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Full Length Research Paper

In vitro screening of antimicrobial activity of extracts of some *macroalgae* collected from Abu-Qir bay Alexandria, Egypt

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Ethanol, methanol and acetone extracts of nine marine *macroalgae* (Rhodophyta, Chlorophyta and Phaeophyta) from Abu-Qir bay (Alexandria, Egypt) were evaluated for antimicrobial activity by agar well diffusion methods against pathogenic microbes (*Bacillus subtilis, Staphylococcus aureus* and *Streptococcus aureus* as gram-positive bacteria, and (*Escherichia coli, Salmonella typhi* and *Klebsiella pneumoniae* as gram-negative bacteria) and one yeast strain *Candida albicans*. All pathogenic microorganisms were obtained from Culture Collection of Botany Department, Faculty of Science, Tanta University. The best results were obtained by acetone extracts with inhibition activity (36.7%), followed by the methanol extracts (32.9%), and then ethanol extracts (30.2%) for all tested microorganisms. The tested species of Chlorophyta were the most active followed by Rhodophyta and Phaeophyta. The most active seaweeds was *Ulva fasciata* (chlorophyceae) against all tested microorganisms.

Key words: Seaweeds, antimicrobial activity, extracts, pathogenic microbes.

INTRODUCTION

Infectious diseases are a major cause of morbidity and mortality worldwide (WHO, 2004). Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-combating strategies to control microbial infections (Sieradzki et al., 1999). Pharmaceutical industries are giving importance to the compounds derived from traditional sources (soil and plants) and less traditional sources like marine organisms (McGee, 2006). The biodiversity of marine ecosystem provides an important source of chemical compounds, which have many therapeutic applications such as antiviral, antibacterial, antifungal and anticancer activities (Caccamese and Azzolina, 1979; Perez et al., 1990; Harada and Kamei, 1997; Siddhanta et al., 1997; Pereira et al., 2004). The ability of seaweeds to produce secondary metabolites of potential interest has been extensively documented (Faulkner, 1993). There are numerous reports of compounds derived from *macroalgae* with a broad range of biological activities, such as antibiotics (antibacterial and antifungal properties), antiviral diseases (Trono, 1999), antitumors and anti-inflammatories (Scheuer, 1990) as well as neurotoxins (Kobashi, 1989). Chemical structure types include sterols (Ahmad et al., 1993), isoprenoids amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid can be counted (Mtolera and Semesi, 1996). The present study was undertaken in order to examine the antimicrobial effects of ethanol, methanol and acetone extracts of 9 marine *macroalgae* species (3 *Rhodophyceae*, 4 *Chlorophyceae* and 2 Phaeophyceae) harvested from area of Abu-Qir, Egypt, South East of the Mediterranean Sea.

MATERIALS AND METHODS

Collection of algae

In this study, 9 species of seaweeds were collected mostly in winter at depth of 1 feet or less for Chlorophyta and 2 - 3 feet for Rhodophyta and Phaeophyta from Rocky Bay of Abu Qir (N 31° 19` E 030° 03`) as shown in Figure 1. All samples were brought to laboratory in plastic bags containing sea water to prevent evaporation.

