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The Seasonal Fluctuation of the Antimicrobial Activity of Some Macroalgae Collected from Alexandria Coast, Egypt

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1. Introduction

Infectious diseases are a major cause of morbidity and mortality worldwide (WHO 2004). Synthetic drugs are not only expensive but are also often with adulterations and side effects. Therefore, there is a need to search for new strategies to control microbial infections (Sieradzki and Tomasz 1999). Pharmaceutical industries are increasingly recognizing the importance of compounds derived from soil plants and other sources such as marine organisms (McGee 2006).

The biodiversity of the 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 concerning compounds derived from macroalgae with a broad range of biological activities, such as antibiotics (antibacterial and antifungal properties), as well as characteristics pertaining antiviral effects (Trono 1999), antitumors and anti-inflammator mechanisms (Scheuer 1990) as well as protections against neurotoxins (Kobashi 1989).

Transplant experiments suggest that environmental conditions are able to alter the concentrations of secondary metabolites although the types of compounds are genetically fixed (Hay 1996). Also, physical stress such as desiccation, UV and visible light and nutrient availability are able to alter secondary metabolites in seaweeds (Watson and Cruz-Rivera 2003).

Alexandria, Egypt has an extensive coast where seaweeds from virtually all groups are present. In our previous work (Osman *et al.* 2010) we tested in vitro the antimicrobial activity of some seaweed collected from Alexandria coast with special reference to the type of solvent used for extraction. In the present work, we aimed to study the seasonal fluctuation in production of the antimicrobial active substances from the collected seaweeds.

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2. Materials and methods

2.1 Collection of algae

In our study, 13 species of seaweeds (6 Rhodophyceae, 4 Chlorophyceae and 3 Phaeophyceae) were collected seasonally at depths of 0.2 m or less for Chlorophyceae and 1 m for Rhodophyceae and Phaeophyceae from Rocky Bay of Abu Qir (N 31º 19` E 030º 03`) (Plate 1). All samples were brought to the laboratory in plastic bags containing sea water to prevent evaporation. Algae were then cleaned from epiphytes and rock debris and given a quick fresh water rinse to remove surface salts. The collected seaweeds were preserved for identification. All seaweeds were identified following the methods of Abbott and Hollenberg (1976), Taylor (1960), and Aleem (1993). The collected species were identified as *Jania rubens* (Linnaeus) Lamouroux, *Corallina elongata* Ellis and Solander *Pterocladia capillacea* (Gmelin) Bornet *ex* Bornet and Thuret, *Galaxaura fragilis* (Ellis et Solanoer) Lamouroux), *Laurencia obtusa* (Hudson) Lamouroux and *Hypnea valentiae* (Turner)Montagne from Rhodophyceae, *Ulva fasciata* Agardh , *Ulva lactuca* (Linnaeus), *Enteromorpha compressa* (Linnaeus) Greville and *Enteromorpha linza* (Linnaeus) Agardh from Chlorophyceae, and *Sargassum vulgare* Agardh, *Padina pavonica* (Linnaeus) Thivy and *Colpomenia sinuosa* (Mertens ex Roth) Derbes and Solier from Phaeophyceae.

