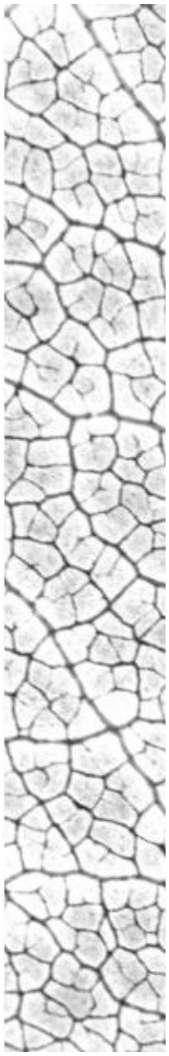

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 11-19 (2020)

Screening for some marine cyanobacteria isolated from Red Sea Coast, Egypt producing antimicrobial activity

Reham G. Elkomy [1*]

[1] Marine Biotechnology and Natural Product Laboratory, National Institute of Oceanography and Fisheries, Alexandria, EGYPT
*Corresponding author: Reham_niof@yahoo.com

Abstract
Marine microalge are known as source of bioactive secondary metabolites**.** In the present work, production of antimicrobial activity by three marine cyanobacteria *Oscillatoria simplicissima, Oscillatoria acutissima* and *Spirulina platensis* and antimicrobial activities of them was investigated against different microorganisms. The effects of pH, temperature and light intensity on the production of antimicrobial activity were tested. Extracts of the algae were prepared using hexane, chloroform, ethanol, methanol and water, then assayed for antimicrobial agents against microorganisms like *Staphylococcus aureus, Micrococus luteus, Serratia marcescens, Salmonella spp., Vibrio spp., Aeromonas hydrophila, Pseudomonas aeruginosa, Escherichia coli* and one species of yeast *Candida albicans*. Data showed that the methanol extract was very effective against bacterial and fungi strains compared to other extract at pH 8.0, 30ºC and 3000 lux for three algal. No antimicrobial activity was detected in the water extracts. This material was produced, maximally, after 12, 14, 12 days of incubation period in aerated culture for *Oscillatoria simplicissima, Oscillatoria acutissima* and *Spirulina* platensis respectively. The results indicated scope for utilizing these microalgae as a source of antimicrobial substances.

Keywords: Antimicrobial activity, marine microalgae, cyanobacteria, Red Sea, optimization

Elkomy RG (2020) Screening for some marine cyanobacteria isolated from Red Sea Coast, Egypt producing antimicrobial activity. Eurasia J Biosci 14: 11-19.

INTRODUCTION

Several recent studies have been revealed that seaweed and algae are potential sources that can be used as antimicrobial products (Al-Saif et al. 2014, Maftuch 2016, Rabia et al. 2013). Microalgae are one of the cheapest organisms that can be used in different biotechnological applications (Demirel et al. 2018). Microalgae has become a very popular source of antibacterial agents and offers numerous advantages for antimicrobial studies due to their huge biodiversity and fast growth rate (Pulz and Gross 2004). Microalgae are new source of structurally - novel and biologically - active compounds in the pharmaceutical industry (Ely et al. 2004, Patra et al. 2009, Tuney et al. 2006). Aquatic microorganisms and algae produce a pool of under investigated secondary metabolites and are potential sources of drug-like compounds to inhibit pathogens (Dussault 2016). Large number of literature have been released about compounds derived from algae with antibacterial activity, such as acrylic acid, halogenated aliphatic compounds, terpenes (Ming 2017). However, the identification of compounds directly responsible for the antimicrobial potential of algae is still a relatively incipient field of research, mainly owing to the new kinds of compounds found in recent years (Amaro et al. 2011, Pina-Perez 2017). Microalgae can produce several compounds like chlorophyll, carotene, phenolics, proteins, and fatty acids (Rao et al. 2010). Under stress conditions, extremophile microalgae might produce unique substances to adapt the changing condition. Thus, with changing environmental requirements, the content of the bioactive compounds might also be changed. It is known that algae had a chemical defence system being synthesized to survive in a competitive environment (Barros et al. 2005). Antibiotic resistance in bacteria and fungi is one of the major emerging health care related problems in the world; it became a greater problem of giving treatment against resistant pathogenic bacteria (Abdel-Raouf et al. 2015, Sieradzki et al. 1999). Extracts from 10 cyanobacteria proved to be active against multidrug resistant *Mycobacterium tuberculosis*, the causative agent of tuberculosis (Rao et al. 2007). Antimicrobial activity depends on both algal species and the solvents used for their extraction (Prakash et al. 2011, Radhika et al. 2012, Reham et al. 2015). The antimicrobial activity of algae extracts is generally assayed using various organic solvents which always provide higher efficiency in extracting compounds for

Received: August 2019
Accepted: October 2019
Printed: October 2019

antimicrobial activity (Cordeiro et al. 2006, Tuney et al. 2006). Analytical methods play important roles in the discovery, development and manufacture of bioactive molecules (Mariswamy et al. 2011). Temperature of incubation (Ame et al. 2003, Issa 1999), pH of the culture medium (Patterson and Boils 1995), and light intensity (Griffiths and Saker 2003) are the important factor influencing antimicrobial agent production. The aim of the present study was discussed about: the antimicrobial activity of three cyanopacteria by different solvent extracts against some pathogenic bacterial and fungal strains and the effects of PH, Temperature and light intensity on the production of antimicrobial activity.