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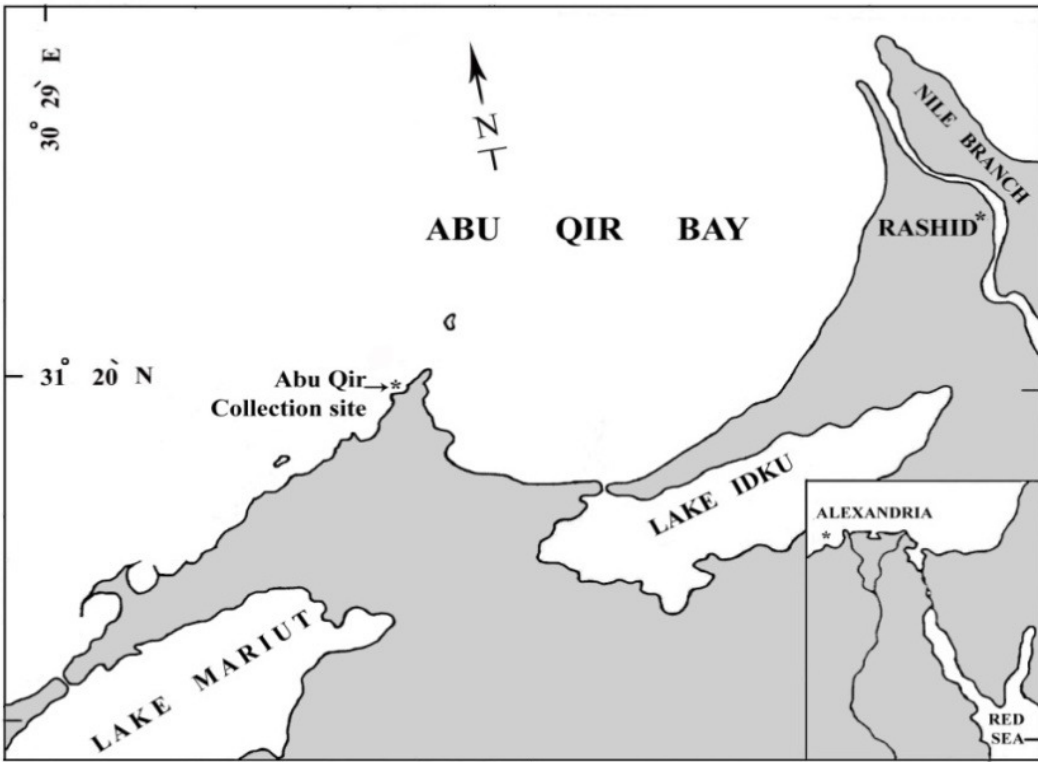

Figure 1. Map showing Rocky Bay of Qir (N 31° 19` E 030° 03`), Egypt, where samples are collected.

The algae then cleaned from epiphytes and rock debris then given a quick fresh water rinse to remove surface salts. Some of the collected seaweeds were preserved for identification. All seaweeds were identified following Abbott and Hollenberg (1976), Taylor (1985) and Aleem (1993). The collected species are the following: *Ulva fasciata* Agardh, *Ulva lactuca* Linnaeus, *Enteromorpha compressa* (Linnaeus) Greville and *Enteromorpha linza* (Linnaeus) Agardh from the Chlorophyta, *Jania rubens* (Linnaeus) Lamouroux, *Corallina elongata* Ellis and Solander and *Pterocladia capillacea* (Gmelin) Bornet *ex* Bornet and Thuret from the Rhodophyta and *Sargassum vulgare* Agardh and *Colpomenia sinuosa* (Mertens ex Roth) Derbes and Solier from the Phaeophyta.

Test micro-organisms

Seven bacterial strains obtained from Culture Collection of Botany Department, Faculty of Science, Tanta University, were tested. They included (*Bacillus subtilis, Staphylococcus aureus* and *Streptococcus aureus*) as gram-positive bacteria, (*Escherichia coli, Salmonella typhi and Klebsiella pneumoniae*) as gram-negative bacteria and one yeast strain *Candida albicans*.

Preparation of the extracts

The clean material was air dried in the shade at room temperature 25 - 30°C on absorbent paper, then grounded to fine powder in an electrical coffee mill. The extraction was carried out with different solvents ethanol 70%, methanol 70% and acetone 70% by soaking the material in the respective solvents (1:15, v/v) on a rotary shaker at 150 rpm at room temperature (25 - 30°C) for 72 h. The extractions with different solvents were carried out individually on samples. The extracts from three consecutive soakings were pooled and filtered using filter paper (Whatman No 4), the obtained filtrate was freed from solvent by evaporation under reduced pressure. The residues (crude extracts) obtained were suspended in the respective solvents to final concentration of 100 mg/ml; the extracts were stored at -20°C in airtight bottle.

Antimicrobial activity test

15 ml of the sterilized media (nutrient agar (CM 3 Oxoid) for bacteria and Sabouraud dextrose agar for yeast) were poured into sterile caped test tubes. Test tubes were allowed to cool to 50°C in a water bath and 0.5 ml of uniform mixture of inocula (10^8 CFU for bacteria and yeast) were added. The tubes were mixed using a vortex mixer vibrating at 1500 - 2000 rounds min^{-1} for 15 - 30 s. Each test tube content was then poured onto a sterile 100 mm diameter Petri dish for solidification (Mtolera and Semesi, 1996).