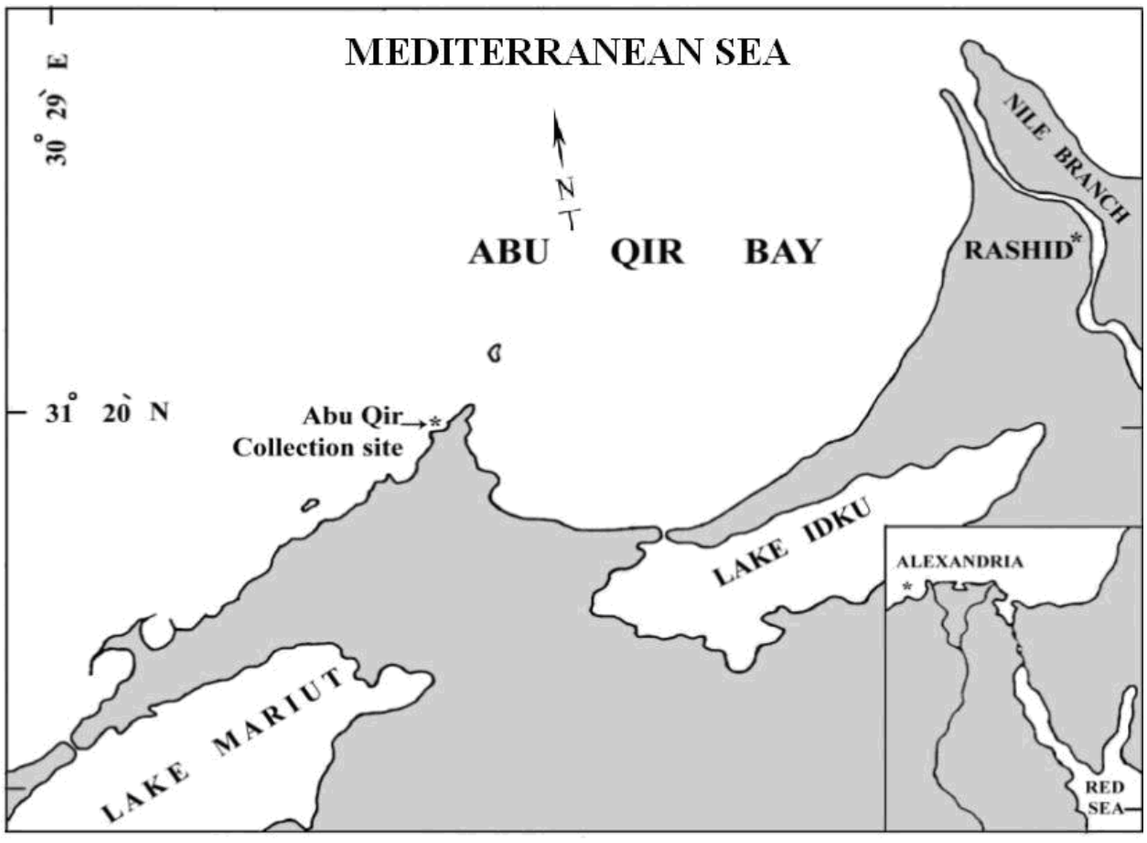

Plate 1. Location of study area

2.2 Test microorganisms

Seven pathogenic microorganisms were isolated from different patients at Tanta university hospital, and primary identified it in microbiology section in Botany Department, Faculty of Science, Tanta University, Egypt. They included (*Bacillus subtilis, Staphylococcus aureus* and *Streptococcus sp.*) as Gram-positive bacteria, (*Escherichia coli, Salmonella typhi and Klebsiella pneumoniae*) as Gram-negative bacteria and one yeast strain *Candida albicans*.

2.3 Determination of the optimum solvent for extraction the antagonistic material from collected seaweeds

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

2.4 Seasonal variation of the antimicrobial activity

To evaluate the possible influence of sampling season on antimicrobial activity, the maximum possible number of different tested seaweeds in each season (winter, spring, summer and autumn) were collected. Seaweeds were collected by hand every 3 months in (viz.,October-2007, January-2008, April-2008 and July-2008), then cleaned, air-dried, ground to a fine powder and extracted for 72 h as previously described using the suitable solvent. Residues were concentrated to 100 mg/ml and stored at -20ºC in an airtight bottle until used. Seasonal variations in air temperature, water temperature and pH value were also measured at the time of each collection.

2.5 Antimicrobial activity test

Fifteen ml of the sterilized media (nutrient agar (Oxoid, Basingstoke, U.K.)) for bacteria and Sabouraud dextrose agar (for yeast) were poured into sterile capped test tubes. Test tubes were allowed to cool to 50°C in a water bath and 0.5 ml of a uniform mixture of inocula (10^8 CFU for bacteria and yeast) were added. Tubes were mixed using a vortex mixer vibrating at 1500-2000 revolutions min^{-1} for 15-30 seconds. Contents from each test tube was then poured into sterile 100 mm diameter Petri dishes for solidification (Mtolera and Semesi 1996).

The antimicrobial activity was evaluated using a well-cut diffusion technique (El-Masry *et al.* 2000*).* Wells were punched out using a sterile 0.7 cm cork borer in suitable media agar plates inoculated with the test microorganism. Approximately 50 µL of various algal extracts 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 hours. To prevent drying, plates were covered with sterile plastic bags and later incubated at 37°C for 24 hours (Mtolera and Semesi 1996). Result was obtained by

measuring the inhibition zone diameter for each well expressed in millimeters. The experiment was carried out three times and mean values were recorded.

