

Full Length Research Paper

Production and characterization of antimicrobial active substance from some macroalgae collected from Abu-Qir bay (Alexandria) Egypt

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Accepted 5 May, 2011

The antimicrobial activity of three different macroalgal species [*Jania rubens* (Linnaeus) Lamouroux; *Ulva fasciata* Delile and *Sargassum vulgare* C. Agardh] belonging to Rhodophyta, Chlorophyta and Phaeophyceae, respectively, were collected seasonally in 2007 to 2008 from Abu-Qir bay (Alexandria, Egypt). The different macroalgal species were tested against pathogenic microbes such as *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*. The influence of sampling season on the antimicrobial activity of the collected seaweeds showed strong activity in spring followed by winter, summer and autumn, respectively. However, the strongest antimicrobial activity was recorded in 70% acetone extract of *U. fasciata* collected during winter against all the tested microorganisms. This extract was purified using column chromatography (CC) and thin layer chromatography (TLC). The nature of this purified antimicrobial material was detected using different chemical analysis (UV, IR, ¹H NMR and MS) which indicated that it is an aromatic compound and has different active groups (-NH₂, -C=O, -NO₂, phenyl ring and -CH₃). The molecular weight of the compound was determined (662) and its structure was characterized as a derivative of phthalate ester [(E)-1-(10-acetamido-2-nitrodec-9-enyl) 2-(10-acetamido-2-nitrodecyl) 4-methylphthalate]. This is the first evidence of the isolation of phthalate esters derivative from green seaweeds (*U. fasciata*) that has broad antimicrobial activity.

Key words: Antimicrobial, pathogenic microbes, season, seaweeds.

INTRODUCTION

Infectious diseases are a major cause of morbidity and mortality worldwide (WHO, 2004). The increase in failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio 1996; Cordell 2000; Scazzocchio et al., 2001). 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-fighting strategies to control microbial infections (Sieradzki and Tomasz, 1999). There are numerous reports of compounds derived from macroalgae with a broad range of biological activities, such as the anti-microbial activities (Reichelt and Borowitzka, 1984; Ballantine et al., 1987; Ballesteros et al., 1992; Vlachos et al., 1996), antiviral diseases (Trono, 1999), antitumors and anti-inflammatory (Scheuer, 1990) as well as neurotoxins (Kobashi, 1989). Subsequent chemical investigations of bioactive extracts led to the discovery of many struc-

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Figure 1. Map of Abu Qir showing collection site.

turally diverse antimicrobial metabolites from marine plants (Blunt et al., 2003, 2004, 2005; Faulkner, 2002). While, marine plant's metabolites have been studied extensively for their biomedical potential, their activities against human pathogens provide little information about their ecological role in antimicrobial activities and chemical structure of these antimicrobial compounds.

Alexandria has an extensive coast where, seaweeds from virtually all groups are present. The aims of this work were the search of novel compounds of potential antimicrobial value extracted from seaweeds, the study of the effect of seasonal variation on antimicrobial production, purification and elucidation of the structure of the antimicrobial compounds.

MATERIALS AND METHODS

Collection of algae

Three species of seaweeds from different divisions (*Jania rubens*, from Rhodophyta, *Ulva fasciata* from Chlorophyta and *Sargassum vulgare* from Phaeophyceae) were collected seasonally by hand in 2007 to 2008 from Rocky Bay of Abu Qir (N 31°19' E 030°03') (Figure 1). All samples were brought to the laboratory in plastic bags containing sea water to prevent evaporation. The algae were cleaned from epiphytes and rock debris and were given a quick fresh water rinse to remove surface salts. After collection, the samples were cleaned, air dried in the shade at room temperature

(25 to 30°C) in the dark on absorbent paper and grounded to fine powder in an electrical coffee mill. The specimen from the collected seaweeds was preserved for identification and all the seaweeds were identified following Abbott and Hollenberg (1976) and Taylor (1985) and Aleem (1993).

Tested micro-organisms

Seven bacterial strains were obtained from the Culture Collection of Botany Department, Faculty of Science, Tanta University. 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*) as yeast.

Preparation of the extracts

The extraction was carried out with 70% acetone. The extraction was carried out by soaking the dried materials in 70% acetone (1:15 v/v) on a rotary shaker at 150 rpm at room temperature (25 to 30°C) for 72 h. 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 resuspended in 70% acetone to a final concentration of 100 mg/ml and then stored at -20°C in airtight bottle.

Antimicrobial activity test

15 ml of the sterilized media (nutrient agar (Oxoid) 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 uniform mixture of inocula (10^8 CFU for bacteria and yeast) were added. The tubes were mixed using a vortex mixer vibrating at 1500 to 2000 rounds min^{-1} for 15 to 30 s. Each test tube's contents were 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 microorganisms. About 50 μl of the different algal extracts were transferred into each well. For each microorganism, controls were maintained where pure solvent was used instead of the extract. All the plates were incubated at 4°C for 2 h to slow the growth of microorganisms and give suitable time for the antimicrobial agent to diffuse. To prevent drying, the plates were covered with sterile plastic bags and incubated at 37°C for 24 h. (Mtolera and Semesi, 1996). The result was obtained by measuring the diameter of the inhibition zone for each well, and expressed as millimeter.

Statistical analysis

The results are presented as mean \pm standard deviation of the mean ($n = 3$). The statistical analyses were carried out using SAS program (1989 to 1996) version 6.12. Data obtained were analyzed statistically to determine the degree of significance between treatments using one and three way analysis of variance (ANOVA) at $P \leq 0.01$ and $P \leq 0.001$ levels of significance.

Column chromatography

Selected active crude extracts (2 g) were fractionated by column chromatography on silica gel (EDWC, 60-120 mesh). Column (2 cm \times 40 cm) was set up in benzene with silica gel (30 to 40 g) and eluted with gradients of solvents from 10:1% of benzene: acetone to 1:10% benzene: acetone (Solomon and Santhi, 2008). The collected fractions were evaporated to dryness with a rotary evaporator and then, the dried samples were dissolved in pure acetone and assayed for their antimicrobial activity. The maximum absorption of the active fractions was measured by spectrophotometer (UV 2101/ pc) using quartz cuvette containing the different fractions. Different active fractions with same absorption maximum were pooled together (Solomon and Santhi, 2008). The active fractions were tested for purity using TLC (thin layer chromatography). The purified fraction was lyophilized and subjected to the following analyses in order to reveal its structure as far as possible:

UV-spectra

The UV-spectra of the tested material were determined using UV2101/pc spectrophotometer. The wavelength ranged from 200 to 800 nm.

