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 in vitro against Gram positive and Gram negative Bacteria (Borowitzkaet al., 1992, Ostensviket al., 1998, Goudet et al., 2007 and Abdel-Raoufet al., 2015a,b). A 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 ethanol extract of *Scenedesmus obliquus*, *Chlorella* sp. and *Nostoc* sp. has antibacterial effect against *Staphylococcus aureus* and *Bacillus cereus*. In the same manner Sammukhet 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. Sammukhet 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 alga 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 (*phormidium formusum, Chlorella marina* and *Navicula f. delicatula*) were isolated from 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*.

And16th day for *Navicula f. delicatula*. Harvesting took place by centrifugation at 4000 rpm for 15 min. The isolated strains 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 *Micrococcus 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 il 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 (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 8th days, then, phase started to decrease, but in static condition the growth...
reached its maximum value after 10th days of incubation. The growth of *chlorella marina* reached its maximum value in aeration condition after 12± days. However, in static culture the maximum was attained after 14± days, Fig. (4). While for *Navicula f. delicatula* the maximum value for growth in static condition reached after 16± 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](image1.png)

![Fig. 5. The effect of aerated and static conditions on growth of *Navicula f. delicatula* measured as chlorophyll (a) mg/g fresh wt](image2.png)

![Fig. 6. The antibacterial activity of (1) *Phormidium formusum* water extract against *Serratia marcescens*, (2) *chlorella marina* ethanol extract against *Staphylococcus aureus* and (3) *Navicula f. delicate* acetone extract against *Pseudomonas aeruginosa*](image3.png)

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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* 10.0 mm 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 *Navicula f. delicatula* represented more activity against

### Table 1.

<table>
<thead>
<tr>
<th>Standard antibiotics</th>
<th>Diameter of inhibition zone(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram (+V) bacteria</td>
</tr>
<tr>
<td></td>
<td><em>S.aureus</em></td>
</tr>
<tr>
<td>Erythromycin 20µg/disc</td>
<td>11.5</td>
</tr>
<tr>
<td>Ampicillin 10µg/disc</td>
<td>11.5</td>
</tr>
<tr>
<td>Microalgal sp. Solvent extracts</td>
<td></td>
</tr>
<tr>
<td><em>Phormidium formusum</em> chloroform</td>
<td>12</td>
</tr>
<tr>
<td>acetone</td>
<td>-</td>
</tr>
<tr>
<td>ethanol</td>
<td>5</td>
</tr>
<tr>
<td>methanol</td>
<td>-</td>
</tr>
<tr>
<td>water</td>
<td>19</td>
</tr>
<tr>
<td><em>chlorella marina</em> chloroform</td>
<td>-</td>
</tr>
<tr>
<td>acetone</td>
<td>-</td>
</tr>
<tr>
<td>ethanol</td>
<td>10</td>
</tr>
<tr>
<td>methanol</td>
<td>7</td>
</tr>
<tr>
<td>water</td>
<td>-</td>
</tr>
<tr>
<td><em>Navicula f. delicatula</em> chloroform</td>
<td>-</td>
</tr>
<tr>
<td>acetone</td>
<td>10</td>
</tr>
<tr>
<td>ethanol</td>
<td>-</td>
</tr>
<tr>
<td>methanol</td>
<td>7</td>
</tr>
<tr>
<td>water</td>
<td>-</td>
</tr>
</tbody>
</table>

- = No inhibitory effect; width 1 to 8 mm = week activity; width 8 to 10 mm = moderate activities; width >10 mm = strong activity
Staphylococcus aureus, Micrococcus 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 Nevicula 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 ahirge 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 8th 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 Serratia arcescens 10 mm diameter of inhibition zone during 12th day of incubation at aerated condition in F/2 medium, while the acetone extract for Nevicula f. delicatula more activity against Staphylococcus aureus, Micrococcus Luteus and Pseudomonas aeruginosa. during 14th day of incubation at aerated condition F/2 medium.

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REFERENCES


14. El-Komy et al.: ANTIMICROBIAL ACTIVITY OF THREE MICROALGAE 2757