The antimicrobial activity was evaluated using well-cut diffusion technique (El-Masry et al., 2000). Wells were punched out using a sterile 0.7 cm cork borer in nutrient agar plates inoculated with the test microorganism. About 50 µl of the different algal extract were transferred into each well. For each microorganism, controls were maintained where pure solvents were used instead of the extract. All plates were subjected to 4°C incubation for 2 h. To prevent drying the plates were covered with sterile plastic bags. The plates were later incubated at 37°C for 24 h. (Mtolera and Semesi, 1996). The result was obtained by measuring the inhibition zone diameter for each well and expressed in millimeter. The experiment was

carried out three times and the mean values were recorded.

Statistical analysis

Data were expressed as mean ± SD. A three way analysis of variance was used to analyze data. $P < 0.001$ represented significant difference between means using (SAS, 1997) computer program of biostatistics.

RESULTS

Antimicrobial activities of crude extracts of nine marine seaweeds species represented by three Rhodophyta (*J. rubens, Cor. elongata* and *Pter. capillacea),* four Chlorophyta (*U. fasciata* , *U. lactuca* , *E. compressa* and *E. linza)* and two Phaeophyta (*S. vulgare* and *C. sinuosa)* were examined against seven test microorganisms (*B. subtilis, S. aureus*, *S. aureus*, *E. coli, K. pneumoniae* and *C. albicans*).

As shown in Figure 2 (a to i) the acetone extracts showed the strongest inhibition against the tested microorganisms with inhibition activity (36.7%), followed by the methanol extracts with inhibition activity (32.9%), whereas, the ethanol extracts showed the weakest inhibition with inhibition activity (30.2%) for all microorganisms. The tested species of Chlorophyta exhibited strongest inhibitory effect against all tested microorganisms followed by Rhodophyta and Phaeophyta. In Chlorophyta *the extract of U. fasciata* was the most active followed by E*. compressa*, *U. lactuca* and *E. linza*. On the other hand *K. pneumoniae* was the most sensitive microorganism for the inhibitory action induced by *U. fasciata* and *E. compressa* with inhibition zones of 24.66-18.7 mm, whereas the lowest inhibition zone for *U. fasciata* was against *S. aureus* (19.33 mm) and for *E. compressa*, it was *S. aureus* (15.1 mm). In *U. lactuca* and E*. linza* extract the lowest inhibition zone was against *B. subtilis* (19-17 mm) .The lowest inhibition activity induced by *U. lactuca* was against *E. coli* (15.5 mm) whereas for *E. linza* was against *S. typhi* (13 mm). On the other hand in Rhodophyta *C. elongata* was the most active extract followed by *Pterocladia capillacea* extract. The most sensitive one for *C. elongata*, *P. capillacea* and *J. rubens* was *K. pneumoniae* with inhibition zone of 19.86 - 17 - 17 mm, and the lowest inhibition zone for *C. elongata* occurred in *S. aureus* (14.33 mm), for *P. capillacea* it was *E. coli* (12.33 mm) and for *J. rubens* it was *S. aureus* (12.1 mm). In Phaeophyta *S. vulgare was* the most effective extract than *C. sinuosa* and the highest inhibition activity for *S. vulgare* was shown against *S. aureus* (15.66 mm) and for *C. sinuosa* it was *S. aureus* and *E. coli* (14 mm) and the lowest inhibition zone for *S. vulgare* and *C. sinuosa* was detected in S*. typhi* (11-12 cm).

The statistical analysis confirms that the antimicrobial activity between different seaweeds, microorganisms and solvents were compared with each other and its interaction by three-way ANOVA and was highly significant (Table1).

DISCUSSION

Seaweeds are considered as such a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, anthelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae (Lindequist and Schweder, 2001; Newman et al., 2003).