The infrared spectra (IR)

Using Perkin-Elmer 1430 infrared spectrophotometer, the molecular structure of the antimicrobial material was partially identified. Since the antimicrobial material is liquid at room temperature, so it can be examined directly as a thin film, "neat", between two clean and transparent NaCl plates. The measurements were carried out at infra red spectra between 400 to 4000 nm.

Nuclear magnetic resonance (^1H NMR) spectra

The sample was dissolved in Dimethyl- d^6 sulfoxide (d^6 DMSO). The different functional groups were identified using NMR (JNMPMX60SI).

Mass spectra (MS)

A mass spectrophotometer (MS-5988) was used. The product was subjected to a steam of high energy of electrons at elevated temperature up to 100°C. The cleavage fragments were yielded which were characterized by mass/charge from mass spectra data.

RESULTS

J. rubens and *U. fasciata* were present in all seasons, whereas, *S. vulgare* was present in spring and summer only. This indicated that a certain level of temperature is required for these species to grow in a massive quantity to facilitate the collection procedures.

Concerning the antimicrobial activities of the different seaweeds collected in the various seasons, the results in Table 1 showed that *U. fasciata* showed stronger activity than *J. rubens* in autumn. *S. aureus* was the most sensitive microorganism to *U. fasciata* extract. However, *K. pneumoniae* was the most sensitive microorganism to *J. rubens* extract.

In winter, the extract of *U. fasciata* also was more active than *J. rubens*, where *K. pneumoniae* was the most sensitive tested microorganisms for *U. fasciata* and *J. rubens* extracts. In spring, the extract of *J. rubens* was more active than *U. fasciata* and *S. vulgare*. With regard to *J. rubens*, it showed high antimicrobial activity against *B. subtilis* whereas, *K. pneumoniae* and *S. aureus* were the most sensitive to *U. fasciata* and *S. vulgare* extracts, respectively.

In summer, the extract of *U. fasciata* exhibited stronger antimicrobial activity than *J. rubens* and *S. vulgare*, respectively. The results showed that the extracts of *U. fasciata* inhibited all the tested microorganisms and *S. aureus* was the most sensitive microorganism to *U. fasciata* and *J. rubens* extracts. However, *B. subtilis* and *K. pneumoniae* exhibited higher activity for *S. vulgare*.

The obtained results show that the highest activity of the different seaweeds extracts were those collected in spring followed by winter, summer and autumn, respectively (Figure 2). The antimicrobial activity of the different species with respect to the different seasons could be arranged in the following order, *U. fasciata* in winter > spring > autumn > summer followed by *J. rubens* in spring > winter > autumn > summer and *S. vulgare* in spring > summer. The stated results indicated that the promising seaweeds for the production of the antimicrobial material was *U. fasciata* (Chlorophyta) that was collected in winter season against all the tested microorganisms. Therefore, we selected this species for further investigation.

The statistical analysis using three-way ANOVA confirmed that the variation in antimicrobial activities in rela-

Table 1. The antimicrobial activity of 70% acetone extract of seaweeds from different seasons against different tested microorganisms, measured as diameter of inhibition zone (mm).

Season	Microorganism	<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>K. pneumoniae</i>	<i>C. albicans</i>
	Seaweed	Diameter of inhibition zone (mm)						
Autumn	<i>J. rubens</i>	13±1	9±0	14.5±0.5	9.5±0.5	9.5±0.5	15.2±1.2	10±0
	<i>U. fasciata</i>	11±0	11±1	15±0	12±1	12±0.5	13.5±0.5	12±0
	<i>S. vulgare</i>	N.P.	N.P	N.P	N.P	N.P	N.P	N.P
Winter	<i>J. rubens</i>	14±0.1	12.1±0.1	13±0	16±0	12.2±0.7	17±0	12.7±0.2
	<i>U. fasciata</i>	22.2±0.2	19.3±1.2	20±1	19.5±0.5	21.8±1.0	24.6±0.5	19.5±0.7
	<i>S. vulgare</i>	N.P.	N.P	N.P	N.P	N.P	N.P	N.P
Spring	<i>J. rubens</i>	18±1	12.7±0.7	14±0	14.3±0.5	15±1	15.2±1.2	14.7±0.2
	<i>U. fasciata</i>	15.7±0.2	12.8±2.0	14.3±1.5	12.2±0.2	12.6±0.5	17.25±2.25	16.5±0.5
	<i>S. vulgare</i>	12±1	15.6±0.3	11.5±0.5	12±1	11±0	11.5±0.5	11.8±0.3
Summer	<i>J. rubens</i>	9.1±1.0	10.5±0.5	12.5±0	9±1	10±0	8.5 ± 0.5	8.2±0.2
	<i>U. fasciata</i>	10.6±0.5	12±1	14.5±1.5	11±1	14±1	12 ± 0.2	12±0
	<i>S. vulgare</i>	8.3±0.7	0±0	8±0	7.5±0	7.5±0.5	8.2 ± 0.7	0±0

N.P. = Not present; (±) standard deviation of the mean (n=3).

tion to seasons, seaweeds and microorganisms, and their interaction on antimicrobial activity was significant at $P \leq 0.001$ for all the treatments.

Purification and characterization of antimicrobial crude extract

Column chromatography

The results showed that 27 fractions were collected and only first four fractions had antimicrobial activity (Table 2). The UV absorption spectrums of these fractions were determined using spectrophotometer (UV 2101/ pc) at range of 200 to 800 nm. The obtained results are shown in Figure 3. The results indicated that the four fractions had

the same absorptions peaks (three absorptions peaks at 333, 405 and 664 nm). Therefore, they pooled together. Thereafter, the different fractions were exposed to purity test using TLC technique (thin layer chromatography).

Thin layer chromatography (TLC)

The result showed that the examined four active fractions had approximately the same R_f value (Retention factor) and the statistical analysis using one-way ANOVA confirmed that the difference of R_f value of the four fractions compared with all the fractions sample was non-significant at $P \geq 0.01$ (Table 3). Therefore, from UV analysis and TLC technique, the four active fractions were pooled together.

Elucidation of the chemical structure of the purified material isolated from 70% acetone of winter collected *U. fasciata* extract

UV spectra of the antimicrobial material

Before measuring the UV spectrum, the different pigments and impurities were removed by filtration using charcoal. Then, the UV spectrum of the purified antimicrobial material isolated from *U. fasciata* was carried out in pure acetone. This spectrum showed one absorption peak at 333 nm, indicating the presence of an aromatic compound (Figure 4).

