Advances in Bioresearch
Adv. Biores., Vol 13 (2) March 2022: 53-58

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.13.2.5358

Advances in Bioresearch

ORIGINAL ARTICLE

In Vitro assessment of antibacterial, antifungal and hemolytic activity of bioactive compounds from microalgae *Nannochloropsis oceanica*

K. Aparnadevi, S. Bragadeeswaran* and R. Ranjithkumar
*Associate Professor, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai. Tamilnadu.
Mail id: drpragathi@gmail.com

ABSTRACT
Marine microalgae are recognized to be novel and rich in bioactive compounds. In the present study, Nannochloropsis oceanica extract (after methanol extraction) was subjected to pharmacological studies like In vitro antibacterial, antifungal and hemolytic assay. The activity against G+ve bacteria was less effective compared to G-ve bacteria. The activity was found to be highly significant against bacteria and moderately significant against fungal strains. The highest hemolytic activity was observed in chicken blood compared to goat and human blood.
Keywords: *Bioactive compounds, antimicrobial activities, N. oceanica, hemolytic unit.*

Received 02.03.2022 Revised 23.03.2022 Accepted 24.03.2022

How to cite this article:
K. Aparnadevi, S. Bragadeeswaran and R. Ranjithkumar. *In Vitro* assessment of antibacterial, antifungal and hemolytic activity of bioactive compounds from microalgae *Nannochloropsis oceanica*. Adv. Biores. Vol 13 [2] March 2022: 53-58.

INTRODUCTION

Microalgae are photoautotrophic organisms that need light as their main source of energy. The growth and proximate composition of microalgae have been widely explored on the basis of the effects of light intensity, temperature, salinity and media nutrients [31, 4]. Microalgae play a key role in aquaculture development as they are the primary food source for a large number of aquatic organisms are widely used to maintain water quality in marine hatcheries as well as food sources [26, 12]. Among the several strains available, *Chaetoceros* spp. and *Nannochloropsis* spp. are widely used in marine hatcheries as food sources as well as to maintain water quality [26, 12]. *Nannochloropsis* sp. is a small green alga that is widely used in the aquaculture industry for growing rotifers (zooplankton) and also been recognized for human diet due to its high nutritional value as an excellent source of proteins, carbohydrates, lipids and vitamins. This microalga is well known as a source of different valuable pigments, such as chlorophyll a, zeaxanthin, canthaxanthin and astaxanthin [24] produced at high levels.

Secondary metabolites from microalgae are associated with toxic, hormonal, antineoplastic and antimicrobial effects [22, 5]. The antimicrobial substances involved may target various kinds of microorganisms, prokaryotes as well as eukaryotes. *Nannochloropsis* is classified under the class Eustigmatophyceae of the Heterokontophyta [1]. The present study was undertaken to examine the antibacterial, antifungal and hemolytic effect of methanolic extracts of *N. oceanica* against human pathogens.

MATERIAL AND METHODS

Microalgal culture: The marine microalgae, *Nannochloropsis oceanica* was isolated, identified and cultured in 'Biotoxinology Laboratory', CAS in Marine Biology, Annamalai University, using Walne medium [24]. The culture was grown at 30 °C, pH between 8.5 to 11 and salinity 25- 32psu with approximate 12:12 light/ light cycle of light intensity. The mass scale production of microalgal species

was cultured by using low cost medium in a glass tank. An amount of 100g of microalgae (*Nannochloropsis oceanica*) was thoroughly washed with demineralized water. The algae were then transferred to an Erlenmeyer flask and extracted with absolute methanol using an ultrasonic homogenizer (BioLogics, Inc., Manassas, VA, USA) for 1 hr at RT, 15 kHz. The mixture was filtered and using a rotary evaporator (Buchi Rotavapor® R-210, Flawil, Switzerland) the solvent was removed. By making use of the freeze-drying technique the mass was lyophilized. The *Nannochloropsis oceanica* extract (AME) was then placed in a desiccators to store it for further evaluation.

Bioassays

Antimicrobial assay: In the present study, the antimicrobial activity of microalgae was compared with antibiotics. Amoxycillin served as an antibacterial standard and Flukonazol (12.5 µg/ml, Pfizer, Turkey) served as an antifungal standard.

