ISSN: 2458-8989

Natural and Engineering Sciences

NESciences, 2021, 6(2): 87- 95 doi: 10.28978/nesciences.970543

Determination of Antibacterial Effect of *Nannochloropsis oculata* Against Some Rainbow Trout Pathogens

I.Tülay Çağatay[1 *], Mehmet Özbaş[2], H. Emre Yılmaz[1], Noha Ali[3]

[1]Akdeniz University, Faculty of Fisheries, Basic Sciences Department, Antalya, Turkey
[2]Akdeniz University, Faculty of Fisheries, Aquaculture Department, Antalya, Turkey
[3]Alneelain University, Agriculture Technology and Fisheries Faculty, Fish Sciences Department, Sudan

Abstract

In this study, antibacterial activity of *Nannochloropsis oculata* which were grown in proper culture condition was searched with their extracts that obtain by using different solvent (methanol, ethanol, chloroform). The antibacterial effects of algal extracts were evaluated by using disc diffusion method against *L. garvieae*, *F. psychrophilum* and *Y. ruckeri*. As the results of the study, it was found that the chloroform extracts of *N. oculata* showed strong antibacterial activities, *F. psychrophilum* was the high resistant strain against to antibacterial effects of the extracts, *L. garvieae* and *Y. ruckeri* were the two most susceptible bacteria strains. The results confirm the possible use of *N. oculata* as a source of antimicrobial compounds or as a health-promoting food for aquaculture.

Keywords:
Nannochloropsis oculata, antimicrobial effect, fish diseases
Article history:
Received 01 March 2021, Accepted 27 March 2021, Available online 12 July 2021

Introduction

The high commercial value of the valuable metabolites accumulated in cellular by biomass obtained from algae with metabolic adaptation ability and the use of some species in environmental applications increases the current interest in microalgae and makes microalgae a field where

* *Corresponding Author: I. Tülay ÇAĞATAY, E-mail*: *tulaycagatay@akdeniz.edu.tr*

biotechnological research is carried out (Cirik & Gökpınar, 2006; Durmaz, 2006). Microalgae synthesize many products such as protein, pigment, polyunsaturated fatty acids (PUFA), vitamins, hydrocarbons, polysaccharides, fluorotannines, terpenes, polyacetylenes and sterols. These important bioactive molecule sources are used as food in human and animal nutrition, as live feed in aquaculture, as a color material, biogas extraction, lipid source and as fertilizer in agriculture (Jones et al., 1987; Yılmaz, 2006; Mata et al., 2010; Duru & Yılmaz, 2013). There are also studies on antibiotic, antiviral, anticancer, antifungal, antimicrobial and anti-inflammatory effects of this group of organisms (Scheuer, 1990; El-Sheekh et al., 2006; Falaise et al., 2016). The antimicrobial properties of microalgae and their widespread availability around the world have also encouraged their research as antimicrobial agents in pharmacological research.

Nannochloropsis species, which are eukaryotic unicellular phytolankton, are also know to be importnat marine resources due to their high protein and PUFA content and non-toxic substances as in cyanobacteria.

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important species of aquaculture in our country. It is important to prevent and treat fish diseases in our country, which ranks first in Europe. Developing fisheries at the desired level and contributing to the country's economy depends on raising fish under suitable conditions and protecting them from diseases. *Flavobacterium psychrophila, Yersinia ruckeri* and *Lactococcus garvieae* are the most common bacterial agents for fish diseases with economic losses and high mortality in rainbow trout farming (Kubilay &Timur, 2001; Altun et al., 2010; Balta et al., 2010; Akaylı et al., 2020).

Some costly antibiotics, synthetic or semisynthetic drugs and vaccines are used extensively by manufacturers for prevention and treatment of these diseases. However, the use of antibiotics brings with it many risks for the environment, fish and human health, and it is reported that it can lead to the development of resistant bacterial strains in fish and humans, and toxic deposits in fish (Romero et al., 2012; Miranda et al., 2018). For this reason, it has been aimed to seek alternative sources against antibiotic applications. In this case, the use of new resources and raw materials that are environmentally friendly, harmless and low economic cost is not only economically important, but also important for the sustainability of the sector and water resources.