The obtained compound was examined for antimicrobial activity. The results showed that the compound still had antimicrobial activity indicating that the compounds which had peak at 405 and

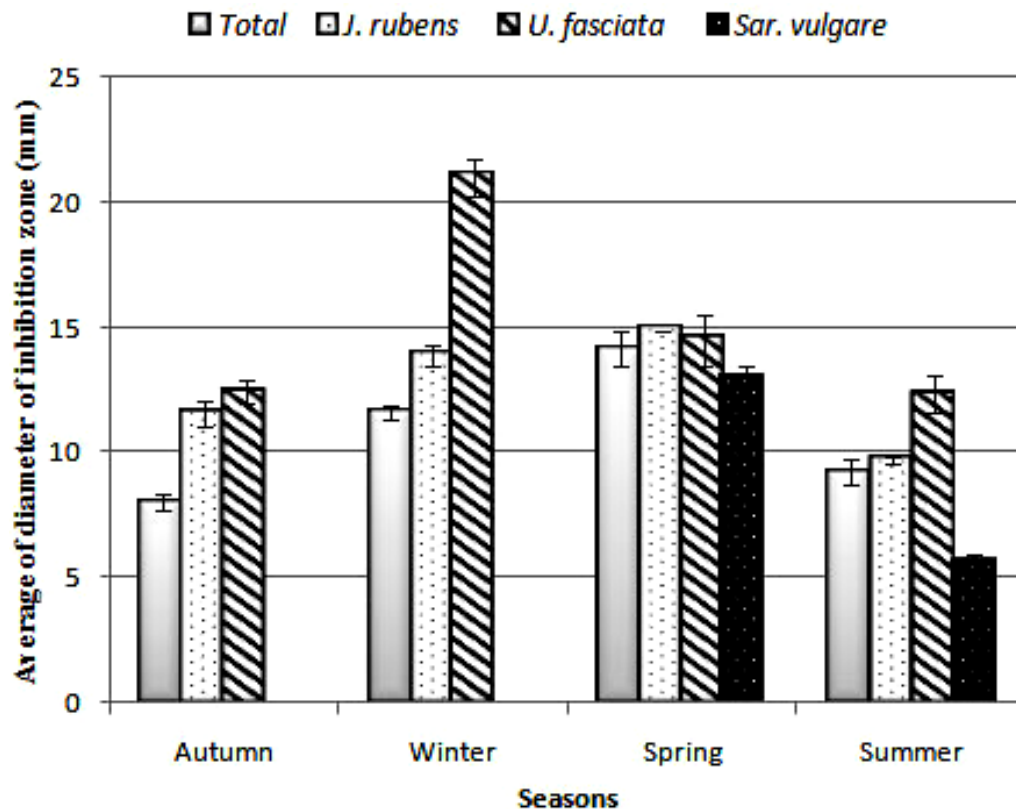


Figure 2. Antimicrobial activity of the different collected seaweeds that were collected in the different seasons.

Table 2. The antimicrobial activities of the different fractions obtained from the silica gel column chromatography against *K. pneumoniae*.

Number of fraction	Diameter of inhibition zone (mm)	Number of fractions	Diameter of inhibition zone (mm)
Fr.1	10 ± 0.023	Fr.15	-
Fr.2	13 ± 0.052	Fr.16	-
Fr.3	15 ± 0.034	Fr.17	-
Fr.4	17 ± 0.029	Fr.18	-
Fr.5	-	Fr.19	-
Fr.6	-	Fr.20	-
Fr.7	-	Fr.21	-
Fr.8	-	Fr.22	-
Fr.9	-	Fr.23	-
Fr.10	-	Fr.24	-
Fr.11	-	Fr.25	-
Fr.12	-	Fr.26	-
Fr.13	-	Fr.27	-
Fr.14	-		

(±)Standard error of the mean (n=3).

664 nm had not any antimicrobial activity. Therefore, we completed our investigation on the purified compound which had peak at 333 nm.

The infrared spectra (IR) of the antimicrobial material

The spectrum was subdivided into different regions, namely,

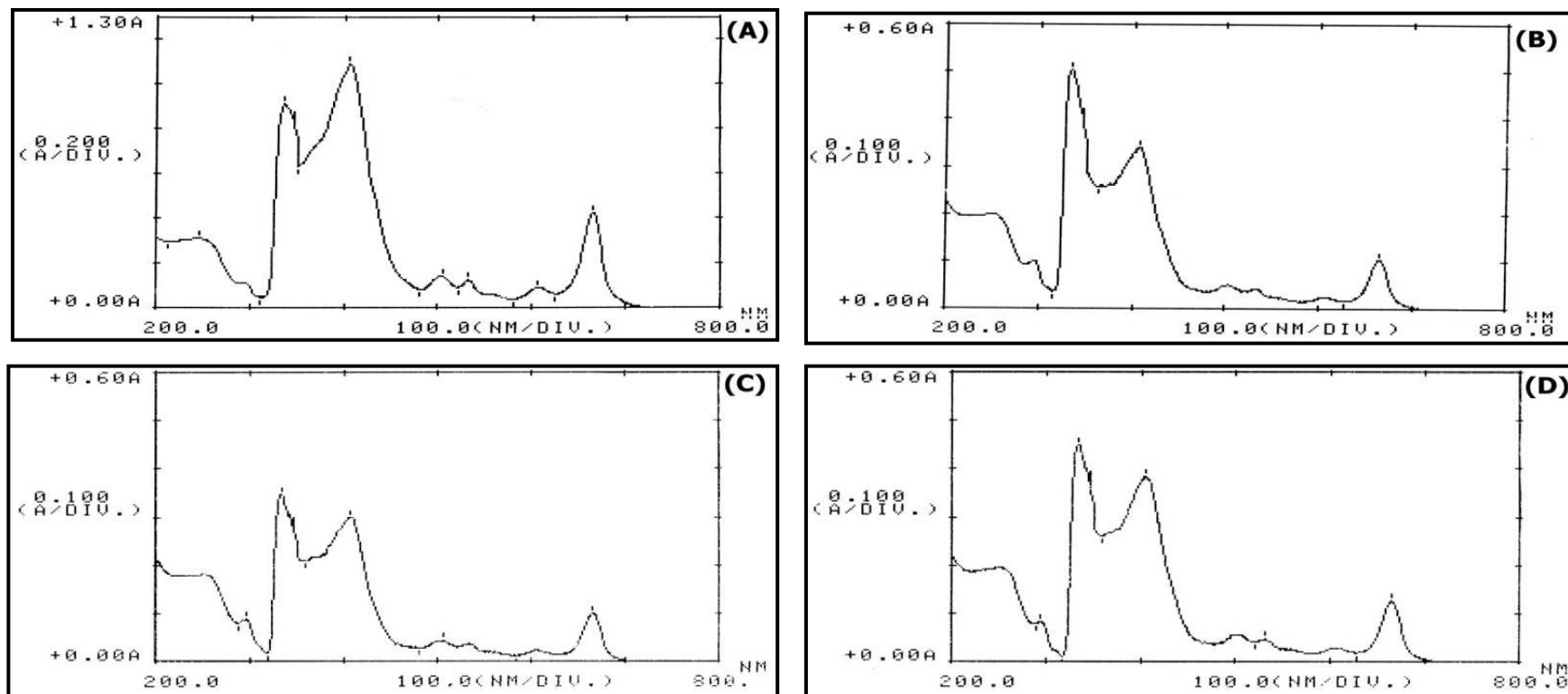


Figure 3. UV spectrophotometer scanning for the different active fractions separated by column chromatography.

