antibacterial agent is diverse worldwide, as they can inhibit the growth of both Gram-negative, and Gram-positive bacteria. Microalgae play an indispensable role in nature as well as in human health, thus leads to suggest that microalgae could be an alternative approach to decrease the emergence of multi-drug resistance pathogens and to restrict the improper use of antibiotics.

REFERENCES

Afrasiabi, S.; Partoazar, N; Chiniforush, N and Goudarzi, R. (2022). The potential Application of Natural Photosensittizers used in Antimicrobial photodybamic Therapy against Oral Infections. Pharmaceuticals, 15(6): 1-11. doi: 10.3390/ph15060767.

Al Bayati, S. S.; Almansoori, D. H. and Guda, M. A.(2022). The study of using Spirulina platensis and Chlorella Vulgaris algae as an antibacterial agent for Escherichia Coli and Proteus Vulgaris. Al-Kufa Uni. Biol. 14 (2): 1-8.

Alebachew, T.; Yismaw, G.; Derabe, A. and Sisay, Z. (2011). Brief Communication Staphylococcus Aureus Burn Wound Infection Among Patients Attending Yekatit 12 Hospital Burn, Ethiop. J. Health. Sci.,22(3): 209–213

Ali, A. H.; Moustafa, E. E.; Abdelkader, S. A.; Hafez, S. S. and Abdallah, S. A. (2020). Antibacterial Potential of Macro and Microalgae Extracts Against Pathogens Relevant To Human Health, Plant Archives.20(2): 9629–9642.

Ali, S. K. and. Saleh, A. M. (2012). Spirulina - An overview, Int. J. Pharm. Pharm. Sci., 4(SUPPL.3): 9–15. doi: 10.1201/9780203025901.

Amaro, H. M.; Barros, R.; Guedes, A. C.; Sousa-Pinto, I. and Malcata, F. X. (2012). Microalgal compounds modulate carcinogenesis in the gastrointestinal tract. Trends in Biotechnology.,31(2):92–98. doi: 10.1016/j.tibtech.2012.11.004.

Anbuchezhian, Z. L. R. and Karuppiah, V. (2016). Prospect of Marine Algae for Production of Industrially Important Chemicals. In: Das, D. (eds) Algal Biorefinery: An Integrated Approach. Springer, Cham. pp.195-217.doi: 10.1007/978-3-319-22813-6_9.

Andrade, L. M.; Andrade, C. J.; Cias, M.; Nascimento, C. A. and Mendes, M. A. (2018). Chlorella and Spirullina micealgae as sources of functional foods, nutraceuticals, and food supplements; an overview. MOJ Food Process Technol., 6(1): 45-58. DOI: 10.15406/mojfpt.2018.06.00144

Barkallah, M.; Slima, A. B.; Elleuch, AF.; Fendri, I.; Pichon, C.; Abdelkafi, S. and Baril, P.(2020). Protective role of spirulina platensis against bifenthrin-induced reprotoxicity in adult male mice by reversing expression of altered histological, biochemical, and molecular markers including microRNAs. Biomol. 10 (5): 753. doi: 10.3390/biom10050753.

Bbosa, G. S.; Mwebaza, N.; Odda, J.; Kyegombe, D. B. and Ntale, M. (2014). Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance, Health (Irvine. Calif., 6(5): 410–425. doi: 10.4236/health.2014.65059.

Benedetti, M.; Vecchi, S.; Barera, V. and Dall'Osto, L. (2018). Biomass from microalgae: The potential of domestication towards sustainable biofactories, Microb. Cell Fact., 17(1): 1–18, doi: 10.1186/s12934-018-1019-3.

Champenois, J.; Marfaing, H. and Pierre, R. (2015). Review of the taxonomic revision of Chlorella and consequences for its food uses in Europe, J. Appl. Phycol.,27(5):1845–1851. doi: 10.1007/s10811-014-0431-2.

Daglia, M. (2012). Polyphenols as Antimicrobial Agents. Curr. Opin. Biotechnol., 23:

174–181.

Darienko, T.; Gustavs, L.; Mudimu, O.; Menendez, C. R.; Schumann, R.; Karsten., U.; Friedl, T. and Proschold, T. (2010). Chloroidium, a common terrestrial coccoid green alga previously assigned to Chlorella (Trebouxiophyceae, Chlorophyta), Eur. J. Phycol.,45(1):79–95. doi: 10.1080/09670260903362820.

Decelle, J.; Romac, S.; Stern, R. F.; Bendif, E.; Zingone, A.; Audic, S.; Guiry, M.; Guillou, L.; Tessier, D.; Le Gall, F.; Gourvil, P.; Dos Santos, A.; Probert, I.; Vaulot, D.; de Vargas, C. and Christe, R. (2015).PhytoREF: A reference database of the plastidial 16S rRNA gene of photosynthetic eukaryotes with curated taxonomy. Mol. Ecol. Resour.,15(6): 1435–1445. doi: 10.1111/1755-0998.12401.

Dell'Anno, M.; Sotira, S.; Rebucci, R.; Reggi, S.; Castiglioni, B. and Rossi, L. (2020). *In vitro* evaluation of antimicrobial and antioxidant activities of algal extracts, Italian J. of Animal Sci., 19(1): 103-113. DOI: 10.1080/1828051X.2019.1703563

de Morais, M. G.; Vaz Bda, S.; de Morais, E. G. and Costa, J. A. (2015). Biologically Active Metabolites Synthesized by Microalgae. Biomed Res Int.2015:835761. doi: 10.1155/2015/835761.