In this study, the antimicrobial effects of *Nannochloropsis oculata* against some bacterial fish disease agents (*Y. ruckeri, F. psychrophila* and *L. garvieae*), which are frequently problematic in rainbow trout farmers, were investigated.

Materials and Method

Nannochloropsis oculata and *Y. ruckeri* ATCC 29473, *F. psychrophilum* NCIMB 1947 and *L. garvieae* ATCC 43921 were obtained from Akdeniz University, Faculty of Fisheries Microbiology Laboratory stock cultures. *N. oculata* were grown in Walne medium with artificial sea water (Walne, 1970) in 1 liter glass flasks, ventilated at 25-30 ^{o}C, in daylight, for 15-20 days. *F. psychrophilum* were incubated on cytophaga agar at 16-18 °C for 6 day, *L. garvieae* were grown brain heart infusion agar (BHIA) and trypticase soy agar (TSA) at 24 ^{o}C for 48 hours and *Y. ruckeri* were grown trypticase soy agar at 20-25 ^{o}C for 24 hours (Austin & Austin, 2012).

Methanol, ethanol and chloroform (Merck, Germany) were used as solvents to prepare the extractions of *N. oculata* (Bhuvaneswari et al., 2013). For the antimicrobial activity experiments,

100 µl of solvent was added for 100 µl of *N. oculata* sample taken from algae medium produced until it reached sufficient cell number ($1.3x10^8$ $/ml^{-1}$cells) and after 4 hours at room temperature, it was disintegrated with a homogenizer. It was centrifuged at 13.000 rpm for 3 minutes and the supernatant were evaporated in vacuum and kept at 4 ^oC until used. Disk diffusion method was used to determine the effect of these extracts on the causative bacteria in fish (Bradshaw, 1992). 6 mm diameter (Oxoid) sterile paper discs were absorbed with 40 µl of extracts and dried at 37 °C for 1 night. After the bacteria was cultivated, discs immersed with algae extracts were placed on the petri dishes simultaneously. As positive control, amoxicillin for *L. garvieae*, ormetoprim-sulfadimethoxine for *F. psychrophilum* and oxytetracycline discs for *Y. ruckeri* were used. At the end of 48 hours of appropriate incubation periods, inhibition zones were measured around the disc. Every experiment was carried out 3 times. Inhibition zones >15 mm were declared as strong, from 8 to 15 mm as moderate and from 1 to 8 mm as weak activities (Bansemir et al., 2006).

Results

In this study, bacteria were grown separately to determine the antibacterial effect of *N. oculata* on *Y. ruckeri*, *F. psychrophilum* and *L. garvieae*, which are the causative agents of rainbow trout disease. *N. oculata* was grown in Walne medium for 25 days. Methanol, ethanol and chloroform were used as solvents to determine the antimicrobial effects of the microalgae used. Disk diffusion method was used to determine the effects of methanol, ethanol and chloroform extracts of *N. oculata* (Fig. 1) against some bacterial fish pathogens, and the results showing the inhibition zones created by the extracts are given in Fig. 2-4. Inhibition zones were recorded millimetrically by measuring the entire edge of the inhibition zone with the disk.