Seaweeds		Solvents (70%)	*B. subtilis*	*Staph. aureus*	*Strept. sp*	*E. coli*
Rhodophyta	*J. rubens*	Ethanol	^{a}12.2±0.3	^{a}11.3±0.3	^{a}11.5±0.5	12.0±1.0
		Methanol	^{ab}12.8±0.7	^{ab}11.8±0.3	^{ab}12.5±0.5	14.0±0.0
		Acetone	^{b}14.0±0.1	^{b}12.1±0.1	^{b}13.0±0.0	16.0±0.0
	F-Value		**11.45***	**7.72$^{(n.s)}$**	**10.5***	**36****
	Cor. elongata	Ethanol	^{a}13.8±0.3	13.2±0.3	^{a}13.0±0.0	^{a}13.0±1.0
		Methanol	^{cb}15.3±0.5	13.7±0.2	^{a}14.0±1.0	^{a}14.3±0.5
		Acetone	^{b}16.2±0.3	15.0±0.0	^{a}14.3±0.5	^{b}19.8±0.1
	F-Value		**23.6****	**53.6****	**3.3$^{(n.s)}$**	**88.6****
	Ptero. capillacea	Ethanol	^{a}11.6±0.5	^{a}10.5±0.5	^{a}11.3±0.5	^{a}13.0±0.0
		Methanol	^{a}13.0±1.0	^{ab}13.0±0.0	^{a}11.8±0.7	^{a}13.6±0.5
		Acetone	^{a}13.3±0.2	^{b}13.5±0.5	^{b}13.0±0.0	^{a}17.0±0.0
	F-Value		**5.3$^{(n.s)}$**	**46.5****	**124****	**3.11$^{(n.s)}$**
Chlorophyta	*U. fasciata*	Ethanol	15.3±0.5	^{a}16.2±0.2	^{a}16.0±1.0	^{a}17.6±1.2
		Methanol	18.0±1.0	^{a}17.0±0.0	^{a}18.6±0.6	^{a}19.2±0.3
		Acetone	22.2±0.2	^{b}19.3±1.1	^{a}20.0±1.0	^{b}24.6±0.5
	F-Value		**78.02****	**16.8***	**6.8$^{(n.s)}$**	**69.8****
	U. lactuca	Ethanol	^{a}14.6±1.7	^{a}12.2±0.7	^{a}13.0±1.0	^{a}13.0±0.0
		Methanol	^{a}16.0±0.0	^{a}12.6±0.5	^{a}13.5±0.5	^{a}13.5±0.5
		Acetone	^{b}19.0±0.0	^{b}17.6±0.7	^{b}15.5±0.5	^{b}15.6±0.5
	F-Value		**14.4****	**55.5****	**10.5***	**31****
	E. compressa	Ethanol	^{a}14.3±0.3	^{a}12.0±0.0	^{a}13.5±0.3	^{a}14.6±0.5
		Methanol	^{cb}16.5±0.8	^{cb}15.5±0.5	^{a}14 ±1.8	^{a}16.5±0.0
		Acetone	^{c}17.1±0.3	^{c}16.0±1.4	^{b}16.6±0.5	^{a}16.6±0.5
	F-Value		**19.8****	**34.2****	**93****	**4.04$^{(n.s)}$**
	E. linza	Ethanol	^{a}14.0 ±1.7	^{a}13.0±1.0	^{a}13.5±0.5	^{a}11.8±0.3
		Methanol	^{a}15.0±0.0	^{a}14.5±0.5	^{a}15.0±1.0	^{ab}12.6±0.5
		Acetone	^{a}17.0±0.0	^{a}15.3±1.2	^{a}15.6±1.5	^{a}13.6±0.5
	F-Value		**99999****	**4.44$^{(n.s)}$**	**3.09$^{(n.s)}$**	**10.1***
Phaeophyceae	*Col. sinuosa*	Ethanol	^{a}11.3±1.5	^{a}11.0±1.1	^{a}11.5±0.5	^{a}11.6±1.5
		Methanol	^{a}11.6±0.5	^{ab}12.0±1.0	^{a}11.8±0.7	^{a}13.0±1.0
		Acetone	^{a}12.5±1.5	^{b}14.0±0.0	^{a}13.0±1.0	^{a}14.0±1.0
	F-Value		**0.6$^{(n.s)}$**	**10.50***	**3.05$^{(n.s)}$**	**2.85$^{(n.s)}$**
	Sar. vulgare	Ethanol	^{a}10.3 ±0.5	^{a}11.0±0.5	^{a}10.2±0.7	^{a}10.0±0.0
		Methanol	^{a}11.0±1.0	^{a}11.2±0.7	^{a}10.5±0.5	^{ab}11±0.5
		Acetone	^{a}12.0±1.0	^{b}15.6±0.3	^{a}11.5±0.5	^{b}12.0±1.0
	F-Value		**2.7$^{(n.s)}$**	**68.8****	**4$^{(n.s)}$**	**9***

Means with the same letter are insignificant using one way analysis of variance (ANOVA).
* Significant at P ≤ 0. 01 ,** Significant at P ≤ 0. 001 and (ns) Non-significant at P ≤ 0. 01 using one way analysis of variance (ANOVA).

Table 1. Antimicrobial activity of different seaweeds extracted with different solvents. (±) standard deviation of the means (n=3)

2.6 Purification of the most active crude extract

The most active crude extract was partially purified using the TLC technique with glass plates (20x20 cm). The flow rate of the active material was determined using different eluent systems. The elution of the active material was made using of the following eluents.

1. Ethyl acetate: methanol: hexane (2:1.5:0.5 v/v) (Wagner and Bladt 1996)
2. Hexane: ethyl acetate (3:2 v/v) (Wendy and Diana 2007)
3. Benzene: ethyl acetate (3:1 v/v) (Sastry and Rao 1995)
4. Butanol: acetic acid: water (4:1:5 v/v) (Partridge 1948)

The developed spot was scrapped off and dissolved in pure acetone. The solution was centrifuged to remove the silica gel and the supernatant was dried under reduced pressure to dryness and stored at 4◦C. The developed spot was examined for the antimicrobial activity using well-cut diffusion technique.

2.7 Statistical analysis

Results are presented as mean ± SD (standard deviation) for three replicates. The statistical analyses were carried out using SAS programming (1989-1996) version 6.12. Data obtained were analyzed statistically to determine the degree of significance between treatments using one, two, and three way analysis of variance (ANOVA) at $P \leq 0.01$ and $P \leq 0.001$ levels of significance.