The antibacterial activity of the prepared *Nannochloropsis oceanica* microalgae was tested against a range of human pathogens which included both gram-positive and negative bacteria and fungi. All the strains were obtained from the Department of Microbiology, Raja Muthiah medical college and hospital, Annamalai University, Annamalai Nagar, India and were maintained on a suitable agar medium at 4°C until testing.

The bacterial pathogens were grown at 37°C in Mueller-Hinton (MH) broth (Himedia Laboratories, Pvt. Ltd, Mumbai, India) and fungal pathogens were grown at 28°C in Sabouraud Dextrose Broth (Himedia Laboratories, Pvt Ltd, Mumbai, India).

The following test bacteria and fungi were used: *Vibrio* sp., (Gram - ve) *Escherichia coli*, (Gram - ve), *Proteus* sp., (Gram - ve), *Klebsiella* sp., (Gram - ve) and MRSA Methicillin-resistant *Staphylococcus aureus*, (Gram +ve) and *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Mucor* sp., and *Rhizopus* sp. After nutrient agar and YEPD (yeast extract peptone dextrose), agar was inoculated with 100 µl of an overnight culture of the test organisms, wells (6 mm)were made. Then extracts (25µl, 50µl and 100 µl) were applied directly into the wells and incubated at appropriate temperatures for 24 hours. The test organisms were prepared by subculturing them overnight in an incubator. The isolated microbes were inoculated using the swab method. The well was cut by using gel puncture. 1ml of the prepared extract was introduced in the hole.

After incubation, the diameter of the inhibition zone was measured with callipers. The measurements were done basically from the edge of the zone to the edge of the well [2].

Antihaemolytic activity of the extract of *Nannochloropsis oceanica*

The crude extracts of *Nannochloropsis oceanica* were assayed for their hemolytic activities on chicken goat bloodand Human blood. Chicken and goat blood were obtained from a nearby slaughterhouse and human blood was collected froma clinical laboratory, using 2.7% ethylene diamine tetra acetic acid (EDTA) solution as an anticoagulant for 5% of the blood volume and brought to the laboratory. The blood was centrifuged thrice at 3000 rpm for 10 minutes with normal saline. This process was repeated thrice and finally concentrated RBC was obtained. 1% erythrocyte suspension was prepared by adding 99 ml normal saline to 1 ml of packed erythrocytes. The micro hemolytic assay was performed in 96-well 'V' bottom microtitre plates. Transferred 100 µl normal saline (pH 7) into a well and 100µl of the microalgal extract was added to the well and mixed thoroughly. Transferred 100µl to the next well and repeated the process till the last well and discarded the 100µl taken from the last well. Then 100 µl of 1%RBC was added to all the wells and gently shaken and allowed to stand for two hours at room temperature. The presence of homogenous red colour suspension in the wells was considered to be a positive reaction due to hemolysis of RBC and a negative reaction was indicated by a compact demarked disc of sediment cell in the bottom of the wells that showed a lack of hemolysis. The reciprocal of the highest dilution at which the hemolysis has taken place is one hemolytic unit (the amount of toxin-producing 50% hemolysis is defined as one hemolytic unit). 100 µl of 1% RBC and 100 µl of distilled water were considered as positive control and 100µl of 1% RBC with 100µl of normal saline served as negative control.

RESULTS AND DISCUSSION

Antibacterial activity

Results are presented as the mean± standard deviation (SD) of three replicates. Agar well diffusion method was carried out to test the antibacterial activities and antifungal activities of methanol extracted *Nannochloropsis oceanica* and the data are tabulated in Table 1 and 2.In the present study *N. oceanica* showed zone of inhibition against all five bacterial pathogens. The highest activity was shown by *Escherchia coli* (4.06±0.06), followed by *Proteus* sp. (3.37±0.06), *Klebsiella* sp. (2.46±0.05), *Vibrio* sp.(2.3±0.05), and least activity were shown by MRSA (1.86±0.1). Fungal strain *Aspergillus flavus* showed highest activity (2.57±0.05) at 100 µl concentration followed by *Rhizopus* sp. (1.8±0.05), *A. niger* (1.57±0.06), *A. fumigatus* (1.43±0.06) and *Mucor* sp. (1.37±0.1).

Marine microalgae are a potential source of new antimicrobial compounds which can be attributed to their genetic diversity. They represent an untapped resource of novel natural products. Like all organisms, they also need to protect themselves against opportunistic attack or pathogenic damage [20]. Till date, many compounds of marine origin are chemically unique with various biological activities have been isolated and are under investigation and are being used to develop new pharmaceuticals [32].