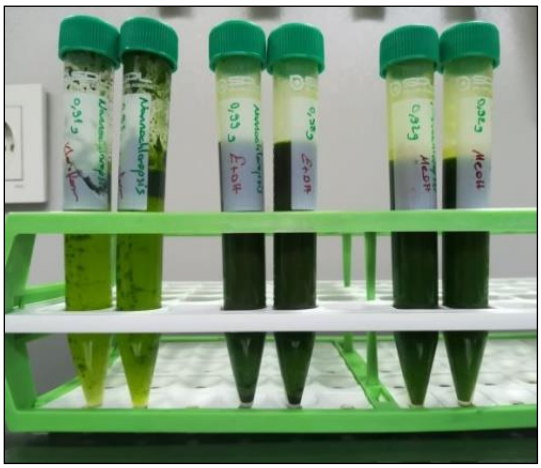

Figure 1. Chloroform, ethanol and methanol extracts of *N. oculata*

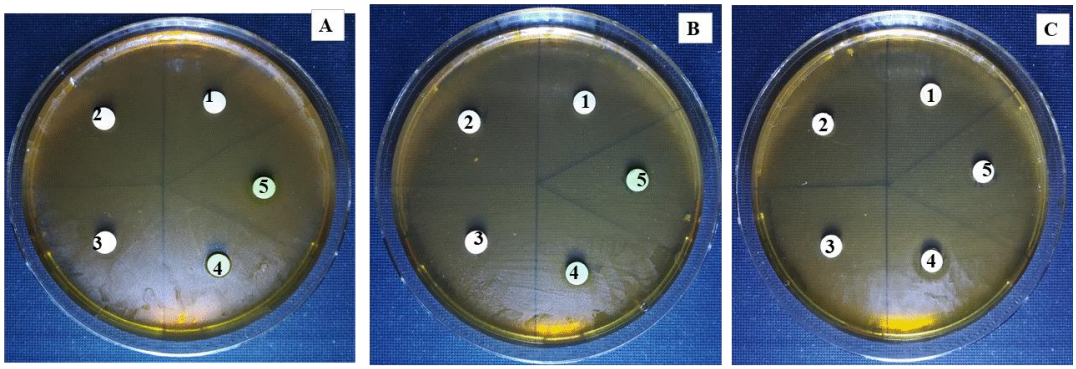

Figure 2. Image of antimicrobial activity zones of ethanol (A), methanol (B) and chloroform (C) extracts of *N. oculata* were applied on *L. garvieae*. 1.3 Negative control (solvent), 2. Positive control (amoxicillin), 4.5. *N. oculata* extracts.

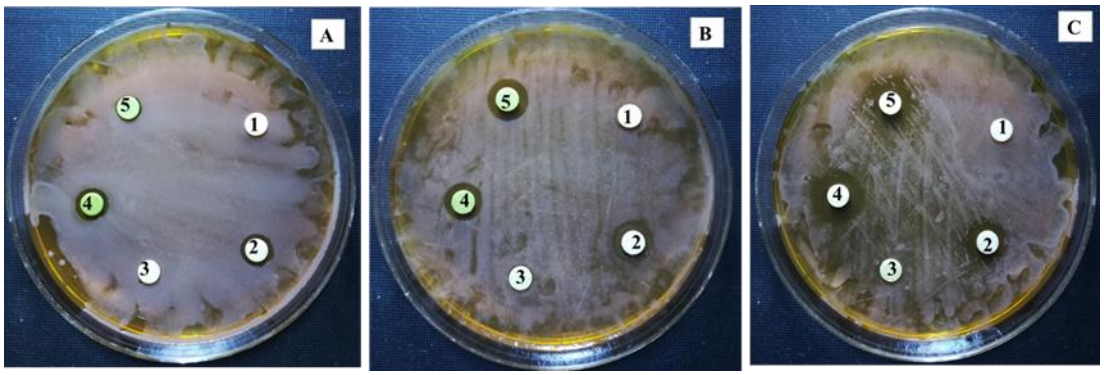

Figure 3. Image of antimicrobial activity zones of ethanol (A), methanol (B) and chloroform (C) extracts of *N. oculata* were applied on *F. psychrophilum*. 1.3 Negative control (solvent), 2. Positive control (ormetoprim-sulfadimethoxine), 4.5. *N. oculata* extracts.