MATERIALS AND METHODS

Algal Isolation

The algal species used in this study were isolated from the Gulf of Aqaba of the Red Sea coast of Alexandria. Samples were grown in the F/2 medium (Guillard 1975, Guillard and Ryther 1962). The medium was then autoclaved at 120 °C for 30 minutes. The culture was incubated at temperature 30±1°C, pH 8 and light intensity 3000 lux. The algae were kept under optimum conditions. The isolated strain was identified according to available literature (Cronberg et al. 2006, Prescott 1968, Tomas et al. 1996).

Measurements of Algal Growth

According to the method described by Strickland and persons (1972). Determination of algal growth as a chlorophyll a. Harvesting took place by centrifugation at 5000 rpm for 15 min. The pigment content in filtered extract was determined by the absorbance at 663 and 645 nm in a 1cm quartz cell against a blank of 80% aqueous acetone by spectrophotometer using the following equation:

$$\text{Chlorophyll a} = 12.7.\ E^{663} - 2.69.\ E^{645}$$

Test Organisms

1. Two gram positive bacteria: ((*Staphylococcus aureus, Micrococus luteus,*).
2. Six gram negative bacteria: (*Serratia marcescens, Salmonella spp., Vibrio spp., Aeromonas hydrophila, Pseudomonas aeruginosa, Escherichia coli*).
3. The unicellular fungus (*Candida albicans).*

These test organisms were deposited as culture collection at Microbiology Lab., National Institute of Oceanography and Fisheries – Alexandria.

Preparation of the Algal Extracts

Three microalgae were grown in F/2 medium at aerated conditions. Microalgae pellets were harvested for growth at stationary phase, the culture centrifuged and the pellets were dried in hot air oven (60°C) till constant weight and used for extraction of antimicrobial agents. Half g of each dried biomass of the three microalgae was extracted in 10 ml each of hexane, chloroform, ethanol and methanol. All of the extracts were preserved at -4° C (Gonzalez Del Val et al. 2001).

Antimicrobial Activity Test

Screening for antibiotic activity of the tested algal extracts was carried out by the agar diffusion assay according to European Pharmacopoeia (1997). One loop full of each test organism was suspended in 3 ml 0.85% sterile NaCl solution, separately. Nutrient agar (Difeco, UK) was inoculated with this suspension of the respective organism and poured into a sterile Petri dish. According to preliminary test for the most effective dose, 10 µl of dimethyl sulfo-oxide (DMSO) Contained 5 mg of each extract was placed on sterilized paper disc (6 mm diameter). The loaded discs were placed apart from each other on the inoculated agar plate aseptically. Sterilized discs that loaded with DMSO only served as negative control and antibiotic discs (Ceprozil and Polymixin) served as positive control. A prediffusion for 3h was carried out at 10°C (Bansemir et al. 2006). Inhibition zones were measured after 24h incubation period at 37°C for bacteria and at 30°C after 48h for the fungus species. After incubation, the diameter of the inhibition zone was measured with calipers and the results were recorded in mm (Attaie et al. 1987).

Effect of Different pH, Temperature and Light Intensity on the Production of Antimicrobial Activity

The F/2 medium (100 ml) was prepared in 250 ml of Erlenmeyer flask. The different growth parameter including pH (5, 6, 7, 8 and 9), temperature (25, 30, 35, 40°C) and light intensity (1000, 2000, 3000 and 4000 Lux) were optimized independently. Then 10 ml of actively growing log phase inoculum was transferred to the culture flask aseptically and reserved under the fluorescent light for 20 days at aerated condition. The antimicrobial activity was determined by disc diffusion method.

Statistical Analysis

The data for various biochemical parameters were analyzed statistically by one-way ANOVA (Duncan 1957).

RESULTS AND DISCUSSIONS

Microalgae Isolated

The algal strains were identified as *Oscillatoria simplicissima, Oscillatoria acutissima* and *Spirulina platensis* (**Fig. 1**). The algal strains were harvested at their exponential phase of growth which is 14th day for *Oscillatoria simplicissima,* 14th day for *Oscillatoria acutissima* and 12th day for *Spirulina platensis* under 30±2°C, pH 8 and 3000 Lux (**Fig. 2**). The most studies on the biochemical production of algal and their analysis were carried out in stationary phase of growth period (Becker 1994).