Table 3. The retention factor of the spots from different fractions.

Sample	R _f
All fraction	0.38
Fraction 1	0.377 ^(n.s.)
Fraction 2	0.4 ^(n.s.)
Fraction 3	0.372 ^(n.s.)
Fraction 4	0.39 ^(n.s.)
F-value	5.67 ^(n.s.)

^(ns) Non significant at $P \geq 0.01$ using one way analysis of variance (ANOVA).

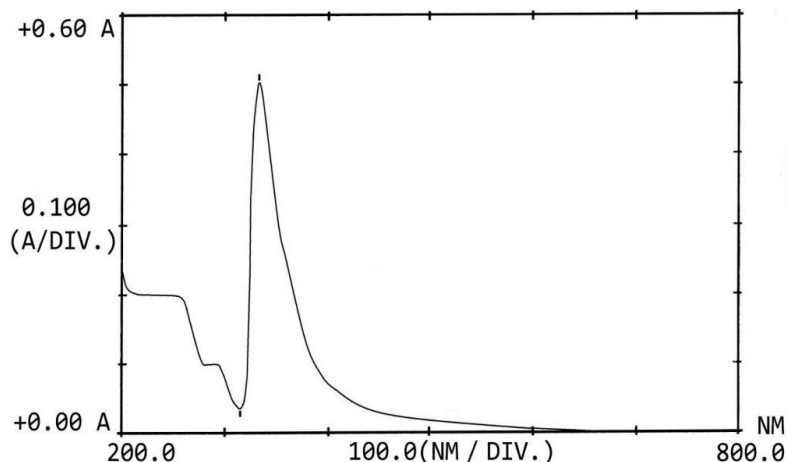


Figure 4. UV spectrum of the antimicrobial material produced by winter collected *U. fasciata* after purification by charcoal.

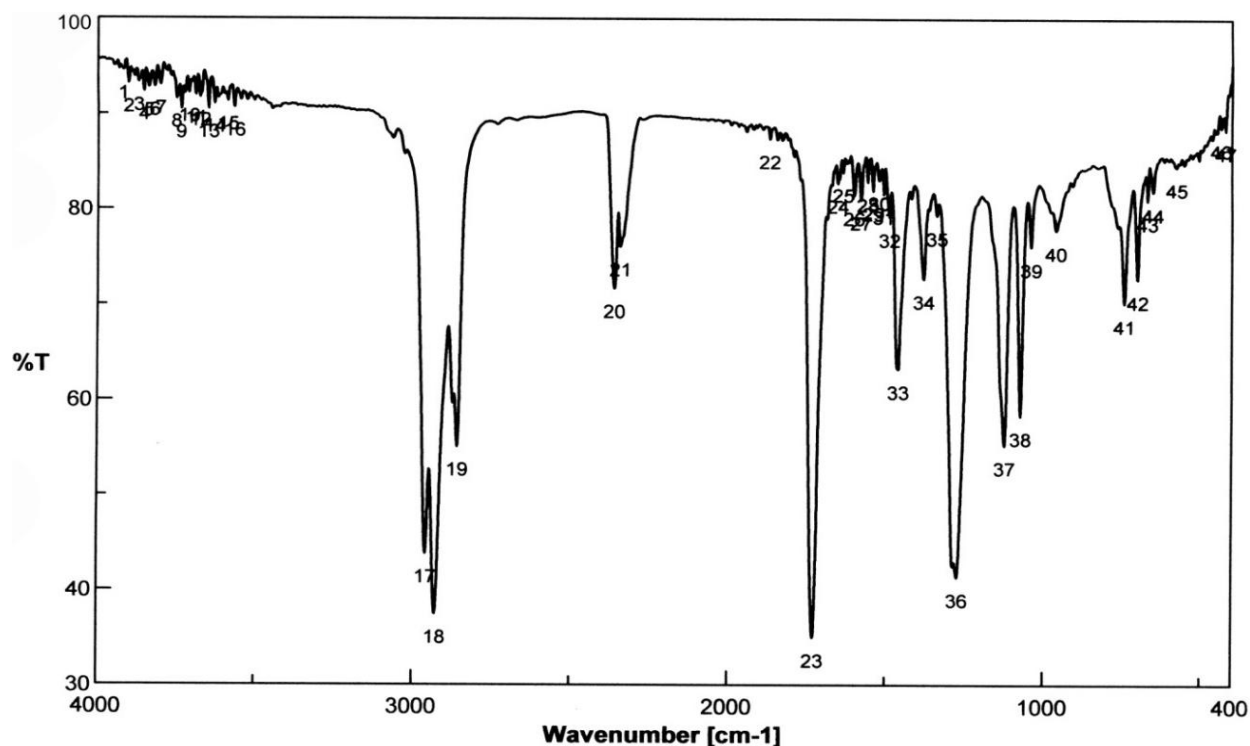


Figure 5. IR spectra of the purified antimicrobial material produced by winter collected *U. fasciata*.

the 2850 to 3050, 1710 to 1780, 1350 to 1470, 1020 to 1390 and 675 to 870 regions. The representative curve is shown in Figure 5.

Absorption in the 2850 to 3050 region: In this region, the ν CH aliphatic and ν CH aromatic strong stretch bands may appear. The IR revealed a strong band at 2958, 2927 and 2857 cm^{-1} , that can be attributed to the stretching vibrations of ν CH aliphatic and at 3000 to 3050 for

ν CH aromatic group.

Absorption in the 1710 to 1780 region: This region comprised one band due to the stretching vibration of the ν C=O of COOR group at 1729 cm^{-1} .

Absorption in the 1020 to 1390 region: This region comprised two bands due to the stretching vibration of the ν C-N at 1122 and 1072 cm^{-1} and in addition, one bands

bands due to the stretching vibration of the u N-O of nitro at 1272.79 cm^{-1} .