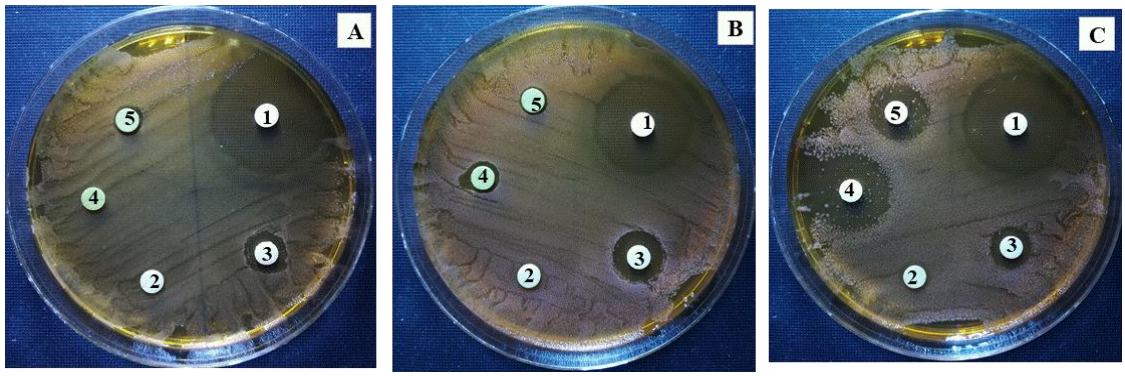

Figure 4. Image of antimicrobial activity zones of ethanol (A), methanol (B) and chloroform (C) extracts of *N. oculata* were applied on *Y. ruckeri*. 1. Positive control (oxytetracycline), 2. Negative control (solvent), 3.4.5. *N. oculata* extracts.

It was seen that the zone diameters formed by *N. oculata* discs extracted with chloroform in all tested bacteria (*L. garvieae*, *F. psychrophilum* and *Y. ruckeri*) were between 11.50 and 19.40 mm and have a higher resistancy than other extracts. The lowest disc diameter was 6,09 mm in the ethanol extract disc applied on *Y. ruckeri*, while the highest disc diameter was 19,40 mm in the chloroform extract disc applied on *F. psychrophilum*. In addition, it is seen that the disc

sensitivities of the extracts of *N. oculata* prepared with methanol and ethanol were close to each other (Tablo 1.).

Table 1. Zone diameters showing the antimicrobial effects of *N.oculata* ethanol (E Ex.), methanol (M Ex.), and chloroform (K Ex.) extracts and positive (PK) and negative (NK) controls on *L. garvieae*, *F. psychrophilum* and *Y. ruckeri.*

Microorganisms	Controls					*Nannochloropsis oculata*		
	M	E	K	NK	PK	E Ex.	M Ex.	K Ex.
L. garvieae	0,00	0,00	0,00	0,00	13,00	10,20	11,90	12,80
	0,00	0,00	0,00	0,00	13,33	10,30	12,80	13,70
	0,00	0,00	0,00	0,00	13,65	10,40	11,95	12,11
Average	0,00	0,00	0,00	0,00	13,33	**10,30**	**12,22**	**12,87**
F. psychrophilum	0,00	0,00	0,00	0,00	13,00	8,80	14,30	19,40
	0,00	0,00	0,00	0,00	12,00	9,00	13,00	17,50
	0,00	0,00	0,00	0,00	11,00	11,40	13,00	19.00
Average	0,00	0,00	0,00	0,00	12,00	**9,73**	**13,43**	**18,45**
Y. ruckeri	0,00	0,00	0,00	0,00	28,70	10,70	13,10	16,40
	0,00	0,00	0,00	0,00	24,80	8,00	11,20	18,50
	0,00	0,00	0,00	0,00	26,60	6,09	8,15	11,50
Average	0,00	0,00	0,00	0,00	26,70	**8,26**	**10,82**	**15,47**