Lipid-soluble extracts from marine *macroalgae* have been investigated as a source of substances with pharmacological properties. Moreover, several different organic solvents have been used to screening algae for antibacterial activity (Mahasneh et al., 1995; Sukatar et al., 2006)

In the current study, acetone was the best solvent for extracting the bioactive compounds; meanwhile it gave the highest antimicrobial activity against the selected pathogens. This result agreed with those of Wefky and Ghobrial (2008) and Fareed and Khairy (2008). In contrast Tüney et al. (2006) investigated that diethyl ether yields higher antimicrobial activity than methanol, acetone and ethanol for extracting 11 seaweeds species from the coast of Urla.

Methanol extracts ranked the second order sustaining high inhibition zone diameters. On the other hand ethanol extracts of all test *macroalgae* have the lowest antimicrobial activity. This could be probably due to the difference in the solubility of bioactive metabolites in the corresponding solvents. Nevertheless, acetone followed by methanol extracts of most test algae showed high antimicrobial activities. However, Das et al. (2005) examined acetone, ethanol and methanol extracts of other algae and showed moderate to high activity against strains of virulent pathogens; *P. florescence, A. hydrophila, .V anguillarum* and *E. coli.*

In relation to taxonomic groups, Reichelt and Borowitzka (1984) and Salvador et al. (2007), screened many species of algae for their antibacterial activity. They reported that the members of the red algae exhibited high antibacterial activity. In contrast, in our study, green algae (chlorophyceae) were the most active species. The present results are in accordance with those of Kandhasamy and Arunachalam (2008) who reported that green algae (chlorophyceae) were the most active division than others and also agreed with that of Fareed and Khairy (2008), who showed that *U. lactua* (*Chlorophyceae*) were more active than *J. rubens* (*Rhodophyceae*). In our study *U. fasciata* (green algae) was more active than other groups of algae screened for their