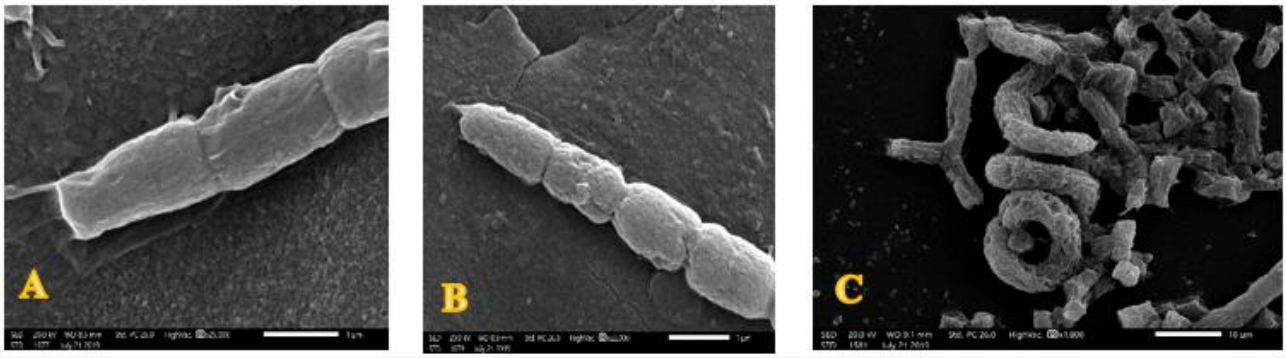

Fig. 1. Scanning electron microscope (SEM) images of (A) *Oscillatoria simplicissima* (B) *Oscillatoria acutissima*(c) *Spirulina platensis*

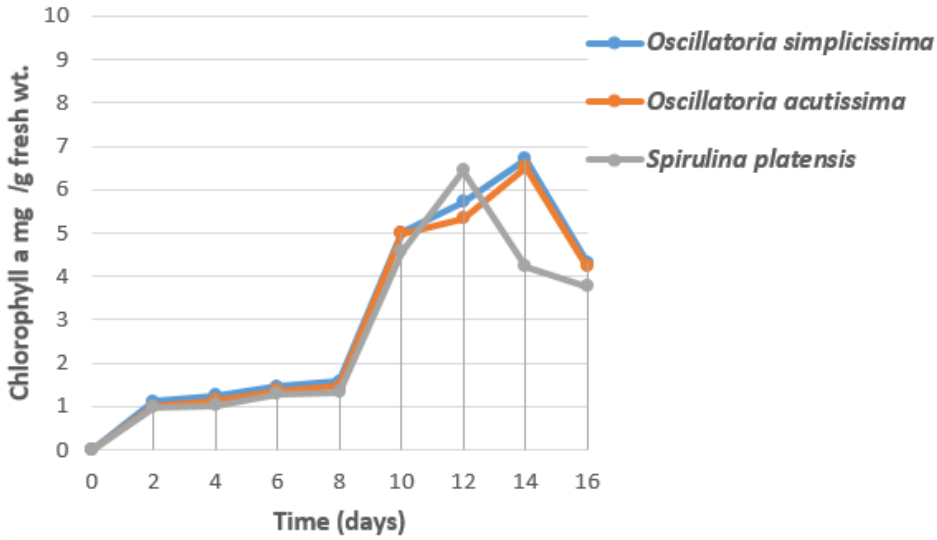

Fig. 2. The growth of Oscillatoria simplicissima, Oscillatoria acutissima and Spirulina platensis measured as chlorophyll (a) mg/g fresh wt

Antimicrobial Activities

The antimicrobial activity was evaluated as the diameters of the inhibition zones formed as a result of disc assay method in case of bacteria and one species of yeast. No antimicrobial activity was detected in the hexane, chloroform and water extracts for *Oscillatoria simplicissima* and *Oscillatoria acutissima.* The methanol extract of three cyanobacteria had more activity against most of the test organisms. The diameter of inhibition zones for methanol extract of *Oscillatoria simplicissima* recorded 1.9 cm in *Staphylococcus aureus.* On the other hand, the hexane and chloroform extracts were not active against all tested microorganisms. The ethanol extract for *Oscillatoria acutissima* showed more activity against *P. aeruginosa* recorded 1.4 cm diameter of inhibition zone. On the other hand; the hexane, chloroform and water extracts were not active against all tested microorganisms. The methanol extract for *Spirulina platensis* represented more activity against *Staphylococcus aureus, Vibrio spp., Pseudomonas aeruginosa, Escherichia coli*. On the other hand; the ethanol and water extracts were not active against all tested microorganism (**Table 1**). In the light of the experimental results concerning the antimicrobial activity of the test microorganisms against standard antibiotics showed that when the effects of extracts obtained from marine microalgae were compared with standard antibiotics used in this study, it was found that the effect of standard antibiotics was more than that of extract of *Oscillatoria acutissima* and *Spirulina platensis*. While the effect of antibacterial agents resulted from extracts of *Oscillatoria simplicissima* was more as compared to standard antibiotics in the same test bacteria which was observed by measuring the zone of inhibition. These results go in harmony with those obtained by Ozdemir et al. (2004) and Tuney et al. (2006). Likewise, in methanol extracts for three cyanobacteria gave the largest inhibition zone tested against bacterial pathogens These results were compatible with the study of Prakash et al. (2011) on the antimicrobial potential of *Oscillatoria sancta* and *Lyngby abirgeiagainst* exhibited antibacterial activity against *S. aureusin* methanol and acetone extracts in accordance with Guedes et al. (2011). In addition, Ostensvik et al., 1998 who observed that aqueous extracts of Microcystis aeruginosa inhibited *B. subtilis*, and Rao et al. (2007). It