Cell extracts and active constituents of various algae have shown to possess antimicrobial activity in vitro against microbial pathogens. A wide range of results of in vitro antifungal activities of extracts of green algae, diatoms and dinoflagellates have also been reported. [3]. The activity against G+ve bacteria was less effective compared to G-ve bacteria [13] which coincided with our results. Methanol have higher antibacterial activity compared to the extracts obtained with other organic solvents [8, 29, 27, 21, 15,6, 7, 28] reported that the methanol extract of seaweeds contains phenolics, alkaloids and amino acids which may responsible for the antimicrobial activity. *N. oceanica* were extracted using methanol as solvent which showed significant results which were confirmed by Manilal *et al.,*[18] and Rangaiah *et al.,* [25] who explained that the methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate. Methanol extract of *Sargassum polycystum* showed more activity against *E.coli, P.vulgaris*, *E.caratovora*, *K. pneumonia* and fungal strain of methanol extract of *A.niger* and *R. stolonifer.* Chloroform extract showed moderate activity with *Sargassum tenerrimum.*

Table 1. Antibacterial activity of *Nannochloropsis oceanica* extracts.

Bacterial Pathogens	25 µl	50 µl	100 µl	50 µl (Positive control)
Escherichia coli	1.8± 0.1	2.56±0.05	4.06±0.06	3.03±0.05
Klebsiella sp.	1.67±0.05	2.17±0.06	2.46±0.05	2.37±0.05
MRSA	1.13±0.06	1.47±0.05	1.86±0.1	1.43±0.06
*Proteus*sp.	2.47±0.05	3.03±0.1	3.37±0.06	2.87±0.1
Vibrio sp.	1.4±0.06	1.9±0.05	2.3±0.05	1.8±0.06

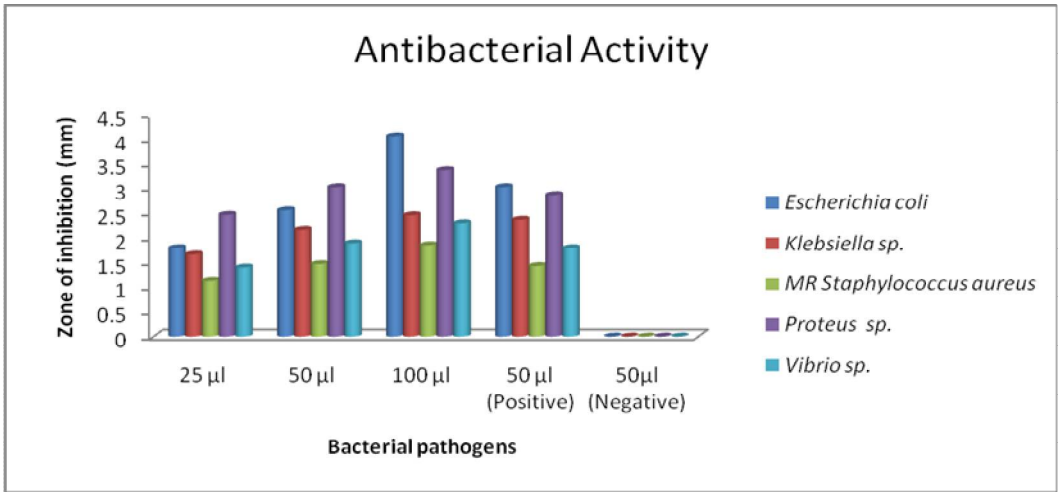

Fig. 1. Antibacterial activity of *Nannochloropsis oceanica* extracts (Positive control – Amoxycillin; Negative control – Distilled water)

Table 2. Antifungal activity of *Nannochloropsis oceanica* extracts

Zone of Inhibition in mm (Mean± SD)				
Fungal Pathogens	25 µl	50 µl	100 µl	50 µl (Positive control)
Aspergillus niger	-	-	1.57±0.06	1.27±0.1
Aspergillus flavus	1.43±0.06	1.97±0.05	2.57±0.05	2.43±0.06
Aspergillus fumigatus	0.87±0.1	1.13±0.1	1.43±0.06	1.53±0.05
Mucor sp.	0.97±0.05	1.27±0.06	1.37±0.1	1.17±0.06
Rhizopus sp.	0.8±0.1	1.4±0.1	1.8±0.05	1.07±0.1