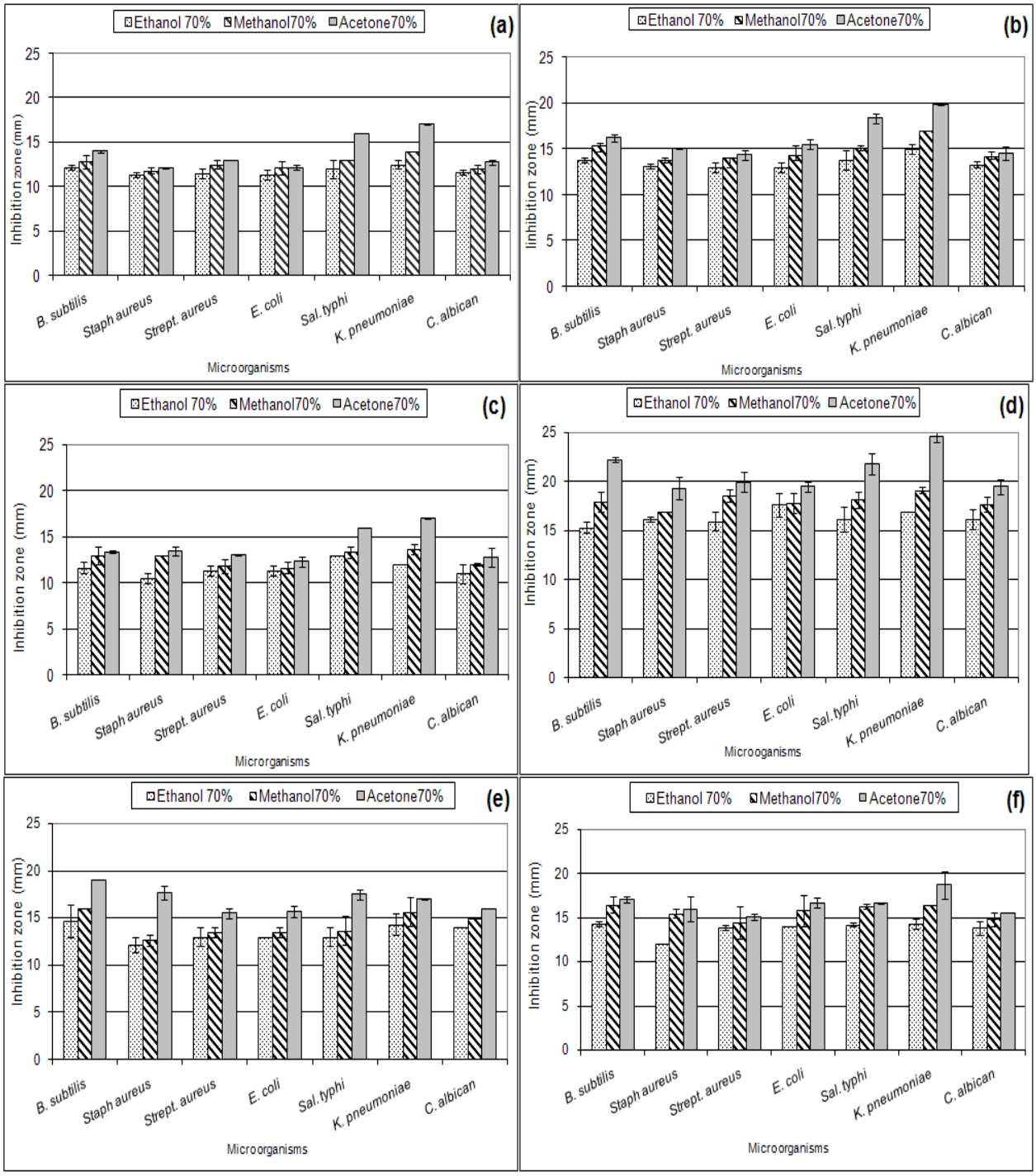

Figure 2. Effect of different solvents on the production of antimicrobial material extracted from different seaweeds. (a) *J rubens;* (b) *Corallina elongate*; (c) *Pterocladia capillacea*; (d) *Ulva fasciata*; (e) *Ulva lactuca*; (f) *Enteromorpha compressa*; (g) *Enteromorpha linza*; (h) *Sargassum vulgare*; and (i) *Colpomenia sinuosa*. Bars represent standard deviation of means(n = 3).

antibacterial activity. *U. fasciata* inhibited the growth of all tested microorganisms and this agreed with Parekh (1978) who reported that the extract of *U. fasciata* was found to inhibit both Gram positive and Gram negative bacteria. Furthermore Selvin and Lipton (2004) reported that the green alga *U. fasciata* exhibited broad spectrum