3. Results

Results of the antimicrobial activity of different organic crude extracts are summarized in Table 1. Acetone (70%) extracts showed the strongest inhibitory effect against the tested microorganisms relative to other solvents with inhibition activity percentage* on 36.7%, followed by 70% methanol extracts with inhibition activity percentage 32.9%, whereas, 70% ethanol extracts showed the weakest inhibition with inhibition activity percentage 30.2% of all tested microorganisms.

$$\text{*Inhibition activity percentage} = \frac{\text{Average diameter of inhibition zone of each solvent}}{\text{Average diameter of inhibition zone of all solvents}} \text{ X } 100$$

The statistical analyses using one way ANOVA confirm that the effect of antimicrobial activities for most treatments were significant (Table 1). Three-way ANOVA confirmed that the variation in the antimicrobial activity in relation to seaweeds, microorganisms, solvents and their interactions were significant at $P \leq 0.001$ (Table 2).

Source	DF	F Value	P-value	R2
Seaweeds	8	325.3	0.0001	
Microorganisms	6	20.4	0.0001	
Solvents	2	387.2	0.0001	
Seaweeds*Microorganisms	48	6.7	0.0001	95.5%
Seaweeds*Solvents	16	4.5	0.0001	
Microorganisms*Solvents	12	3.9	0.0001	
Microorganisms*Solvents* Seaweeds	96	1.73	0.0002	

Table 2. Three-way analysis of variance (ANOVA) of different seaweeds extracts against different microorganisms using different solvents.

The results for the acetone extracts from each season are summarized in Figure 1, 2, 3 and 4. However, data of environmental parameters are reported in Table 3.

Environmental Parameters	Seasons			
	Autumn (2007)	**Winter (2008)**	**Spring (2008)**	**Summer (2008)**
Air temperature in ◦C	26.3	19	24	31.3
Water temperature in ◦C	20.4	14	14	21.5
pH value	8.0	7.9	7.5	8.1

Table 3. Environmental parameters of the sampling site in each collection time.

In autumn (October, 2007) four seaweeds samples were collected (2 Rhodophyceae *and* 2 Chlorophyceae). The results show that the red seaweeds extract exhibited stronger antimicrobial activity than the green. For red seaweeds, *Cor. elongata* was most active and exhibited the most inhibition *for K. pneumoniae with* an inhibition zone of 15.7 mm. With respect to green seaweeds, *U. fasciata* showed the strongest activity which exhibited most inhibition for *Strept. sp* with inhibition zone of 15 mm (Fig. 1).

In winter (January, 2008) five samples of seaweeds were collected (3 Rhodophyceae and 2 Chlorophyceae). Results demonstrated that extract of green seaweeds was more active than red seaweeds. The highest antimicrobial activity of the collected green seaweeds species was observed in *U. fasciata,* which had the strongest inhibition against *K. pneumoniae* with an inhibition zone diameter of 24.6 mm. *However, the red seaweeds, Cor. elongate* showed the highest antimicrobial activity than others. *The most sensitive microorganism for Cor. elongate was K. pneumoniae* showing an inhibition zone of 19.8 mm *(Fig*. 2)

In spring (April, 2008) nine seaweeds samples were collected (3 Rhodophyceae, 4 Chlorophyceae and 2 Phaeophyceae). The obtained data show that the extract of tested red seaweeds was more active than green and brown seaweeds, respectively. The most active specie was *Cor. elongata. Sensitivity responses showed that K. pneumoniae* exhibited the *highest sensitivity for Cor. elongata* extract *with* an inhibition zone of 21.6 mm. Among the green seaweeds, obtained results show that *U. fasciata* has the strongest antimicrobial action. *K. pneumoniae* exhibited *higher sensitivity for U. fasciata* with an inhibition zone of 17.2 mm. Concerning brown seaweeds, results show that *Sar. vulgare extract showed higher antimicrobial activity* than *Col. sinuosa*, where *Staph. aureus* exhibited *higher sensitivity for Sar. vulgare* with an inhibition zone of 15.6 mm. (Fig. 3)

In the summer (June, 2008) ten seaweeds were collected (5 Rhodophyceae, 3 Chlorophyceae and 2 Phaeophyceae). The extracts of collected green seaweeds exhibited the strongest antimicrobial activity followed by red and brown species. The results show that the most active one was that from *U. fasciata*. The most sensitive microorganism for *U. fasciata* extract was *Strept. sp.* with an inhibition zone of 14.5 mm. With regard to red seaweeds, the obtained data show that the most active one was *Gal. fragilis*; however, *Can. albicans* exhibited *higher sensitivity for Gal. fragilis* with an inhibition zone of 12.0 mm. Concerning brown seaweeds, the obtained results showed that *P. pavonia was the strongest* antimicrobial activity which exhibited the highest inhibitory effect against *Sal. typhi* with an inhibition zone of 12 mm. (Fig. 4). The statistical analyses using two-way ANOVA confirmed that the variation in antimicrobial activities in relation to seaweeds, microorganisms and their interactions were significant at Pr≥0.001 for all treatments (Table 4).