Table 1. Antimicrobial activity of the investigated hexane, chloroform, ethanol, methanol and water extracts of three cyanobacteria using the agar plate by diffusion assay

Standard antibiotics		Diameter of inhibition zone(cm)								
		Gram (+V) bacteria			Gram (-V) bacteria					Fungal sp.
		S. aureus	*M. Luteus*	*S. marcescens*	*Salmonella spp.*	*Vibrio spp.*	*A. hydrophila*	*P. aeruginosa*	*E. coli*	*C. albicans*
Cefprozil		1.0± 0.003	1.0 ± 0.06	1.0±0.03	1.1 ± 0.03	0.8±0.03	2.0± 0.03	1.0 ± 0.03	1.5 ± 0.03	1.2±0.03
Polymixin										
Microalgal sp.	Solvent extracts	2.0 ± 0 .03	1.5 ± 0.03	1.5±0.03	1.5± 0.03	1.0±0.03	2.3 ± 0.03	1.3± 0.03	1.6± 0.03	1.6±0.03
Oscillatoria simplicissima	Hexane	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	-	-	-
	Ethanol	1.1 ± 0.03	-	-	-	1.1±0.03	1.2± 0.03	1.2 ± 0.03	-	-
	Methanol	1.9± 0.03	1.0± 0.03	1.3± 0.03	-	1.7±0.03	1.3± 0.03	1.5 ± 0.03	1.1±0.03	1.8±0.03
	Water	-	-	-	-	-	-	-	-	-
Oscillatoria acutissima	Hexane	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-			-
	Ethanol	1.3± 0.03	-	0.9±0.03	-	0.6±0.03	-	1.4± 0.03	0.8±0.01	-
	Methanol	0.9 ± 0.03	1.0 ± 0.03	-	-	1.3±0.03	1.0± 0.03	-	1.0±0.03	0.6±0.03
	Water	-	-	-	-	-	-	-	-	-
Spirulina platensis	Hexane	0.9± 0.03	-	-	-	1.2±0.01		0.8 ± 0.01	-	-
	Chloroform	1.0 ± 0.01	0.9 ± 0.03	0.9±0.03	-	1.2±0.01	-	0.9± 0.01	0.9±0.01	1.0±0.03
	Ethanol	-	-	-	-	-	-	-	-	-
	Methanol	1.5± 0.03	0.8 ± 0.01	1.0±0.01	-	1.4±0.03	-	1.5± 0.03	1.5±0.06	0.5±0.01
	Water	-	-	-	-	-	-	-	-	-

- = No inhibitory effect; width 0.1 to 0.8 cm = week activity; width 0.8 to 1.0 cm = moderate activities; width >1,0 cm = strong activity. Data are expressed in the form of mean ±SD

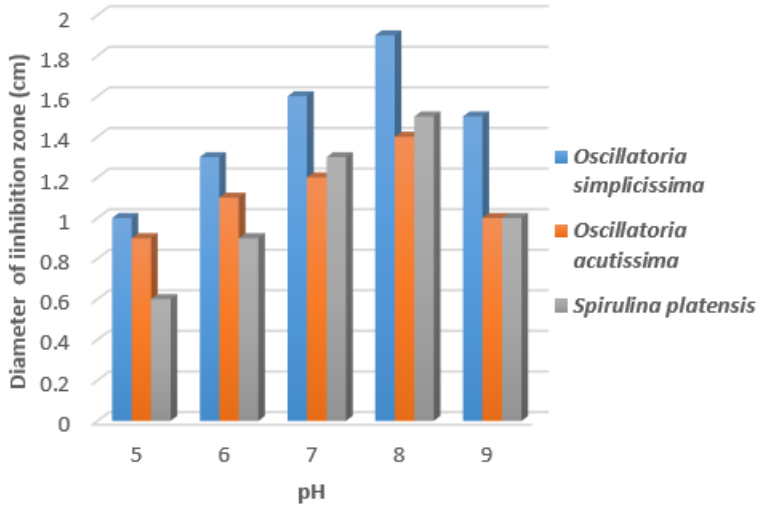

Fig. 3. Effect of different pH on antimicrobial activity production by *Oscillatoria simplicissima, Oscillatoria acutissima* and *Spirulina platensis*

is worth mentioning that the extracts obtained from various solvents used in this study had antibacterial and antifungal activities and so of these extracts could be more effective than antibiotics. Emerging resistance to antibiotics has raised serious concerns regarding the next source of new chemical entities that can meet the challenge of continually emerging drug resistance. Although considerable progress is being made within the fields of chemical synthesis and engineered biosynthesis of antimicrobial compounds, nature still remains the richest and the most versatile source for new antibiotics (Pina-perazl et al. 2017). Some recent studies reveal seaweed and algae as a potential source of antimicrobial products (e.g. Alghazeer et al. 2013, Al-Saif et al. 2014, Mendes et al. 2013).