Discussion

Interest in microalgae technology has increased due to the valuable metabolites and fat content that microalga species accumulate in the cell. Due to their rich nutrient content, protein, polyunsaturated fatty acids and carotenoids, they are used in feeding zooplankton and fish larvae. In addition to being a food source in aquaculture, it has been shown that they can be used in different areas such as biodiesel production due to its high oil content, removing industrial wastes such as heavy metals, animal feed, fertilizer, natural food coloring, food additives and cosmetics industry (Aksu & Dönmez 2003). It has been reported that phenolic compounds obtained from microalgae have inhibitory and antimicrobial effects on bacterial growth, depending on their structural properties and their amount in the environment (Abd El-Baky et al., 2009; Heuer et al., 2009; Vijayabaskar & Vaseela 2012).

In this study, the antibacterial effects of *N. oculata* extracts prepared with three different solvents (methanol, ethanol, chloroform) against *L. garvieae*, *F. psychrophilum*, *Y. ruckeri*, which are the causative agents of bacterial fish disease, were determined. Chloroform extracts of the eukaryotic microalgae *N. oculata* produced the highest antimicrobial activity due to the strong inhibition zone against *Y. ruckeri*, methanol extracts showed the moderate activity and ethanol extracts showed weak antibacterial activity in all test bacteria. In the literature, there are studies showing that chloroform has better antimicrobial activity than methanol and benzene extracts, consistent with our findings (Sastry & Rao 1994). In a study conducted by Tüney & et al., (2006) with algae, no significant difference was found between the effects of acetone, methanol and ethanol extracts of algae species used. Özdemir & et al., (2004) stated in their study that methanol extracts of *S. platensis* showed more antimicrobial activity against *Streptococcus faecalis*,

Staphylococcus epidermidis and *C. albicans* compared to dichloromethane, ethyl acetate and petroleum ether extracts.

In recent years, there has been an increase in studies on the use of new antimicrobial sources to treat of human pathogens for other animal pathogens. *Nostoc muscorum, Oscillatoria angustissima, Anabaena variabilis, Phormidium* sp. and *Synechocystis* sp. have been shown to produce antimicrobial products and antibiotics (Bloor & England, 1989; Issa, 1999; Katırcıoğlu et al., 2006). In Özdemir & others's (2001) study showed that *S. platensis* had antimicrobial activities against human pathogens such as *Pseudomonas* sp., *Proteus* sp., *Salmonella* sp., *Streptococcus* sp., *Klebsiella* sp. Especially studies conducted with *N. oceania* and *Isochrysis galbana* species show that the water-soluble polysaccharides produced by these organisms can be used as antimicrobial agents (Hafsa et al., 2017). Taniguchi et al. (2011) and Sharifah & Eguchi (2011) showed for the first time that *Nannochloropsis* sp. has an antimicrobial effect to Vibrio species that cause fish diseases Vibriosis.

Nowadays, the increase of resistant pathogens against conventional chemotherapeutic agents makes the use of these compounds useless. In this case, algae are extremely important in terms of being less toxic and more effective with drug-like physiological activity as they contain extremely useful antimicrobial agents, as well as being environmentally friendly therapeutic models for drugs. New and sustainable disease treatment methods should be developed due to the development of resistance to vaccines and antibiotics among conventional treatment methods and their high cost.

As a result, it has been shown in this study that *N. oculata* is a source with high antimicrobial activity against some pathogens. These findings suggest that this alga can be used as an agent as an agent to lead studies on the prevention and treatment of fish diseases. The production performance of fish farms will increase and the cost will also decrease, when *N. oculata* is used as an alternative chemotherapeutic resource that is environmentally friendly and has a low carbon footprint and low cost as an alternative to antibiotics.

Acknowledgment

This study was supported by the Scientific Research Coordination Unit of Akdeniz University with the project number FBA-2018-3558.

Author Contributions

All author contributions are equal for the preparation research in the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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