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Antimicrobial Activity of Three Microalgae Isolated from Mediterranean Sea Coast, Egypt

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Chloroform, acetone, ethanol and methanol extracts in addition to water extract of three microalgae (*Phormidium formusum*, *Chlorella marina* and *Navicula f. delicatula*) isolated from Mediterranean - Sea, coast (Egypt) were evaluated for their antibacterial and anti-fungal activities against *Staphylococcus aureus*, *Micrococus luteus, Serratia marcescens, Pseudomonas aeruginosa, Escherichia coli* and unicellular fungus (*Candida albicans*). Data showed that some extracts recorded strong inhibitory activities as compared to standard antibiotics.

Key words: Antibacterial, Antifungal, Marine microalgae, Mediterranean Sea.

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 (Sieradzki, *et al.*, 1999). 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, Tuneyet *al.,* 2006, Abdel-Raouf and Ibraheem 2008). Microalgae and cyanobacteria offer numerous advantages for antimicrobial investigations because of their enormous biodiversity and fast growth rate (Pulz and Gross, 2004, Reham G. *et al.,* 2013).The cell extracts and active constituents of various algae shown to have antibacterial activity invitro against Gram positive and Gram negative Bacteria (Borowitzka*et al.,* 1992, Ostensvik*et al.,* 1998, Goud*et al*., 2007 and Abdel-Raouf*et al.,* 2015a,b). A wide range of *in vitro* antifungala ctivities 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*.,

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2007). Najdenski *et al.,* 2013 stated that ethanol 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. He found that 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). The purpose of this work to evaluate the antimicrobial activity of cell extracts of cyanobacteria (*phormidium formusum),* green microalgae (*Chlorella marina)* and diatom (*(Navicula f. delicatula*) in vitro against two gram-positive genera, three gram-negative bacteria and the unicellular fungus (*Candida albicans).*

MATERIALS AND METHODS

Isolation and purification of algal isolates

The algal strains (*phormidiumformusum, Chlorella marina and Navicula f. delicatula*) were isolatedfrom three different locations, namely, El-Agamy (west of Alexandria), Eastern harbor (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) under at 28±2°C, PH 8.0 and light intensity 2000 lux. The algal strains were harvested at their exponential phase of growth which is 10th day for *phormidium formusum,* 14th day for *chlorella marina.*

And16thday for*Navicula f. delicatula*. Harvesting took place by centrifugation at 4000 rpm for 15 min. The isolatedstrains were identified according to (Tomas C. *et al.*, 1996, Prescott1968 and Cronberg G. *et al.,* 2006).

Test Organisms

1. Two gram positive bacteria: (*Staphylococcus aureus and Micrococus Luteus*).
2. Three gram negative bacteria: (*Serratiamarcescens, 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 cultures were centrifuged and the pellets were dried in hot air oven (60°C) till constant weight and used for extraction of antimicrobial agents. Have gram of each dried biomass of the three microalgae was extracted in 10 ml each of chloroform, acetone, ethanol, methanol and water. 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 microalgal 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 of 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 ìl 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 the inoculated 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 static and aeration condition on the growth of three microalgae

About 5 ml of the preculture two microalgal were transferred into Erlenmayer flasks (100 ml) containing 50 ml of F/2 medium. The flasks were incubated under static conditions (without shaking and aeration) and aeration conditions (two microalgal culture was agitated continuously to Prevent the settling of the cells at the bottom of the flasks, and bubbled with dry sterile air), for different periods (2 to 20 days).