Effect of Different Initial pH, Temperature and Light Intensities on Antimicrobial Activities Production by *cyanobacteria*

One optimum pH 8.0 was recorded for antimicrobial agent production from three cyanobacteria **(Fig. 3**). The diameter of inhibition zone recorded (1.9cm) for ***Oscillatoria simplicissima*** against *S. aureus,* while in ***Oscillatoria acutissima*** **and** ***Spirulina platensis*****,** the diameter of inhibition zone recorded (1.4 and 1.5cm respectively) against *P. aeruginosa*. It has been well documented by the earlier researcher (Richmond A.2000, Renaud SM. et al., 1991, Renaud SM. et al., 1995, and Borowizka MA. et al., 1990). The pH of the medium is very important for growth of microorganisms, for the character of their metabolism and hence for the biosynthesis of antimicrobial products as secondary metabolites. *Scytonema ocellatum* was found to exhibit

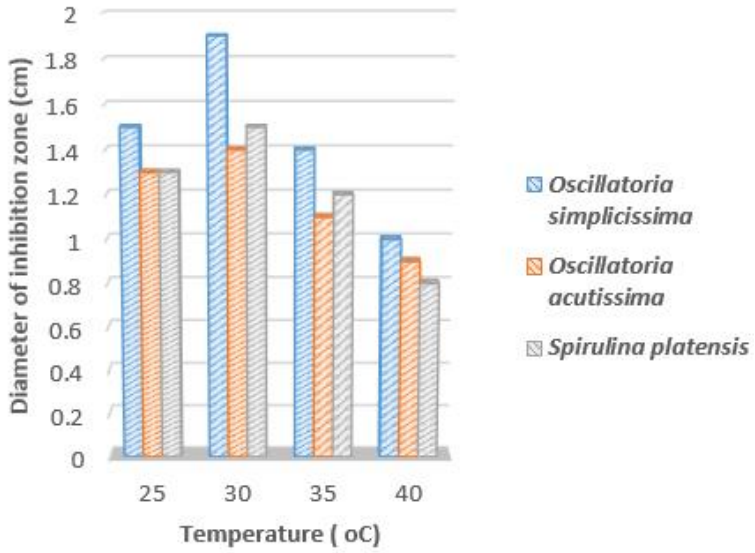

Fig. 4. Effect of different temperature on antimicrobial activity production by *Oscillatoria simplicissima, Oscillatoria acutissima* and *Spirulina platensis*

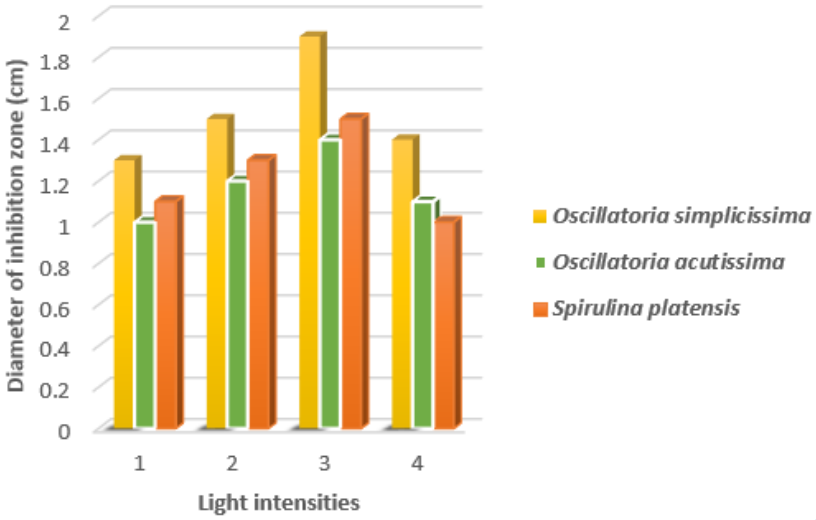

Fig. 5. Effect of different light intensity on antimicrobial activity production by *Oscillatoria simplicissima, Oscillatoria acutissima* and *Spirulina platensis*

maximal scytophycin productivity at pH 8.0–8.5 (Patterson and Boils 1995).

Temperature is an environmental factor which indirectly affects growth of microalgae and antimicrobial activity (Huang et al. 2008). The results recorded in (**Fig. 4**) revealed that the diameter of inhibition zone recorded (1.9 cm) after 14th days of incubation for ***Oscillatoria simplicissima***. While in ***Oscillatoria acutissima*** the diameter of inhibition zone recorded (1.4cm) at the same temperature 30 after 14th days of incubation. Ame *et al.* (2003) found that production of higher amounts of the bioactive toxin, microcystin by cyanobacteria was favored at temperature more than 30 ^{o}C, although maximum cylindros peropsin production was attained by the cyanobacterium Cylindros permopsis raciborskii at 30 ^{o}C (Griffiths and Saker, 2003). Lehtimaki et al. (1997) found that low temperatures (7, 10, 16 ^{o}C) gave low measurements for nodularin production by cyanobacteria, while the highest production was attained at high temperatures. The relationship between temperature and growth of microalgae is linear (Takemura et al. 1985). Temperature determines the activity and reaction rates of intracellular enzyme, which will have an influence on algal photosynthesis, respiration intensity, affect the growth of microalgae and to limit its distribution (Tan et al. 2009).