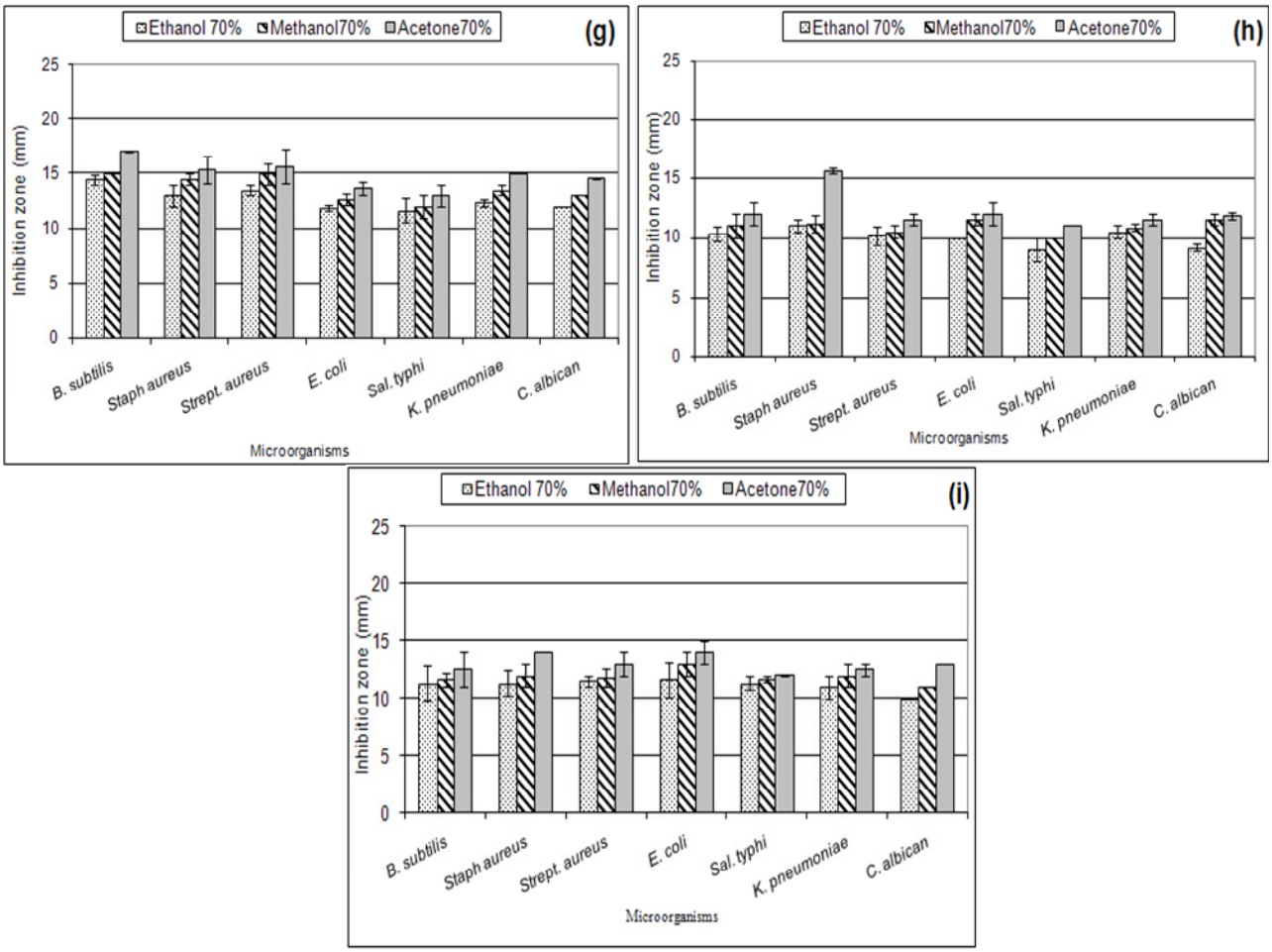

Figure 2. Contd.

Table 1. Three-way analysis of variance (ANOVA) of different seaweeds extracts against different microorganisms using different solvents.

Source	DF	F Value	Pr > F	R^2
Seaweeds	8	648.64	0.0001	95.56%
Microorganisms	6	53.61	0.0001	
Solvents	2	724.15	0.0001	
Seaweeds*Microorganisms	48	12.83	0.0001	
Seaweeds*Solvents	16	10.63	0.0001	
Microorganisms*Solvents	12	8.20	0.0001	

antibacterial activity whereas the red alga *Hypnea musciformis* showed narrow spectrum antibacterial activity. In contrast, Lima-Filho et al. (2002) reported that *U. fasciata* have not any antimicrobial activity against tested organisms. However, the results obtained by the aforementioned authors suggest the production of antimicrobial substances by the same species varies (Pesando, 1990); this remarkable differences may be due to several factors. First of all, this can be because of the intraspecific variability in the production of secondary metabolites, occasionally related to seasonal variations and these variations are seen in other published reports (Moreau et al 1988; Lima-Filho et al., 2002). Secondly, there may also be differences in the capability of the extraction protocols to recover the active metabolites and differences in the assay methods that would result in

different susceptibilities of the target strains (Perez et al., 1990; Gonzalez et al., 2001). Finally there may be difference in the stage of active growth or sexual maturity (Pratt et al., 1951; Chesters and Stott, 1956; Burkholder et al., 1960).

The chemical nature of active principles in lipid-soluble extracts of marine algae is not so far totally identified. Rossel and Srivasta, (1987) associated antibiotic activity from 10 Xantophyta to the presence of unsaturated fatty acids, organic acids and phenol compounds. Our preliminary results suggest that the acetone extract of *U. fasciata* is the most effective extract against Gram-positive, Gram-negative bacteria as well *C. albicans*. This hypothesis will be further investigated. Finally we conclude that *macroalgae* from Abu Qir coast in Alexandria are potential sources of bioactive compounds and the nature of these antibiotics must be investigated.

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