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Optimization of Potential Antimicrobial Activity by *Chlorella marina* and *Navicula f. delicatula*

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In the present investigation two marine microalgae *(Chlorella marina* and *Navicula f. delicatula)* had been chosen for optimization their antibacterial and antifungal activities. The effects of pH, temperature and light intensity were tested for this purpose. Different solvent extracts (chloroform, acetone, ethanol, methanol and water) of two microalgae were tested by agar well diffusion method for their antibacterial and antifungal agent (*Staphylococcus aureus, Micrococus luteus, Serratia marcescens*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and unicellular fungus (*Candida albicans*). Data showed that the ethanol was the best organic solvent for extraction of active material, for *Chlorella marina* at pH 8.0, 25ºC, 3000 lux and 35 psu. This material was produced, maximally, after 12 days of incubation in aerated culture. While in *Navicula f. delicatula* the acetone was the best organic solvent for extraction of active material, at pH 8.0, 20ºC, 1000 lux and 35 psu after 14 days of incubation in aerated culture.

Key words: Optimization, Antimicrobial activity, Microalgae, Marine.

Microalgae play a key role in the productivity of oceans. Marine organisms produce pharmacologically important diverse group of natural products (Ravikumar S. *et al*., 2010 and Krishnakumar *et al*., 2011) that include algae, which produce novel and unexplored sources of potentially useful bioactive compounds that might represent useful leads in the development of new pharmaceutical agents (Iwamoto *et al*., 2001). Biologically active compounds from natural resources have always been of great interest to scientists working on different diseases (Hemraj Upmanyu *et al*., 2012 and Abdel-Raouf and Ibraheem 2008). Algae have been used in traditional medicine for a long time and also some algae have bacteriostatic, bactericidal, antifungal, antiviral and antitumor activity (Justo *et al*., 2001). Microalgae are rich source of structurally novel and biologically active metabolites. So it has been studied as potential bioactive compounds of interests in the pharmaceutical industry (Rangaiah *et al*., 2010 and Ely *et al*., 2004). Antibiotic resistance in bacteria and fungi isone of the major emerging health care related problems in the world; it became a greater problem of giving treatment against resistant pathogenic bacteria (Sieradzki, *et al.*, 1999 and Abdel-Raouf *et al*., 2015a, b). One approach to antibiotic resistance is the discovery of novel antimicrobial compounds for clinical application (Desbois*et al*., 2008 and 2009). Algal organisms are rich source of structurally novel and biologically active secondary and primary metabolites which may be potential bioactive compounds of interest in the pharmaceutical industry (Ely *et al.,* 2004 and Tuney*et al.,* 2006). A

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wide range of *in vitro* antifungal activities have also been reported from extracts of green algae, diatoms and dinoflagellates(Ely *et al.,* 2004) and from *Nostoc* sp. (Kim, 2008). Extracts from 10 cyanobacteria proved to be active against multidrug resistant *Mycobacterium tuberculosis*, the causative agent of tuberculosis (Rao *et al*., 2007). Najdenski *et al.* 2013 stated that ethanolic extract of *Scenedesmus obliqus*, *Chlorella* sp. and *Nostoc* sp. has antibacterial effect against *Staphylococcus aureus* and *Bacillus cereus.* In the same manner Sanmukh *et al.* (2014) explored bioactive compounds of a group of microalgae with emphasizing on the *Chlorella* sp. which showed antibacterial effect against *Staphylococcus* sp. Beenaand Krishnika (2011) tested antibacterial activity of *Scenedesmus* sp. isolated from a natural pond against three pathogenic bacteria with different solvents, the aqueous and methanol extracts gave better results. Sanmukh *et al*. (2014) explored microalgae for their bioactive compounds and affirmed promising applications encompassing antibacterial, antiviral, and antifungal activities; also he stated that the application of bioactive compounds derived from algae will prove beneficial and much more effective as compared with traditional treatment methods. Antimicrobial activity depends on both algal species and the solvents used for their extraction (Prakash *et al.,* 2011, Radhika *et al*., 2012 and Ibraheem *et al*., 2014). The antimicrobial activity of algae extracts is generally assayed using various organic solvents which always provide a higher efficiency in extracting compounds for antimicrobial activity (Cordeiro *et al*., 2006 and 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 (Issa, 1999; Ame *et al*., 2003), pH of the culture medium (Patterson and Boils, 1995), phasphate concentration (Banker and Carmeli, 1998 and light intensity (Griffiths and Saker, 2003) are the important factor influencing antimicrobial agent production. The aim of the present study was to study the antimicrobial activity of two microalgae green algae *(chlorella marina*) and diatom *(Navicula f. delicatula)* 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

Isolation and purification of algal isolates

The algal strains (*Chlorella marina and Navicula f. delicatula*) were isolated from two different locations, namely, El-Agamy (west of Alexandria) and Baltim (East of Alexandria) in the Mediterranean coast of Egypt Figure 2.