Absorption in the 870 to 675 region: This region comprised 3 bands due to the stretching vibration of the u phenyl ring substitution band at $742,700$ and 6698 cm^{-1} .

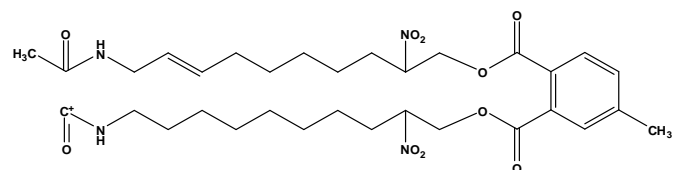
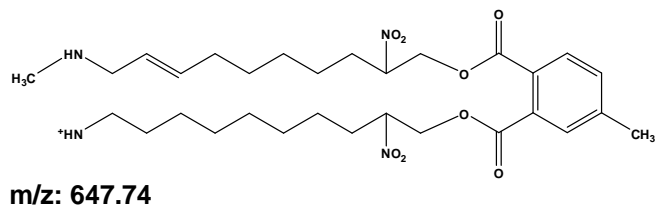
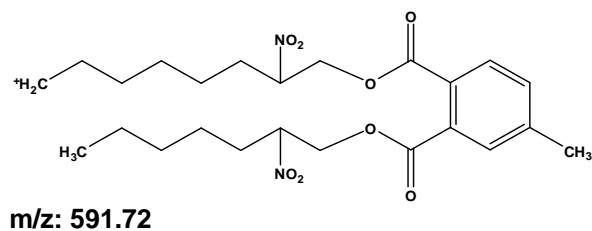
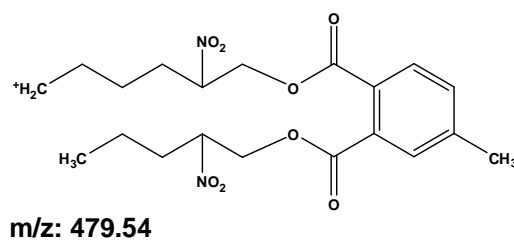
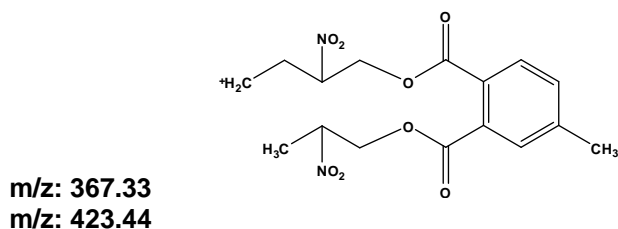
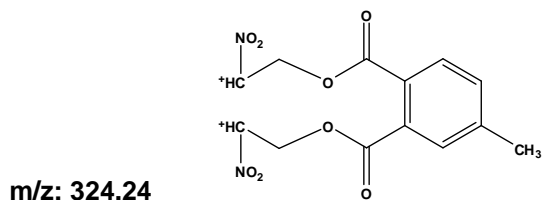
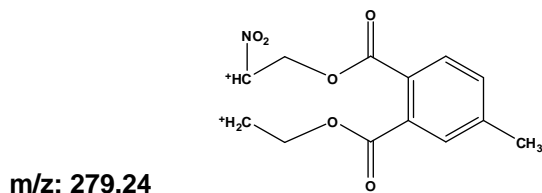
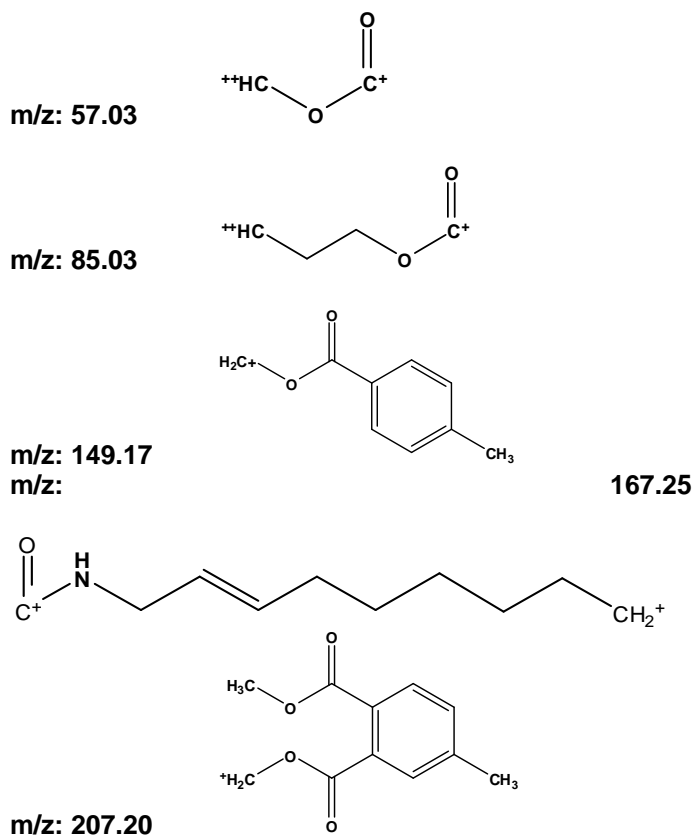
Proton magnetic resonance spectra

The ^1H NMR spectrum of the compound investigated was measured using in dimethyl- d^6 sulfoxide (d^6 DMSO) as solvent. The characteristic signals in ^1H NMR spectrum are represented graphically in Figure 6.

The signals were at δ 7.68 ppm (s, 2H, 2NH), at δ 6.89 to 7.47 ppm (m, 3H of three aromatic protons), δ 5.320 ppm (s, 2H, CH=CH), δ 3.998 and 4.126 ppm (d, 2H, CH- NO_2), δ 3.342 ppm (s, 4H, 2 O- CH_2), δ 1.26, 1.6, 2.5, 2.73 ppm (s, 28H, 14 CH_2) and at δ 1.9 and 2.1 (9H, 3- CH_3).