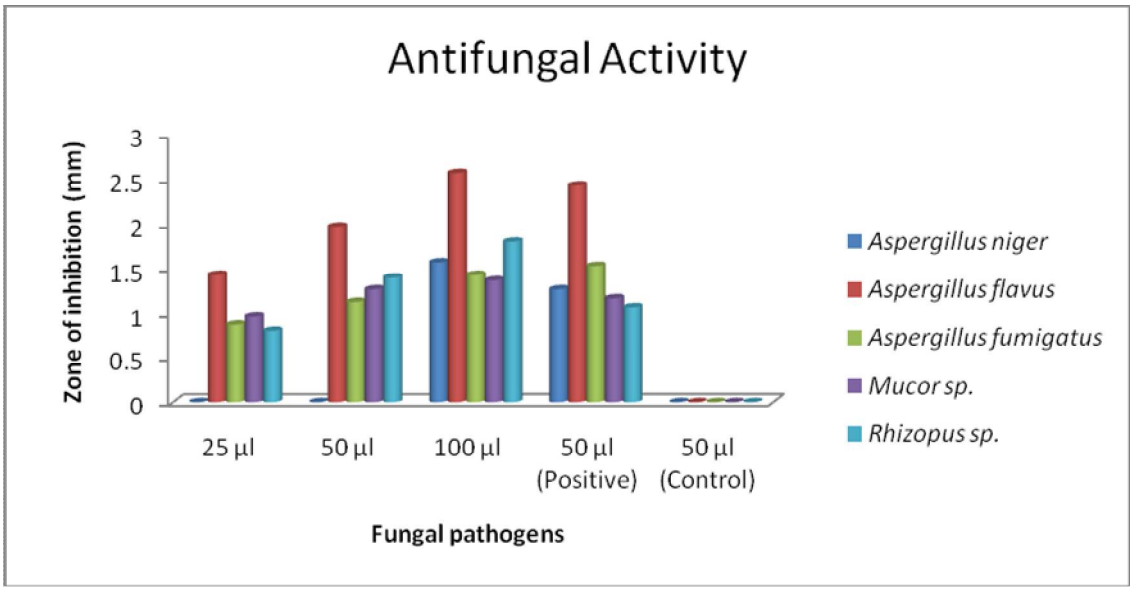

Fig. 2. Antifungal activity of *Nannochloropsis oceanica* extracts (Positive control-fluconazole; Negative control- distilled water)

Hemolytic activity

The micro algae, ***Nannochloropsis oceanica*** after methanol extraction produced notable hemolytic activity on chicken, goat and human (O + ve) erythrocytes. The highest hemolytic activity was observed in chicken blood (32 HU).

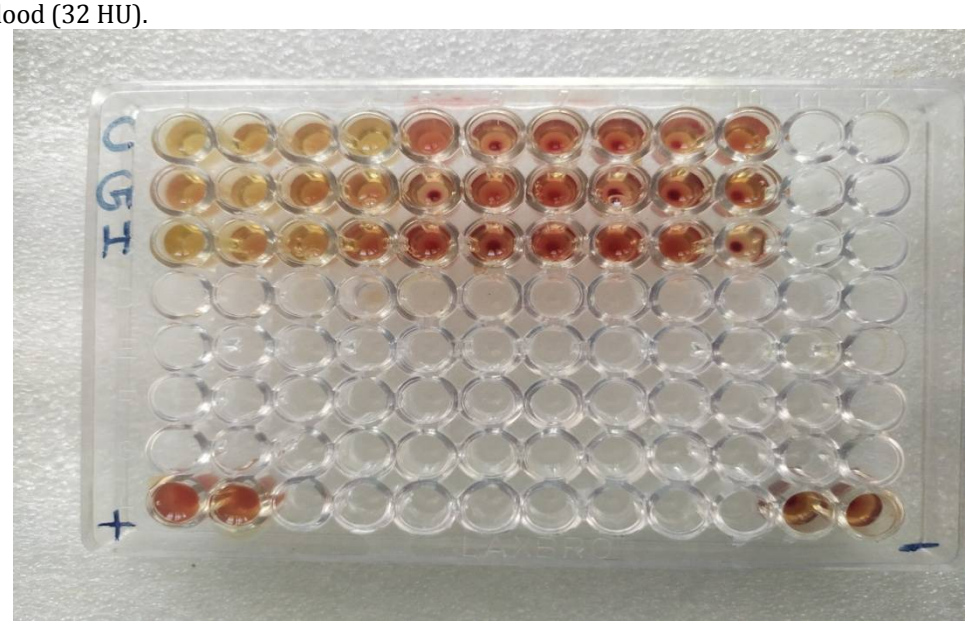

Fig. 3. Hemolytic assay of methanol extracted *Nannochloropsis oceanica* on chicken, goat and Human (O +ve) erythrocytes.