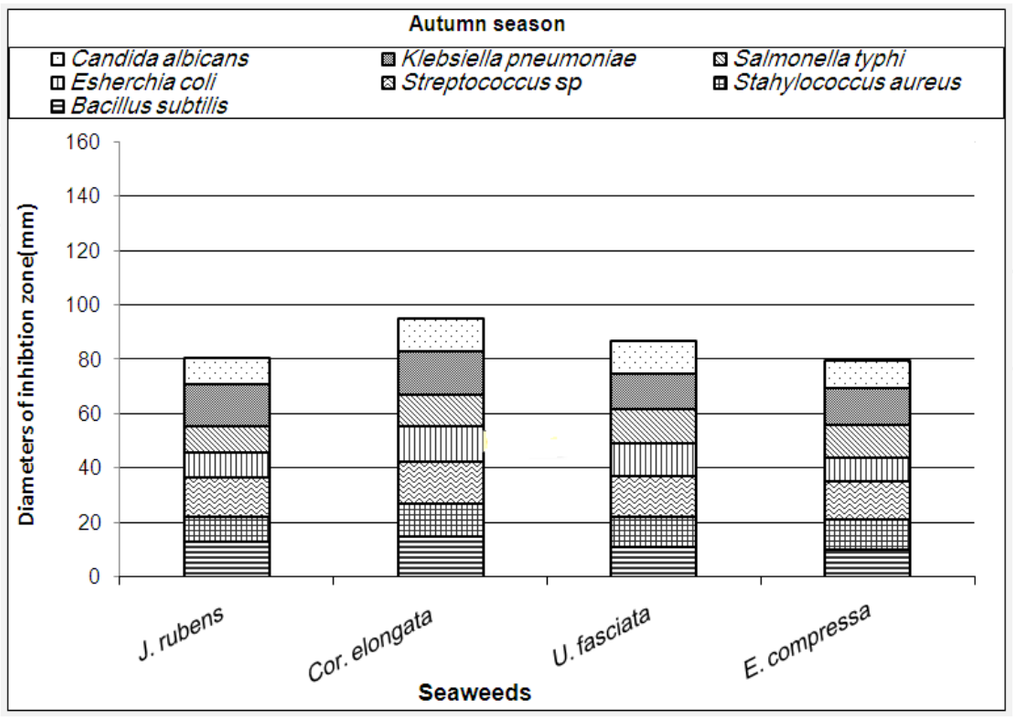

Fig. 1. Diameter of inhibition zone of autumn collected seaweeds against each tested microorganism.

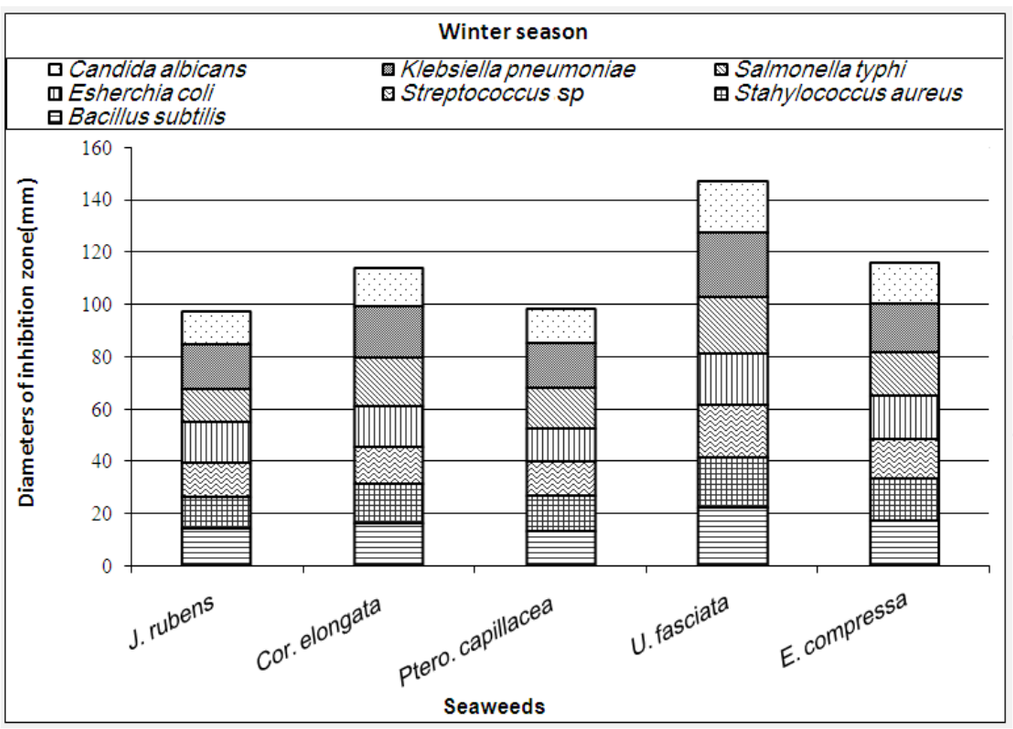

Fig. 2. Diameter of inhibition zone of winter collected seaweeds against each tested microorganism.