Mass spectra of the antimicrobial material

The mass spectrum fragmentation pattern of the compound investigated is shown in Figure 7. It reveals the presence of peak at m/z 662 of relative abundance characteristic of the parent compound. According to the earlier mentioned chemical analysis, the chemical structure of the purified antimicrobial material isolated from *U. fasciata* was:



According to the stated data, the suggested structure of the isolated antimicrobial substance should be as the following:

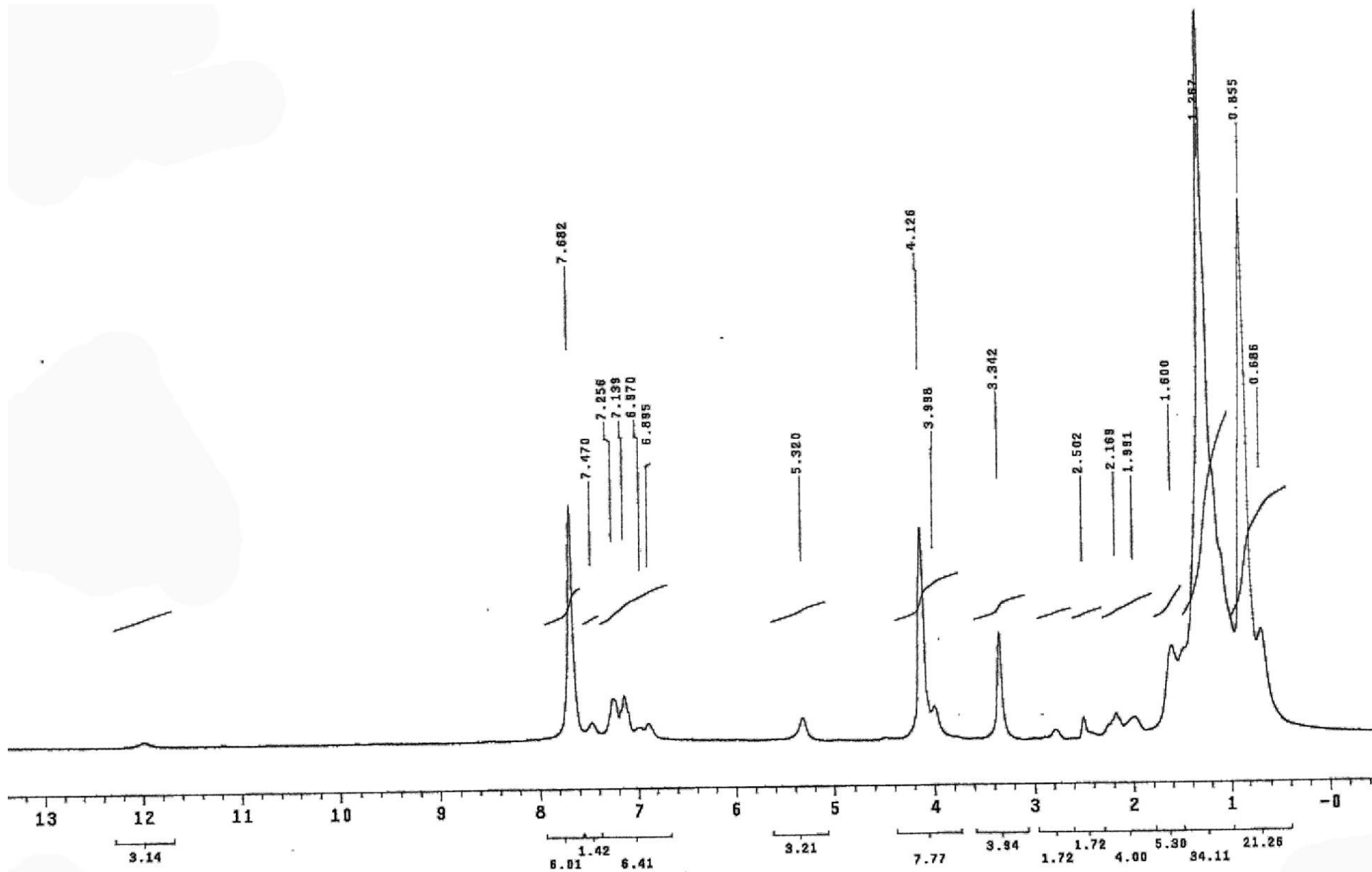


Figure 6. Proton magnetic resonance of the antimicrobial material produced by winter collected *U. fasciata*.

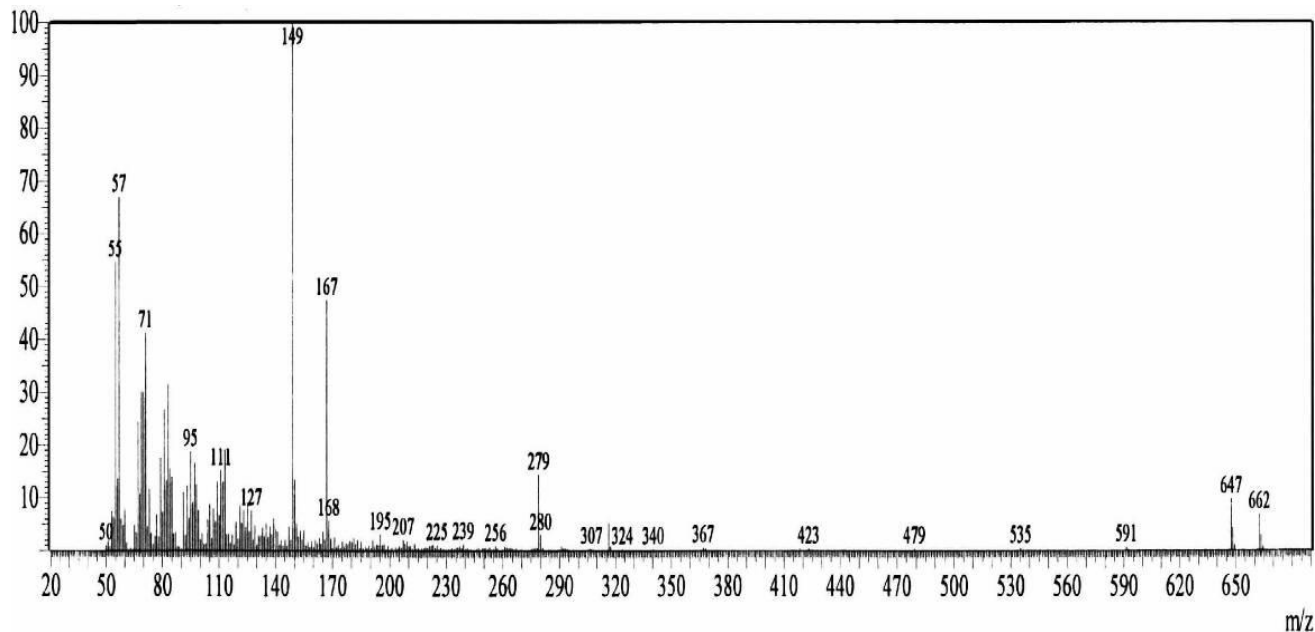
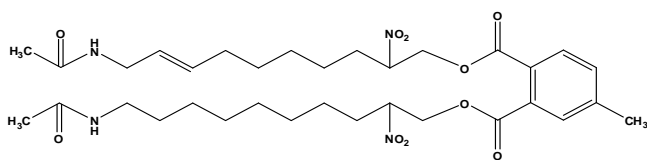


Figure 7. Mass spectra of the antimicrobial material produced by *U. fasciata*.