Statistical analysis

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

RESULTS AND DISCUSSION

Effect of static and aerated conditions on growth of microalgal by chlorophyll (a) mg/g fresh wt

It is evident from Fig. (3) That the growth of *phormidium formusum* in aeration condition increased and reached its maximum value at stationary phase after 8^{the} days, then, phase started to decrease, but in static condition the growth

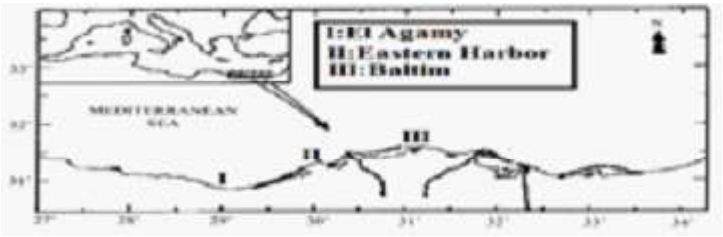

Fig. 1. Locations for isolated microalgae I- *Navicula f. delicatula* (El Agamy), II- *Phormidum formusum(Eastern Harbor)* and III- *Chlorella marina (Baltim)*

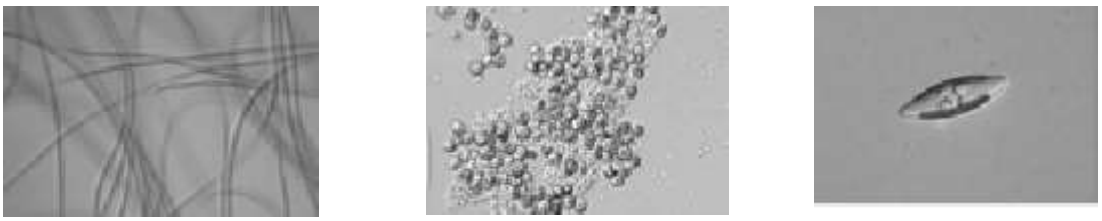

Fig. 2. The Electron microscope images of (1) *Phormidium formusum*, (2) *Chlorella marina* and (3) *Navicula f. delicate*

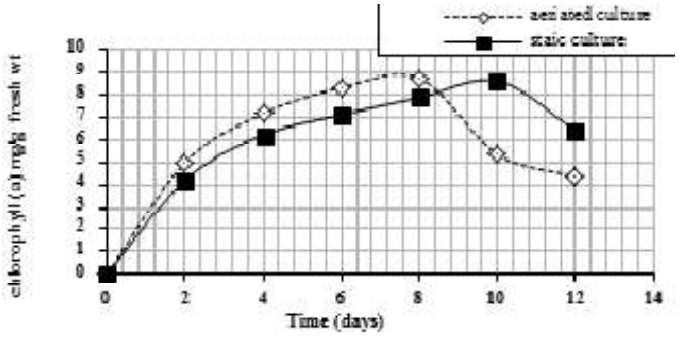

Fig. 3. The effect of aerated and static conditions on growth of *phormidium formusum* measured as chlorophyll (a) mg/g fresh wt

reached its maximum value after 10^{the} days of incubation. The growth of *chlorella marina* reached its maximum value in aeration condition after 12^{the} days. However, in static culture the maximum was attained after 14^{the} days, Fig. (4). While for *Navicula f. delicatula.* the maximum value for growth in static condition reached after 16^{the} days while in aeration condition reached after

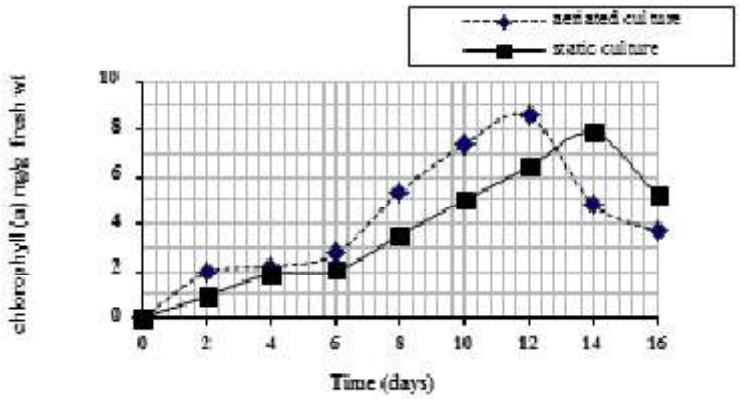

Fig. 4. The effect of aerated and static conditions on growth of *chlorella marina* measured as chlorophyll (a) mg/g fresh wt