Light is an essential key for growth of microalgae. when light intensity was changed effectiveness of bioactive compounds also varied. In **Fig. 5,** it was clearly seen that under 3000 Lux light intensity, three cyanobacteria produced the most effective bioactive compounds against all the microorganisms tested. At stationary phase, the diameter of inhibition zone for ***Oscillatoria simplicissima, Oscillatoria acutissima***

and *Spirulina platensis* recorded (1.9, 1.4 and 1.5 cm) respectively. Microalgae uses light to process the photosynthetic, but the light energy cannot be stored by microalgae, so the light should be supplied sustainably. The microalgae cannot use all the supplied light because microalgae cannot absorb all the photons, and too much light will cause light inhibition for the surface layer of microalgae. Through the photosynthetic process, for autotrophic microalgae to convert carbon dioxide in the air into organic compounds, visible light is the main source of energy (Carvalho et al. 2011) since the chlorophylls, phycobilins and carotenoids in microalgae can be absorbed in the visible light range. High light intensity can cause strong harmful effects in the cell due to excessive stimulation of the photochemical apparatus (Skjånes et al. 2013). Under high light stress, chlorophyll can interact with oxygen to cause the formation of reactive oxygen species (ROS). Algae had defense systems against these ROS, of these, increasing to produce carotenoids was the most common one. On the other hand, Seepratoomrosh et al. (2016) demonstrated that under low light conditions, the chlorophyll content of D. tertiolecta was higher than in high light condition. In a study done by Simionata et al. (2011), under low light, cells of *Nannochloropsis gaditana* accumulated more chlorophyll than it was in high light. Since chlorophyll is essential for light harvesting (Li et al. 2009), in low light the increment of the pigment was expected. It was known that carotene had antioxidant properties, while, phenolic compounds as a photo protective response were be useful as antimicrobial (Skjånes et al. 2013).

CONCLUSION

The results of the present investigation on selected species of marine cyanobacteria indicated scope for deriving biologically active compounds which are effective in inhibiting the growth of the pathogenic bacteria both Gram-positive and Gram-negative. Further the Red sea marine environment has potential to return pharmaceutically useful microalgae which can be harnessed for the development of drugs for use in management of human pathogens, cancer, tumor, AIDS and many human degenerative diseases. There is great scope for further investigations toward drug development.

ACKNOWLEDGEMENT

We did not receive any grants from funding agencies in the public, commercial, or not-for-profit sectors for this work.

REFERENCES

Abdel-Raouf N, Al-Enazi NM, Al-Homaidan AA, Ibraheem IBM, Al Othman MR, Hatamleh AA (2015) Antibacterial b-amyrin isolated from Laurencia microcladia. Arabian Journal of Chemistry, 8: 32-37. https://doi.org/10.1016/j.arabjc.2013.09.033

Alghazeer R, Whida F, Abduelrhman E, Gammoudi F, Naili M (2013) In vitro antibacterial activity of alkaloid extracts from green, red and brown macroalgae from western coast of Lybia. African Journal of Biotechnology, 12: 7086-7091.

Al-Salif SSA, Abdel-Raouf N, El-Wazanani HA, Aref IA (2014) Antibacterial substances from marine algae isolated from Jeddah coast of Red Sea, Saudi Arabia. Saudi Journal of Biological Sciences, 21: 57-64. https://doi.org/10.1016/j.sjbs.2013.06.001

Amaro HM, Guedes AC, Malcata FX (2011) Science against microbial pathogens: communicating current research and technological advances A. Mendez-Vilas (Ed.), Antimicrobial activities of microalgae: an invited review: 1272-1280.

Ame MV, Diaz M, Wunderline DA (2003) Occurance of toxic cyanobacterial blooms in San Roque Reservoir (Cordoba, Argentina): a field and chemometric study. Inc. Environ. Toxicol, 18: 192-198. https://doi.org/10.1002/tox.10114

Attaie R J, Whalen K M, Arner M A (1987) Inhibition of growth of S. aureusduring production of acidophilus yogurt.J. Food Protec, 50: 224-228. https://doi.org/10.4315/0362-028X-50.3.224

Bansemir A, Blume M, Schröder SU, Lindequist U (2006) Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture; 252: 79-84. https://doi.org/10.1016/j.aquaculture.2005.11.051

Barros MP, Pinto E, Sigaud-Kutner TCS, Cardozo KHM, Colepicolo D P (2005). Rhythmicity and oxidative/nitrosative stress in algae. Biological Rhythm Research, 36(1/2): 67-82. https://doi.org/10.1080/09291010400028666

Becker EW (1994) Microalgae: biotechnology and microbiology. Cambridge: University press: 1-293.

Borowitzka MA, Borowitzka LJ, Kessly D (1990) Effect of salinity increase on carotenoids accumulation in the green alga Dunaliella salina. Journal of Applied Phycology, 2: 111-119. https://doi.org/10.1007/BF00023372

Carvalho PA, Silva OS, Baptista MJ, Malcata FX (2011) Light Requirements in Microalgal Photobioreactors: An Overview of Biophotonic Aspects. Appl Microbiol Biotechnol, 89: 1275-1288. https://doi.org/10.1007/s00253-010-3047-8

Cordeiro RA, Gomes VM, Carvalho AFU, Melo VMM (2006) Effect of Proteins from the Red Seaweed Hypnea musciformis (Wulfen) Lamouroux on the Growth of Human Pathogen Yeasts. Brazilian Archives of Biology and Technology, 49(6): 915-921. https://doi.org/10.1590/S1516-89132006000700008

Cronberg G, Annadotter H (2006) Manual on Aquatic cyanobacteria. ISSHA.