(*E*)-1-(10-acetamido-2-nitrodec-9-enyl), 2-(10-acetamido-2-nitrodecyl) 4-methyl phthalate. Chemical formula was $C_{33}H_{50}N_4O_{10}$; exact mass was 662.35; molecular weight was 662.77; elemental analysis was C: 59.80, H: 7.60, N: 8.45 and O: 24.14. This was the suggested chemical structure of the purified antimicrobial material isolated from *U. fasciata*.

DISCUSSION

This study is an endeavor for the study of the production, purification and structure elucidation of the antimicrobial compounds isolated from some species of seaweeds. Three species of seaweeds were tested for their capacity to produce antimicrobial substance. These species were collected from Abu-Qir, Alexandria coast, Egypt.

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 screen algae for antibacterial activity (Mahasneh et al., 1995; Sukatar et al., 2006).

In this study, 70% acetone was used for extracting the bioactive compounds from different tested seaweeds and gave antimicrobial activity against all selected pathogens. This results are in accordance with those obtained by Wefky and Ghobrial (2008) and Fareed and Khairy (2008).

It is well known that some species of macro-algae possess antibacterial activities against pathogenic bacteria (Kumar and Rengasamy, 2000; Selvin and Lipton, 2004; Tüney et al., 2006; Karabay-Yavasoglu et al., 2007; Salvador et al., 2007; Chiheb et al., 2009). The results reported by the earlier mentioned authors are in accordance with our data, which demonstrated that all the tested seaweeds had antimicrobial activity against the tested microorganisms. However, Salvador et al. (2007) and Gonzalez del Val (2001) detected that some seaweeds have not any antimicrobial activity in all the seasons. These differences in activity may be due to different developmental stages, locality, extraction methods, etc. Also, the antimicrobial activity depends on both algal species and efficiency on extraction of their active(s) principle(s).

In relation to the 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, the green alga (*U. fasciata*) was the most active one. These results are in agreement with the results of Kandhasamy and Arunachalam (2008) who reported that green algae (Chlorophyta) were the most active taxa than others and Fareed and Khairy (2008) which showed that *U. lactua* (Chlorophyta) was more active when compared with *J. rubens* (Rhodophyta).

Some pure compounds isolated from seaweeds have been identified as natural antimicrobial compounds.

However, the relationship between their ecologic role and antimicrobial activities is not fully understood in many studies. Chemical defenses can be very specific or very broad, depending on the method of extraction, the orga-

nisms, season of algal collection, different growth stages of the plant, experimental methods, etc.

In our study, we focused on the possibility that the antimicrobial activity of the tested seaweeds will also fluctuate seasonally. As regards to seasonal variation of bioactivity, for all of the tested seaweeds, spring was the season with the highest activity against the 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 the extracts which have antibacterial activity in late winter and early spring.

In this study, *U. fasciata* was the most effective seaweed species, having antibacterial activity throughout the year compared with other seaweeds screened for their antibacterial activity. *U. fasciata* inhibited the growth of all the tested microorganisms and this result is in agreement with Parekh (1978), who reported that the extract of *U. fasciata* was found to be inhibiting for both gram positive and gram negative bacteria. Also, Selvin and Lipton (2004) reported that the green alga *U. fasciata* exhibited broad-spectrum antibacterial activity.

The results showed that *U. fasciata* extracts of winter collection exhibited stronger antimicrobial effects followed by spring season than the other seasons and this agreed with Stirk and Reinecke (2007), who demonstrated that *U. fasciata* collected in winter and spring seasons were active against the tested organisms compared with 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 with summer and autumn collections. The former represents the peak growing and reproductive season, while the later is the stasis and senescence period of *U. fasciata* growth. The highest 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 some worker (Hornsey and Hide, 1974; Daly and Prince, 1981; Moreau et al., 1984; Rao and Indusekhar, 1989; Muñoz, 1992).

In this study, the antimicrobial material extracted from *U. fasciata*, which was collected from column chromatography was purified with charcoal and subjected to UV analysis. It showed maximum absorption spectra at 333 nm. Accordingly, the composition of the active antimicrobial material was suggested to contain aromatic ring.

The infrared (IR) spectroscopy indicated the presence of many functional groups in the antimicrobial material which were ν CH aliphatic at 2958, 2927 and 2857 cm^{-1} , ν CH aromatic at 3000 to 3050, ν C=O of COOR group at 1729 cm^{-1} , ν C-N at 1122 and 1072 cm^{-1} , ν N-O of nitro at 1272 cm^{-1} and ν phenyl ring substitution band at 742.46, 700.033 and 669.178 cm^{-1} .

The ^1H NMR spectrum showed two protons of two (NH), three protons of aromatic protons, two protons of (CH=CH), two protons of two (CH-NO₂), two protons of

two (O-CH₂), 28 protons of 14 (CH₂-) and nine protons of three (-CH₃). The mass spectroscopy of our antimicrobial material indicated that the molecular weight was 662.

According to the obtained data, the compound is an aromatic compound having the following structure C₃₃H₅₀N₄O₁₀ [(E)-1-(10-acetamido-2-nitrodec-9-enyl) 2-(10-acetamido-2-nitrodecyl) 4-methylphthalate]. This is the first evidence for the isolation of phthalate esters derivative from green seaweeds (*U. fasciata*) and that has broad antimicrobial activity. However, some worker isolated it from brown algae. Cho et al. (2005) isolated di-*n*-octylphthalate which has antifouling activities from the brown seaweed *Ishige okamurae*. Also, Ganti et al. (2006) isolated phthalic acid from *Sargassum confusum*.

These previous reports led us to suggest that the origin of the phthalate esters was natural and can be synthesized by living organisms not derived from artificial products or a contaminant from environment.

Finally, we conclude that macroalgae from Abu Qir coast in Alexandria could be considered as potential sources of bioactive compounds and the production of these compounds are affected by seasons. Our study indicated that the activities of different seaweeds for the production of antimicrobial substances in various seasons could be arranged in the following seasons spring > winter > summer > autumn and we obtained the highest activity from the winter collected *U. fasciata*. Thus, the production of the antimicrobial substance by seaweeds is season and species dependant. The structure of this antimicrobial material is C₃₃H₅₀N₄O₁₀ [(E)-1-(10-acetamido-2-nitrodec-9-enyl) 2-(10-acetamido-2-nitrodecyl) 4-methylphthalate], which is a strong anti-microbial activity against the tested human pathogenic microorganisms.