Samples were grown in F/2 medium (Guillard and Ryther 1962, Guillard 1975). The algal strains were harvested at their exponential phase of growth which is 12$^{\underline{\text{the}}}$ day for *chlorella marina* and 14$^{\underline{\text{the}}}$ for *Navicula f. delicatula* at aerated condition. Harvesting took place by centrifugation at 4000 rpm for 15 min. The isolated strains were identified according to (Tomas C. *et al.*, 1996, Prescott1968, Cronberg G. *et al.,* 2006).

Test Organisms

1. Two gram positive bacteria: (*Staphylococcus aureus and Micrococus Luteus*).
2. Three gram negative bacteria: (*Serratiam arcescens, Pseudomonas aeruginosa, and E.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

The two microalgae were grown in F/2 medium at aerated conditions. We make harvest for growth at stationary face, the culture centrifuged and the pellets were dried in hot air oven (60°C) till constant weight and used for extraction of antimicrobial agents. 0.5 g of each dried biomass of the two microalgae was extracted in 10 ml each of chloroform, acetone, ethanol, methanol and aqueous. 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 respectiveorganism and poured into a sterile Petri dish. According to preliminary test for the most effective dose, 10 ¼l of dimethyl sulfo-oxide (DMSO) Contained5 mg of each extract was placed on sterilized paper disc (6 mm diameter). The loaded discs were placed apart from each other on theinoculated agar plate aseptically. Sterilized discs that loaded with DMSO only served as negative control and antibiotic discs (Erythromycin and Ampicillin) served as positive control. A pre-diffusion 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 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(5,6,7,8,9,10) ,temperature (20, 25, 30, 35, 40°C) and light intensity(1000, 2000, 3000 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.

Statistical analysis

The data were statistically analyzed by applying one-way ANOVA.

RESULTS AND DISCUSSIONS

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 fungi. Table 1 showed that the ethanol extracts for *chlorella marina* showed more activity against *Staphylococcus aureus* and *Serratia marcescens* 10.0mm diameter of inhibition zone). On the other hand; the water, chloroform and aceton extract was not active against all tested microorganisms. The aceton extract for *Nevicula F. delicatula* represented more activity against *Staphylococcus aureus, Micrococus Luteus and Pseudomonas aeruginosa*. On the other hand; the water, chloroform, and ethanol extract was not active against all tested microorganisms. 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 *chlorella marina* and *Nevicula f. delicatula*. These results go in harmony with those obtained by Ozdemir *et al*. (2004) and Tuney *et al*. (2006). With the study of Prakash *et al*. (2011) on the antimicrobial potential of *Oscillatoria sancta* and *Lyngbya birgei* against *S. aureus*. *Scenedesmus* exhibited antibacterial activity against *S. aureus* in 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).

Effect of pH, temperature and light intensity on the production of antimicrobial activity

One optimum PH (8.0) was recorded for antimicrobial agent production from two microalgal

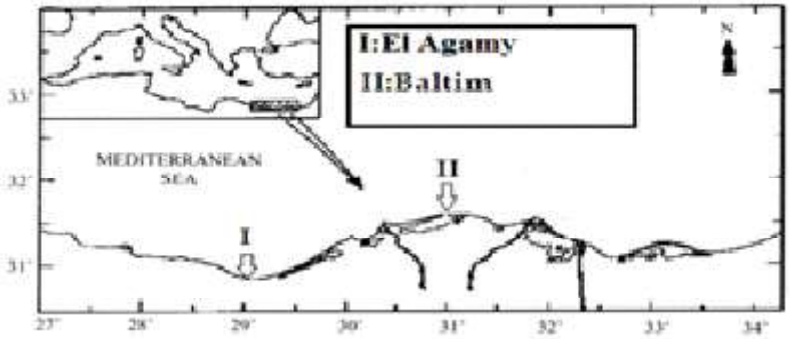

Fig. 1. Locations for isolated microalgae I- *Navicula f. delicatula* (El Agamy and II- *Chlorella marina (Baltim)*

genera (Fig.3). No antimicrobial activity could be detected at PH values below 5.0 or above 10.0.The highest growth of *chlorella marina* was reached at pH 8 and the diameter of inhibition zone recorded (12mm). While in *Navicula f. delicatula* the diameter of inhibition zone recorded (13mm). 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 themedium 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 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 highest growth of *chlorella marina* was reached at temperature 25°C and the diameter of inhibition zone recorded (12 mm) after 12th days of incubation. While in *Nevicula F. delicatula* the diameter of inhibition zone recorded (13mm) at temperature 20