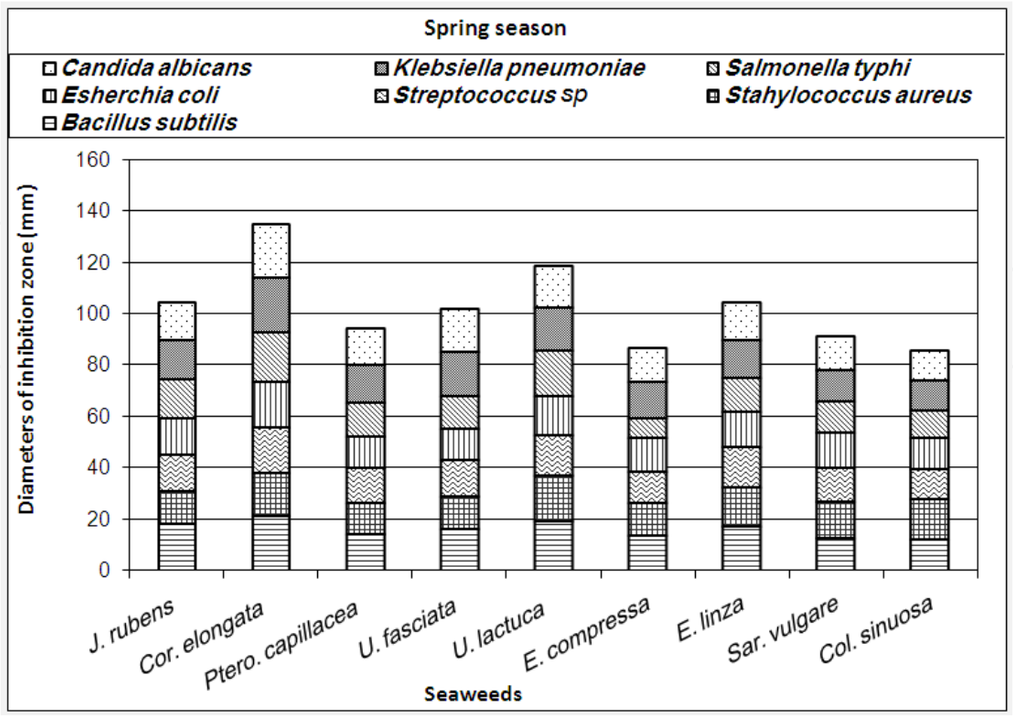

Fig. 3. Diameter of inhibition zone of spring collected seaweeds against each tested microorganism.

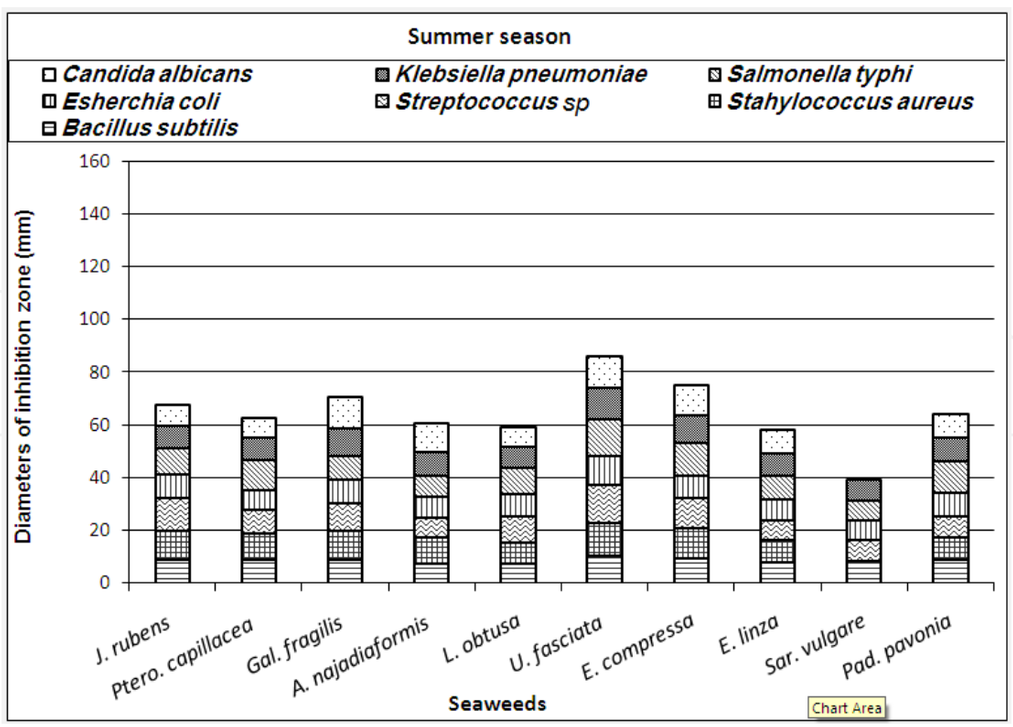

Fig. 4. Diameter of inhibition zone of summer collected seaweeds against each tested microorganism.