Table 3. Hemolytic activity of methanol extracted *Nannochloropsis oceanica* on chicken, goat and Human (O +ve) erythrocytes.

Sample	Haemolytic unit (HU)		
	Chicken blood	Goat blood	Human blood (O^{+ve})
Nannochloropsis oceanica	32	16	16

CONCLUSION

Finally, we conclude that microalgae *Nannocloropsis oceanica* from Vellar estuary, southeast coast of India are potential sources of bioactive compounds and should be investigated for natural antibiotics.

ACKNOWLEDGEMENT

The authors are grateful to the Director, CAS in Marine Biology and the authorities of Annamalai University (India) for the facilities provided.

REFERENCES

1. Andersen RA, Brett RW, Potter D, Sexton JP., (1998). Phylogeny of the Eustigmatophyceae based upon 18S rDNA, with emphasis on Nannochloropsis. Protist. 149:61–74.
2. Attaie, R. Whalen, J. Shahani K.M. and Amer M.A., (1987). Inhibition of growth of *S. aureus* during production of acidophilus yogurt. Journal of Food Protection 50, 224-228.
3. Borowitzka, M. A., (1995). Microalgae as sources of pharmaceuticals and other biological active compounds. J. Appl. Phycol., 7, 3-15.
4. Brown MR, Jeffery SW, Volkman JK, Dunstan GA (1997). Nutritionalproperties of microalgae for mariculture. Aquaculture, 151: 315-331.
5. Carmichael W.W., (1992). Cyanobacteria secondary metabolites-the cyanotoxins. Journal of Applied Bacteriology 72,445-459.
6. Cox, S., Abu-Ghannam, N. and Gupta, S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. International Food Research Journal, 17: 205-220.
7. Devi, K.P., Suganthy, N., Kesika, P. and Pandian, S.K. (2008). Bioprotective properties of seaweeds: in vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. BMC Complementary and Alternative Medicine, 8: 38-48.
8. Febles, C.I., Arias, A., Gil-Rodriguez, M.C., Hardisson, A. and Sierra Lopez, K.J. Elnabris, A.A. Elmanama and W.N. Chihadeh A. (1995). In vitro study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). Anuario del Estudios Canarios, 34: 181-192.
9. Hibberd, D.J., (1980). Eustigmatophytes. In cox. E.R. (Ed.) Phytoflagellates. Developments in Marine Biology. Vol 2. Elsevier/ North Holland New York, pp 319-334.
10. Hibberd, D.J., (1981). Notes on the taxonomy and Nomenclature of the classes Eustigmatophyceae and Tribophyceae (Synonym Xanthophyceae) Bot. J. Limn. Soc. 82; pp 93-119.
11. Karlson, B., Potter, D., Kuylenstierna, M., & Andersen, R. A. (1996). Ultrastructure, pigment composition, and 18S rRNA gene sequence for *Nannochloropsis granulata* sp. nov. (Monodopsidaceae, Eustigmatophyceae), a marine ultraplankter isolated from the Skagerrak, northeast Atlantic Ocean. *Phycologia*, *35*(3), 253-260.
12. Khatoon H, Yusoff FM, Banerjee S, Shariff M, Mohamed S (2007). Use of periphytic cyanobacterium and mixed diatoms coated substrate forimproving water quality, survival and growth of *Penaeus monodon* Fabricius postlarvae. Aquaculture, 271: 196-205.
13. Kolanjinathan K., Stella, D., 2009. Antibacterial Activity of Marine macro algae against HumanPathogens.Recent Research in Science and Technology, 1(1): 020–022.
14. Krienitz, L., Hepperle, D., Stich, H. B., & Weiler, W. (2000). *Nannochloropsis limnetica* (Eustigmatophyceae), a new species of picoplankton from freshwater. *Phycologia*, *39*(3), 219-227.
15. Kumar, G.S., Sarada, D.V.L. and Rengasamy, R. (2008). Seaweed extracts control the leaf spot disease of the medicinal plant Gymnemasylvestre Indian Journal of Science and Technology, 1(3): 1-5.
16. Lavanya, R. and Veerappan, N. (2011). Antibacterial Potential of six seaweeds collected from Gulf of Mannar of Southeast Coast of India. Advances in Biological Research, 5(1): 38-44.
17. Lubián, L. M. (1982). *Nannochloropsis gaditana* sp. nov., una nueva Eustigmatophyceae marina. *Lazaroa*, *4*, 287-293..
18. Manilal A, Sujith S, Selvin J, Shakir C, Kiran GS. (2009). Antibacterial activity of Falkenbergiahillebrandii (Born) from the India coast against human pathogens. FYTON 78:161-166.
19. Marvin W. Fawley Ian Jameson and Karen P. Fawley (2015). The phylogeny of the genus Nannochloropsis (Monodopsidaceae, Eustigmatophyceae), with descriptions of N. australis sp. nov. and Microchloropsis gen. nov. Phycologia Volume 54 (5), 545–552.
20. Mayali, X and Azam, F., (2004). Algicidal bacteria in the sea and their impact on algal blooms, J. Eukaryo. Microbiol., 51, 139-144.
21. Meenakshi, S., ManickaGnanambigai, D., Tamil mozhi, S., Arumugam, M. and Balasubramanian, T. (2009). Total flavanoid and in vitro antioxidant Antibacteria from seaweedsof Gaza 91 activity of two seaweeds of Rameshwaram coast. Global Journal of Pharmacology, 3 (2): 59-62.
22. Patterson, G.M.L., Larsen . L.K. and Moore R.E., (1994). Bioactivenatural products from blue-green algae. Journal of AppliedPhycology 6, 151-157.
23. Pietra F. (1997). Secondary metabolites from marine microorganisms:bacteria, protozoa, algae and fungi. Achievementsand prospects. Natural Product Reports 15, 453-464.
24. Radakovits R., Jinkerson R.E., Fuerstenberg S.I., Tae H., Settlage R.E., Boore J.L. & Posewitz M.C. (2012). Draft genome sequence and genetic transformation of the oleaginous alga: *Nannochloropsis gaditana*. Nature Communications 3: 686. DOI: 10.1038/ncomms1688.
25. Rangaiah GS, Lakshmi PA, Sruthi keerithia K. (2010). The antimicrobial activity of the crude extracts of *Chlorophyceacan* Seaweeds *Ulva, Caulerpa* and *Spongontorpha* spp. against clinical and phyto pathogens. Drug Invent. Today :2:311-314.