El-Chaghaby, G.; Rashad, S.; Abdel-Kader, S. F.; Rawash, E. A. and Abdul Monee, M. (2019). Assessment of phytochemical components, proximate composition and antioxidant properties of Scendesmus obliquus, Chlorella vulgaris and Spirullina platensis algae extracts. Egyptian J. Aqua. Biolo. Fisheries., 23 (4): 521-526.

Frazzini, S. Scaglia E.; Dell'Anno, M. Reggi, S.; Panseri, S.; Giromini C.; lanzoni, D.; Sgoifi Rossi, C. A. and Rossi, L. (2022). Antioxidant and Antimicrobial Activity of Algal and Cyanobacterial Extracts: An In Vitro Study. Antioxidants, 11(5): 992. doi: 10.3390/antiox11050992.

Ghosh, S.; Sarkar, T., Pati, S.; Kari, Z. A., Edinur, H. A. and Chakraborty, R. (2022). Novel Bioactive Compounds From Marine Sources as a Tool for Functional Food Development, Front. Mar. Sci., 9. doi: 10.3389/fmars.2022.832957.

Gullberg, E.; Cao, S.; Berg, O. G.; IIback, C.; Sandergren, L.; Hughes, D. and Andersson, D. I. (2011). Selection of resistant bacteria at very low antibiotic concentrations, PLoS Pathog.,7 (7):1–9. doi: 10.1371/journal.ppat.1002158.

Hadi, J. and Brightwell, G. (2021). Safety of alternative proteins: Technological, environmental and regulatory aspects of cultured meat, plant-based meat, insect protein and single-cell protein. Foods,10 (6). doi: 10.3390/foods10061226.

Hassan, S.; Meenatchi, R.; Pachillu, K.; Bansal, S.; Brindangnanam, P.; Arockiaraj, H,; Kiran, G. S. and Selvin, J. (2022). Identification and characterization of the novel bioactive compounds from microalgae and cyanobacteria for pharmaceutical and nutraceutical applications. J. Basic Microbiol.,62(9):21–23. doi: 10.1002/jobm.202100477.

Hoseini, M.; Khosravi-Darani,K. and Mozafari, M. R. (2013). Nutritional and Medical Applications of Spirulina Microalgae,Mini-Reviews Med. Chem., 13(8):1231–1237. doi: 10.2174/1389557511313080009.

Johansen, J. R.; Bohunicka, M.; Lukesova, A.; Hrckova, K.; Vaccarino, M. A. and Chesarino, N. M. (2014). Morphological and molecular characterization within 26 strains of the genus Cylindrospermum (Nostocaceae , Cyanobacteria), with descriptions of three new species. J Phycol., 50 (1): 187-202. doi: 10.1111/jpy.12150.

Kovač, D.; Simeunović, J.; Babić, O.; Mišan, A. Č. and Milovanović, I. L. (2013). Algae in food and feed, Food Feed Res.,40(1): 21–31.

Lari, A. R.; Bahrami, H. H. and Alaghehbandan, R. (1998). Pseudomonas infections in Tohid Burn Center, Iran, Burns, 24 (7): 637–641, 1998, doi: 10.1016/S0305-4179(98)00090-4.

Luo, W.; Pflugmacher, S.; Pröschold, T.; Walz, N. and Krienitz, L. (2006). Genotype versus Phenotype Variability in Chlorella and Micractinium (Chlorophyta, Trebouxiophyceae), Protist,157 (3):315–333. doi: 10.1016/j.protis.2006.05.006.

Rani, K.; Sandal, N. and Sahoo, P. K. (2018). A comprehensive review on chlorella-its composition, health benefits, market and regulatory scenario. Pharma Innov. J.,7(7): 584–589.

Reusch, T. B. H. (2014). Climate change in the oceans: Evolutionary versus phenotypically plastic responses of marine animals and plants, Evol. Appl.,7(1):104–122. doi: 10.1111/eva.12109.

Sauvé, S.; Bernard, S. and Sloan, P. (2016). Environmental sciences, sustainable development and circular economy: Alternative concepts for trans-disciplinary research, Environ. Dev.,17: 48–56. doi: 10.1016/j.envdev.2015.09.002.

S. Sukhikh, S.; Prosekov, A.; Ivanova, S.; Maslennikov, P.; Andreeva, A.; Budenkova, E.; Kashirskikh, E.; Tcibulnikova, A.; Zemliakova, E.; Samusev, I. and Babich, O. (2022). Identification of Metabolites with Antibacterial Activities by Analyzing the FTIR Spectra of Microalgae, 12(1395):1–18. https://doi.org/ 10.3390/life12091395

Vahdati, S. N.; Lashkari, A.; Navasatli, S. A.; Ardestani, S. K. and Safavi, M. (2021). Butylated hydroxyl-toluene, 2,4-Di-tetr-butylphenol, and phytol of Chlorella sp. Protect the PC12 cell ine against H_2O_2-induces neurotoxicity. Biomed. Pharmacother., 145 (10): 112415. doi: 10.1016/j.biopha.2021.112415.

Wang,J.; Zhang, Q.; Zhang, Z. and Li, Z.(2008). Antioxidant activity of Sulfated polysaccharide fractions extracted from Laminaria Japonica. Int. J. Biol. Macroml., 42: 127-132.