Seasons	Source	DF	F Value	P-value	R2
Autumn	Seaweeds	3	44.1	0.0001	92.7%
	Microorganisms	6	72.1	0.0001	
	Seaweeds*Microorganisms	18	8.3	0.0001	
Winter	Seaweeds	4	528.9	0.0001	97.7%
	Microorganisms	6	112.7	0.0001	
	Seaweeds*Microorganisms	24	9.3	0.0001	
Spring	Seaweeds	8	145.3	0.0001	93.0%
	Microorganisms	6	26.0	0.0001	
	Seaweeds*Microorganisms	48	7.7	0.0001	
Summer	Seaweeds	9	206.3	0.0001	96.2%
	Microorganisms	6	42.1	0.0001	
	Seaweeds*Microorganisms	54	26.8	0.0001	

Table 4. Two-way analysis of variance (ANOVA) of seasonal collected seaweeds extracts against different microorganisms

3.1 Seasonal variation of antimicrobial activity

Species of green and red seaweeds were found and collected in four seasons whereas brown seaweeds were collected only in spring and summer. The highest activity of the different seaweeds extracts were those collected in spring, followed by winter, summer and autumn, respectively (Fig. 5). According to the taxonomic group level, the most active extracts were

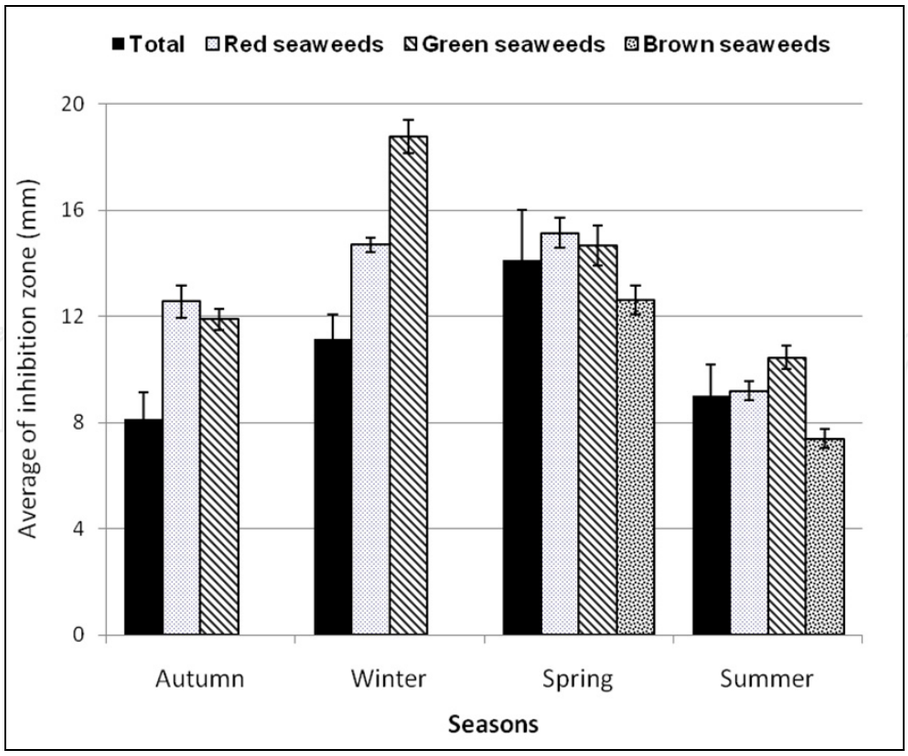

Fig. 5. Seasonal variation of antimicrobial activity of different seaweeds extracts.

the green seaweeds. The antimicrobial activity of the different species with respect to different seasons could be arranged in the following order, green seaweeds in winter > spring > autumn > summer followed by red seaweeds in spring > winter > autumn > summer and brown seaweeds in spring > summer. The above-mentioned results indicate that the promising seaweed for production of antimicrobial antagonistic material was winter-collected *U. fasciata* (Chlorophyceae).

The suitable solvent system used in the TLC technique was ethyl acetate: methanol: hexane (2:1.5:0.5 v/v). The obtained result show that there are three spots formed with different R_f values (0.5, 0.6, 0.6 and 0.7). All spots were examined against *K. pneumoniae* and the most active one had an R_f value of 0.53 with an inhibition zone of 30 mm. This spot is currently undergoing further analysis to determine the nature and identify of the active constituents.

The statistical analyses conducted using three-way ANOVA confirmed that the effects of different seasons, seaweeds, microorganisms and their interactions on antimicrobial activity were highly significant.

4. Discussion

The present study showed that 70% acetone could be considered a good solvent for extracting the bioactive substance in the studied seaweeds against the tested microorganisms. However, Tüney *et al.* (2006) reported that diethyl ether was the best solvent for extracting the bioactive compounds of 11 seaweeds species from the coast of Urla, which agreed with Wefky and Ghobrial (2008) and Fareed and Khairy (2008).