26. Riquelme CE, Avendaño-Herrera RE (2003). Microalgae and bacteria interaction in the aquatic environment and their potential use inaquaculture. Rev. Chil. Hist. Nat. 76: 725-736.
27. Seenivasan, R., Indu, H., Archana, G. and Geetha, S. 2010. The Antibacterial Activity of Some Marine Algae from South East Coast of India. American- Eurasian Journal of Agricultural & Environmental Science, 9(5): 480-489.
28. Sharifah, E. N., & Eguchi, M. (2011). The phytoplankton *Nannochloropsis oculata* enhances the ability of *Roseobacter clade* bacteria to inhibit the growth of fish pathogen *Vibrio anguillarum. PLoS One*, *6*(10), e26756.
29. Sidharta, A.K., Mody, K.H., Ramavat, B.K., Chauhan, V.D., Garg, H.S., Goel, A.K., Doss, M.J., Srivastava, M.N., Patnaik, G.K. and Kamboj, V.P. 1997.Bioactivity of marine organisms: Part VIII-Screening of some marine flora of western coast of India. Indian Journal of Experimental Biology, 35(6): 638-643.
30. Suda, S., Atsumi, M., & Miyashita, H. (2002). Taxonomic characterization of a marine *Nannochloropsis* species, *N. oceanica* sp. nov. (Eustigmatophyceae). *Phycologia*, *41*(3), 273-279
31. Thompson PA, Guo MX, Harisson PJ (1992). Effects of variation intemperature. I. On the biochemical composition of eight species ofmarine phytoplankton. J. Phycol. 28: 481-488.
32. Tuney, I., Cadirci, B.H., Unal, D. & Sukatar, A.,(2006). Antimicrobial activities of the extracts of marine algae from the cpast of Urla (Zmir, turkey), Tur,J.Biol., 30, 1-5.