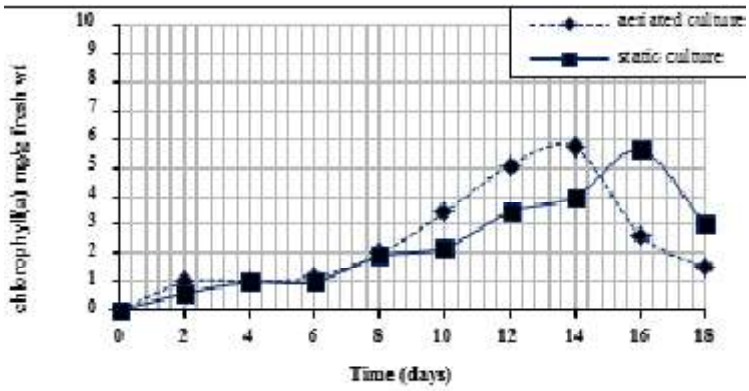

Fig. 5. The e effect of aerated and static conditions on growth of *Navicula f. delicatula* measured as chlorophyll (a) mg/g fresh wt

Fig. 6. The antibacterial activity of (1) *Phormidium formusum* water extract against *Serratia marcescens*, (2) *chlorella marina* ethanol extract against *Staphylococcus aureus* and (3) *Nevicula f. delicate* acetone extract against *Pseudomonas aeruginosa*

14the days, Fig.(5).

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. The water extract of *Phormidium formusum* had more activity against most of the test organisms. The diameter of inhibition zones for water extract 19.0 mm in *Staphylococcus aureus* but in *Serratia marcescens* the diameter of inhibition zones was 16.5 mm. On the other hand, the acetone and methanol extracts were not active against all tested microorganisms. The ethanol extract 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 acetone extract was not active against all tested microorganisms. The acetone extract for *Nevicula f. delicatula* represented more activity against

Fig. 7. The antibacterial activity of *Phormidium formusum* water extract against *Serratia marcescens* as compared with the control antibiotic Erythromycin

Table 1. Antibacterial and antifungal activity of the investigated chloroform, acetone, ethanol, methanol and water extracts of three 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*
Erythromycin20µg / disc		11.5	14	10.5	11	15.5	-
Ampicillin10ìg/ disc		11.5	12	10.5	11.5	11	-
Microalgal sp.	Solvent extracts						
Phormidium	chloroform	12	10	10	9	10	9
formusum	acetone	-	-	-	-	-	-
	ethanol	5	8	-	-	-	8
	methanol	-	-	-	-	-	-
	water	19	-	10	-	16.5	-
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

Staphylococcus aureus, Micrococus Luteus and Pseudomonas aeruginosa. On the other hand; the water, chloroform, and ethanol extract was 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 chlorella and *Nevicula f. delicatula*. While the effect of antibacterial agents resulted from extracts of *Phormidium formusum* 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 aqueous extract of *Phormidium formusum*, ethanol extract of *chlorella marina* and acetone extract of *Navicula f. delicatula* 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 abirgei*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). It 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. However these microalgae are potential sources of bioactive compounds and should be investigated for natural antibiotics.

It is intended that the present work will contribute to an understanding and determining the factors that controls the bioactive compounds produced by some microalgae. So these bioactive compounds need further studies to identify the chemical structures of these active compounds and to examine their beneficial effect for inhibition of some pathogenic bacteria and fungi.

CONCLUSION

The water extract of *Phormidium formusum* had more activity against most of the test organisms. The diameter of inhibition zones for water extract 19.0 mm in *Staphylococcus aureus* but in *Serratia marcescens* the diameter of inhibition zones was 15.0 mm during 8^{the} day of incubation at aerated condition in F/2 medium. On the other hand the ethanol extract for *chlorella marina* showed more activity against *Staphylococcus aureus* and *Serratiam arcescens* 10 mm diameter of inhibition zone during 12^{the} day of incubation at aerated condition in F/2 medium, while the aceton extract for *Nevicula f. delicatula* more activity against *Staphylococcus aureus, Micrococus Luteus and Pseudomonas aeruginosa.* during 14^{the} day of incubation at aerated conditiin F/2 medium.

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