Macroalgae are already well-documented as possess antibacterial activities against pathogenic bacteria (Kumar and Rengasamy 2000; Lipton 2004; Tüney *et al.* 2006; Karabay-Yavasoglu *et al.* 2007; Salvador *et al.* 2007; Chiheb *et al.* 2009). The results reported by the above-mentioned authors are in accordance with our data, which demonstrated that the collected seaweeds have antimicrobial activity against the tested microorganisms. In contrast to our results, Salvador *et al.* (2007) detected that some seaweeds such as *Ptero. capillacea* showed no antimicrobial activity in any seasons. Gonzalez del Val (2001) also demonstrated that the extract of *Enter. compressa* showed no antimicrobial activity against the tested microorganisms. Perez *et al*. (1990) observed that the extract of *U. lactuca* had no antibacterial activity. These differences in activity may be due to different seaweeds developmental stages, locality and extraction methods, *etc.*

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 family exhibited high antibacterial activity. In contrast, in our study, green algae (Chlorophyceae) were the most active species. The present results agreed with the results of Kandhasamy and Arunachalam (2008) who reported that green algae (Chlorophyceae) were more active taxa than others and also agreed with Fareed and Khairy (2008) who showed that *U. lactua* (Chlorophyceae) were more active when compared with *J. rubens* (Rhodophyceae).

Some pure compounds from algae have been identified as natural antimicrobial; however, the relationship between their ecologic role and their antimicrobial activity is not fully understood in many studies which were based on the screening of antimicrobial activities

from macroalgae. These studies determined that the range of chemical defenses can differ from narrow to broad spectrum, depending on the extraction method, the algae species, the collected season of the algae, algal growth phases, *etc.* The variation in the production of secondary metabolites has been reported for a variety of marine algae (Hay 1996). In the present study, we focused on the possibility that antimicrobial activity will fluctuate seasonally. Abu Qir (Alexandria, Egypt) was chosen as a sample site, so geographical and spatial variation was eliminated.

As regards seasonal variation of bioactivity, for all of the tested subdivisions, spring was the season with the highest activity against test microorganisms, followed by winter. These results are in accordance with those obtained from Atlantic samples by Hornsey and Hide (1974), from Mediterranean samples by Khaleafa *et al.* (1975) and Stirk and Reinecke (2007) who reported that seasonal variation in antibacterial activity was observed with extracts which have antibacterial activity in late winter and early spring. This is in contrast to studies carried out by Rao and Parekh (1981), and Arun, Kumar and Rengasamy (2000) using Indian samples, and from Mediterranean samples by Martí *et al.* (2004) who demonstrated the most active season was autumn. Salvador *et al.* (2007), nevertheless, demonstrated that autumn and spring were the seasons with the highest percentage of active taxa against at least one test microorganism (69% and 67% respectively), followed by winter (56%) and summer (50%).

It is worthy to mention that Abu Qir Bay is a very important productive area of the Mediterranean Sea on the Egyptian coast, since it receives nutrient-rich brackish water from Lake Edku as well as the El-Tabia pump station. The obtained data in this study demonstrated that environmental parameters (air and water temperature and pH) showed insignificant correlations with antimicrobial activities of tested seaweeds. Moreover, Shams El-Din *et al.* (2007) studied the nutrient concentration in Abu Qir and found that the correlation coefficient between nutrients and the natural components in some seaweeds were not significant, which may be due to the water deterioration, resulting from the acute eutrophication and the increase of pollution stress in the bay.

In the present study *U. fasciata* (green seaweeds) was the most effective seaweeds species, having antibacterial activity throughout the year compared to other seaweeds screened for antibacterial activity. *UIva fasciata* inhibited the growth of all tested microorganisms, which agreed with Selvin and Lipton (2004) reported that the green alga *U. fasciata* exhibited broad-spectrum antibacterial activity.

These results show that *U. fasciata* extracts of the winter collection exhibited stronger antimicrobial effects followed by spring season (more so than summer or autumn) which agrees with Stirk and Reinecke (2007) who demonstrated that *U. fasciata* collected in winter and spring seasons were more active against tested organisms when compared to other seasons. This may be influenced by the seasonal variation as extracts of *U. fasciata* from winter and spring collection were more potent as compared to the summer and autumn collection, the former representing the peak growing and reproductive season, while the later is the stasis and senescence period for *U. fasciata* growth. The better antimicrobial action of winter collection is possibly due to the elevated biochemical constituents during the growing and reproductive phase of the *U. fasciata*. This hypothesis is further strengthened by Hornsey and Hide (1974), Daly and Prince (1981), Moreau *et al.* (1984) and Rao and Indusekhar (1989)

Finally, we conclude that macroalgae from Abu Qir coast in Alexandria are potential sources of bioactive compounds. The production of these compounds could be affected by seasonal variation and should be investigated for natural antimicrobial properties. Furthermore, *U. fasciata* collected in the winter could be considered the most active species for production of antagonistic materials. Thus, the suitable season for collection of seaweeds producing antimicrobial activity must be taken in consideration.

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