Demirel Z, Yılmaz FF, Ozdemir G, Dalay MC (2018) Influence of Media and Temperature on the Growth and the Biological Activities of Desmodesmus protuberans (FE Fritsch & MF Rich) E. Hegewald. Turkish Journal of Fisheries and Aquatic Sciences, 18: 1195-1203. https://doi.org/10.4194/1303-2712-v18_10_06

Duncan BD (1957) Multiple range tests for correlated and heteroscedastic means. Biometrics, 13: 359-364. https://doi.org/10.2307/2527799

Ely R, Supriya T, Naik CG (2004) Antimicrobial activity of marine organisms collected off the coast of South East India. Journal of Experimental Marine Biology and Ecology, 309: 121-127. https://doi.org/10.1016/j.jembe.2004.03.010

European Pharmacopoeia (1997) Mikrobiologische Wertbestimmung von Antibiotika, Diffusions method. Deutscher-Apotheker-Verlag, Stuttgart (6th Ed.).

Gonzalez del Val, Platas G, Bailio A (2001) Screening of antimicrobial activities in red, green and brown macroalgae from (Gran Canaria, Spain). Int. Microbiol, 4: 35-40.

Griffiths DJ, Saker ML (2003) The Palm island mystery disease 20 years on: a review of research on cyanotoxin cylindrospermopsin. Inc. Environ. Toxicol; 18: 78-93. https://doi.org/10.1002/tox.10103

Guedes A C, Catarina R, Barbosa H M, Amaro C I, Pereira F X M (2011) Microalgal and cyanobacterial cell extracts for use as natural antibacterial additives against food pathogens. International Journal of Food Science and Technology, 46(4): 862-870. https://doi.org/10.1111/j.1365-2621.2011.02567.x

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: WL Smith and MH Chanley (eds.), Culture of Marine Invertebrate Animals. Plenum Press, New York: 26-60. https://doi.org/10.1007/978-1-4615-8714-9_3

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonulaconfervacea Cleve. Can. J. Microbiol., 8: 229-239. https://doi.org/10.1139/m62-029

Issa AA (1999) Antibiotic production by the cyanobacteria Oscillatoria angustissima and Calothrix parietina. Environ. Toxicol. Pharm. 8: 33-37. https://doi.org/10.1016/S1382-6689(99)00027-7

Lehtimaki J, Moisander P, Sivonen K, Kononen K (1997) Growth, nitrogen fixation and nodularin production by two Baltic Sea cyanobacteria. Appl. Environ. Microbiol, 63(5): 1647-1654.

Li Z, Wakao S, Fischer BB, Niyogi KK (2009) Sensing and responding to excess light. Annual Review of Plant Biology, 60: 239-260. https://doi.org/10.1146/annurev.arplant.58.032806.103844

Maftuch I, Adam A, Zamzami I (2016) Antibacterial effect of Gracilaria verrucosa bioactive on fish pathogenic bacteria. Egyptian Journal of Aquatic Research, 42: 405-410. https://doi.org/10.1016/j.ejar.2016.10.005

Mariswamy Y, Gnaraj WE, Johnson M (2011) Chromatographic finger print analysis of steroids in AervalanataL by HPTLC technique. Asian Pacific Journal of Tropical Biomedicine, 1(6): 428-433. https://doi.org/10.1016/S2221-1691(11)60094-4

Mendes M, Pereira R, Sousa-Pinto I, Carvalho AP, Gomes AM (2013) Antimicrobial activity and lipid profile of seaweed extracts from the North Portuguese Coast. International Food Research Journal, 20: 3337-3345.

Ming L, Yixiang MJ, Cao L, Guang-Ming Q, Chen L, Sun H, Chen H (2017) Antibacterial activity and mechanisms of depolymerized fucoidans isolated from Laminaria japonica, Carbohydrate Polymers, 172: 294-305. https://doi.org/10.1016/j.carbpol.2017.05.060

Ostensvik O, Skulberg OM, Underdal B, Hormazabal V (1998) Antibacterial properties of extracts from selected planktonic fresh water cyanobacteria—a comparative study of bacterial bioassays. Journal of Applied Microbiology, 84: 1117-1124. https://doi.org/10.1046/j.1365-2672.1998.00449.x

Ozdemir G, Karabay N, Dolay M, Pazarbasim B (2004) Antibacterial activity of volatile extracts of Spirulina platensis. Phytother. Res., 18(9): 754-757. https://doi.org/10.1002/ptr.1541

Patra JK, Patra AP, Mahapatra NK, Thatoi N, Das S, Sahu RK, Swain GC (2009) Antimicrobial activity of organic solvent extracts of three marine macroalgae from Chilika Lake, Orissa, India. Malaysian Journal of Microbiology, 5: 128-131.