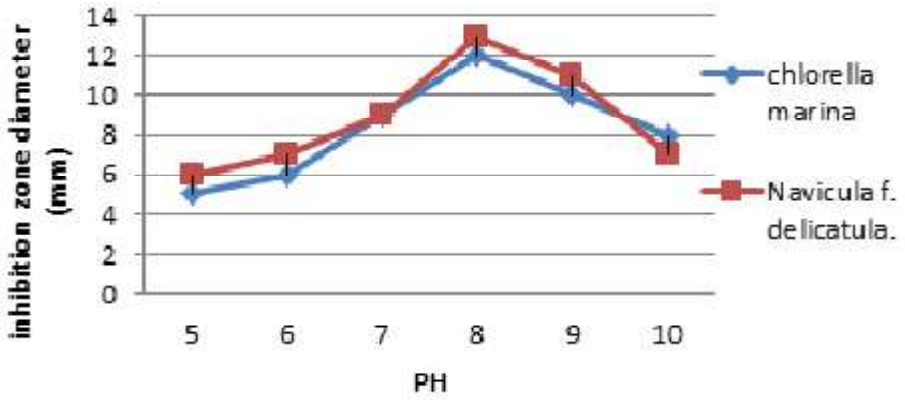

Fig. 2. Effect of different pH on antimicrobial activity production of *chlorella marina* & *Navicula f. delicatula.*

Table 1. Antibacterial and antifungal activity of the investigated chloroform, acetone, ethanol, methanol and water extracts of two microalgal genera using the agar plate by diffusion assay method.

Standard antibiotics		Diameter of inhibition zone(mm)					
		Gram (+V) bacteria		Gram (-V) bacteria			Fungal sp.
		S.aureus	*M.luteus*	*P.aeruginosa*	*E.coli*	*S.marcescens*	*C.albicans*
Erythromycin 20µg / disc		10.5	14	10.5	11.0	15.5	-
Ampicillin 10µg/ disc		11.5	15	10.5	11.5	11	-
Microalgal sp.	Solvent extracts						
chlorella	chloroform	-	-	-	-	-	-
marina	acetone	-	-	-	-	-	-
	ethanol	10		9		10	
	methanol	7	-	-	-	5	6
	water	-	-	-	-	-	-
Navicula f.	chloroform	-	-	-	-	-	-
delicatula	acetone	10	9	10	-	11	8
	ethanol	-	-	-	-	-	-
	methanol	7	7	8	-	8	5
	water	-	-	-	-	-	-

- = No inhibitory effect; width 1 to 8 mm = week activity; width 8 to 10 mm = moderate activities; width > 10 mm = strong activity.

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 23 C, although maximum cylindrosperopsinproduction was attained by the cyanobacterium Cylindros permopsis raciborskii at 20 C (Griffiths and Saker, 2003). Lehtimaki et al. (1997) found that low temperatures (7, 10, 16 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. 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

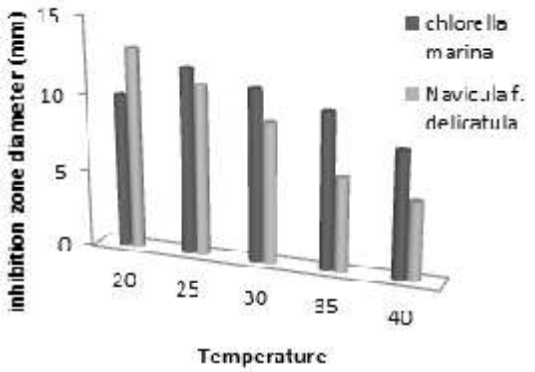

Fig. 3. Effect of different temperature on antimicrobial activity production of *chlorella marina*& *Navicula f. delicatula*

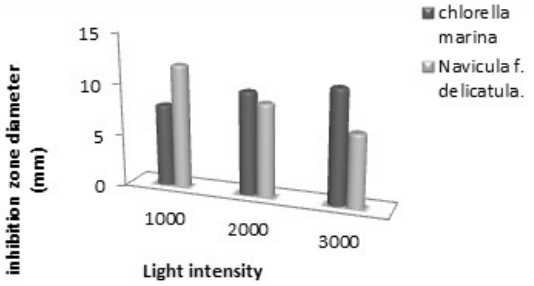

Fig. 4. Effect of different light intensity on antimicrobial activity production of *Chlorella marina*& *Navicula f. delicatula*

visible light range. At stationary phase, the data indicated that the light intensities affected the values of the growth and the diameter of inhibition zone Fig. (5), the highest growth of *chlorella marina* was reached at 3000 lux and the diameter of inhibition zone recorded (11 mm). While in *Nevicula F. delicatula* the diameter of inhibition zone recorded (12mm) at 1000 Lux. On other hand, it was found that the diameter of inhibition zone recorded (7.0 mm) at 3000 Lux for *Nevicula F. delicatula* but for *chlorella marine* the diameter of inhibition zone recorded (8.0 mm) at 1000 Lux.

When the light intensity above a certain value, continue increasing in light intensity level will decrease the microalgae growth rate actually, this is called photo inhibition phenomenon.

CONCLUSION

The ethanol extract for *chlorella marina* showed more activity against *Staphylococcus aureus* and *Serratiam arcescens* 10 mm diameter of inhibition zone with pH 8.0 in 35psu of salinity at 25°C and light intensity 3000 lux during 12the day of incubation at aerated condition in F/2 medium On the other hand the aceton extract for *Nevicula F. delicatula* more activity against *Staphylococcus aureus, Micrococus Luteus and Pseudomonas aeruginosa.* with pH 8.0 in 35psu of salinity at 20C and light intensity 1000 lux during 14the day of incubation at aerated condition in F/2 medium.

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