Patterson G M L, Boils C M (1995) Regulating of scytophycin accumulation in cultures of Scytonema ocellatum II. Nutrient requirement. Appl. Microbiol. Biotechnol, 43: 692-700. https://doi.org/10.1007/BF00164775

Pina-Perez MC, Rivas A, Martinez A, Rodrigo D (2017) Antimicrobial potential of macro and microalgae against pathogenic and spoilage microorganisms in food. Food Chemistry. https://doi.org/10.1016/j.foodchem.2017.05.033

Prakash JW, Johnson M and Solomon J (2011) Antimicrobial activity of certain fresh water microalgae from Thamirabarani Asian Pacific Journal of Tropical Biomedicine, 1(2): 170-173. https://doi.org/10.1016/S2221-1691(11)60149-4

Prescott GW (1968) The Algae: A review. Boston, Mass.: Houghton Mifflin Co.Pulz O and Gross W. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol., 65: 635648.

Pulz, O, Gross W (2004) Valuable products from biotechnology of microalgae. Applied Microbiological Biotechnology, 65: 635-648. https://doi.org/10.1007/s00253-004-1647-x

Rabia A, Fauzi W, Entesar A, Fatiem G, Mahboba N (2013) In vitro antibacterial activity of alkaloid extracts from green, red and brown macroalgae from western coast of Libya. African Journal of Biotechnology, 12: 7086-7091.

Radhika D, Veerabahu C, Priya R (2012) Antibacterial activity of some selected seaweeds from the Gulf of Mannar Coast, South India. Asian Journal of Pharmaceutical and Clinical Research; 5(4): 8990.

Rao A R., Reddy AH, Aradhya SM (2010) Antibacterial properties of Spirulina platensis, Haematococcus pluvialis, Botryococcus braunii micro algal extracts. Current Trends in Biotechnology and Pharmacy, 4(3): 809-819.

Rao M, Malhotra S, Fatma T, Rattan A (2007) Antimycobacterial activity from cyanobacterial extracts and phytochemical screening of methanol extract of Hapalosiphon. Pharmaceutical Biology; 45: 88-93. https://doi.org/10.1080/13880200601105319

Reham G, Ibraheem IBM, Mohammed S, Rabab M (2015) Optimization of Potential Antimicrobial Activity by Chlorella marina and Navicula f. delicatula. Journal of pure and applied microbiology, 9(4): 2725-2732.

Renaud SM, Parry DL, Luong-Van T, Kuo C, Padovan A, Sammy N (1991) Effect of light intensity on proximate biochemical and fatty acid composition of Isochrysis sp. And Nannochloropsis oculata for use in tropical aquaculture. Journal of Applied Phycology, 3: 43-53. https://doi.org/10.1007/BF00003918

Renaud SM, Zhou HC, Parry DL, Thinh LV, Woo KC (1995) Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae Isochrysis sp., Nitzschia closterium, Nitzschia paleacea, and commercial species Isochrysis sp. (clone T.ISO). Journal of Applied Phycology, 7: 595-602. https://doi.org/10.1007/BF00003948

Richmond A (2000) Microalgal biotechnology at the turn of the millennium: a personal view. Journal of Applied Phycology., 12(3-5): 441-451. https://doi.org/10.1023/A:1008123131307

Seepratoomrosh J, Pokethitiyook P, Meetam M, Yokthongwattana K, Yuan W, Pugkaew W, Kangvansaichol K (2016) The Effect of Light Stress and Other Culture Conditions on Photoinhibition and Growth of Dunaliella tertiolecta. Applied Biochemistry and Biotechnology, 178: 396-407. https://doi.org/10.1007/s12010-015-1882-x

Sieradzki K, Robert RB, Haber SW, Tomasz A (1999) The development of vanomycin resistance in patient with methicillin resistant S. aureus. The New England Journal of Medicine, 340: 517-523. https://doi.org/10.1056/NEJM199902183400704

Simionato D, Sforza E, Carpinelli EC, Bertucco A, Giacometti GM, Morosinotto T (2011) Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation. Bioresource Technology, 102: 6026-6032. https://doi.org/10.1016/j.biortech.2011.02.100

Skjånes K, Rebours C, Lindblad P (2013) Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. Critical Reviews in Biotechnology 33(2): 172-215. https://doi.org/10.3109/07388551.2012.681625

Strickland JDH, Persons TR (1972) A practical handbook of seawater analysis. 2nd ed. Bulletin 167. Ottawa: Fisheries Research Board of Canada: 1-311.

Takemura N, Iwkume T, Rusuno M (1985) Photosynthesis and Primary Production of Microcystis agruginosa in Lake Kasumigaura. Journal of Plankton Research, 7(3): 303-312. https://doi.org/10.1093/plankt/7.3.303

Tan X, Kong F, Yu Y, et al. (2009) Effects of Enhanced Temperature on Algae Recruitment and Phytoplankton Community Succession. China Environmental Science, 29(6): 578-582.

Tomas C, Hasle GR, Syverts EE, Steidinger K, Tangen K (1996) Identifying Marine Diatoms and Dinoflagellates. Elsevier. https://doi.org/10.1016/B978-012693015-3/50005-X

Tuney I, Cadirci BH, Unal D, Sukatar A (2006) Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). Turkish Journal of Biology, 30: 171-175.