REFERENCES

- Abbott IA, Hollenberg IG (1976). Marine algae of California Stanford University press.
- Aleem AA (1993). The marine Algae of Alexandria, Egypt. Aleem AA (Ed.), Faculty of science, University of Alexandria, Egypt.
- Ballantine DL, Gerwick WH, Velez SM, Alexander E, Guevara P (1987). Antibiotic activity of lipid-soluble extracts from Caribbean marine algae. *Hydrobiol.*, 151/152: 463–469.
- Ballesteros E, Martin D, Uriz MJ (1992). Biological activity of extracts from some Mediterranean macrophytes. *Bot. Mar.*, 35: 481–485.
- Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2003). Marine natural products. *Nat. Prod. Rep.*, 20: 1–48.
- Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2004). Marine natural products. *Nat. Prod. Rep.*, 21: 1–49.
- Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2005). Marine natural products. *Nat. Prod. Rep.*, 22: 15–61.
- Chiheb I, Riadi H, Martinez-Lopez J, Dominguez SJF, Gomez VJA, Bouziane H, Kadiri M (2009). Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. *Afr. J. Biotechnol.*, 8(7): 1258-1262.
- Cho JY, Choi JS, Kang SE, Kim JK, Shin HW, Hong YK (2005). Isolation of antifouling active pyroglutamic acid, triethyl citrate and di-*n*-octylphthalate from the brown seaweed *Ishige okamurae*. *J. Appl. Phycol.*, 17: 431–435.
- Colombo ML, Bosisio E (1996). Pharmacological activities of *Chelidonium majus* L (Papaveraceae). *Pharmacol. Res.*, 33: 127-134.

- Cordell GA (2000). Biodiversity and drug discovery a symbiotic relationship. *Phytochemistry*, 55: 463-480.
- Daly JEL, Prince JS (1981). The ecology of *Sargassum pteropleuron* Grunow (Phaeophyceae, Fucales) in the waters off South Florida. III. Seasonal variation in alginic acid concentration. *Phycology*, 20: 352-357.
- El-Masry HA, Fahmy HH, Abdelwahed ASH (2000). Synthesis and antimicrobial activity of some new benzimidazole derivatives. *Molecule*, S: 1429-1438.
- Fareed MF, Khairy HM (2008). *In vitro* Antimicrobial Activities of Seaweeds Collected from Abu-Qir Bay Alexandria, Egypt. *World Appl. Sci. J.*, 5(4): 389-396.
- Gonzalez del Val A, Platas G, Basilio A (2001). Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int. Microbiol.*, 4: 35-40.
- Ganti VS, Kim KH, Bhattarai HD, Shin, HW (2006). Isolation and characterisation of some antifouling agents from the brown alga *Sargassum confusum*. *J. Asian Nat. Prod. Res.* 8: 309-315.
- Hornsey IS, Hide D (1974). The production of antimicrobial substances by British marine algae I. Antibiotic producing marine algae. *Br. Phycol. J.*, 9: 353-361.
- Kandhasamy M, Arunachalam KD (2008). Evaluation of in vitro antibacterial property of seaweeds of southeast coast of India. *Afr. J. Biotechnol.*, 7(12): 1958-1961.
- Karabay-Yavasoglu NU, Sukatar A, Ozdemir G, Horzum Z (2007). Antimicrobial activity of volatile components and various extracts of the red alga *Jania rubens*. *Phytother. Res.*, 21: 153-156.
- Khaleafa AF, Kharboush AM, Metwalli AA, Mohsen F, Serwi A (1975). Antibiotic fungicidal action from extracts of some seaweeds. *Bot. Mar.*, 18: 163-165.
- Kumar KA, Rengasamy R (2000). Evaluation of Antibacterial Potential of Seaweeds Occurring along the Coast of Tamil Nadu, India against the Plant Pathogenic Bacterium *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Dye. *Bot. Mar.* 43(5): 409-415.
- Mahasneh I, Jamal M, Kashashneh M, Zibdeh M (1995). Antibiotic activity of marine algae against multi-antibiotic resistant bacteria. *Microbios.*, 83(334): 23-26.
- Moreau J, Pesando D, Caram B (1984). Antifungal and anti bacterial screening of Dictyotales from the French Mediterranean coast. *Hydrobiol.*, 116/117: 521-524.
- Mtolera MSP, Semesi AK (1996). Antimicrobial activity of extracts from six green algae from Tanzania. *Curr. Trends Mar. Bot. Res. East Afr. Regul.*, 211-217.
- Muñoz A (1992). *Drogas del mar. Sustancias biomédicas de algas marinas*. Servicio de Publicaciones Intercambio Científico, Universidad de Santiago de Compostela, Santiago de Compostela.
- Parekh KS (1978). Antimicrobial and Pharmaceutically Active Substances from Marine Sources. Ph.D. Thesis, Saurashtra University, Rajkot, India.
- Rao CK, Indusekhar VK (1989). Seasonal variation in chemical constituents of certain brown seaweeds and seawater from Saurashtra coast. I. Carbon, nitrogen and phosphorus. *Mahasagar*. 22: 63-72.
- Salvador N, Gomez-Garreta A, Lavelli L, Ribera MA (2007). Antimicrobial activity of Iberian macroalgae. *Sci. Mar.*, 71: 101-113.
- SAS (1996). Copyright (c) 1989-1996 by SAS Institute Inc., Cary, NC, USA. SAS (r) Proprietary Software Release 6.12 TS020.
- Scazzocchio F, Comets MF, Tomassini L, Palmery M (2001). Antibacterial activity of *Hydrastis canadensis* extract and its major isolated alkaloids. *Planta Med.*, 67: 561-563.
- Scheuer PJ (1990). Some marine ecological phenomena: chemical basis and biomedical potential. *Science*, 248: 173-177.
- Selvin J, Lipton AP (2004). Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the peninsular coast of India. *J. Marine Sci. Technol.*, 12(1): 1-6.
- Sieradzki KSW, Tomasz A (1999). Inactivation of the methicillin resistance gene *mecA* in vancomycin-resistant *Staphylococcus aureus*. *Micro. Drug. Resist.* 5: 253-257.
- Solomon RDJ, Santhi VS (2008). Purification of bioactive natural product against human microbial pathogens from marine seaweed *Dictyota acutiloba* J. Ag. DOI 10.1007/s11274-008-9668-8.
- Stirk WA, Reinecke DL (2007). Seasonal variation in antifungal, antibacterial and acetylcholinesterase activity in seven South African seaweeds. *J. Appl. Phycol.*, 19: 271-276.
- Sukatar A, Karabay-Yavasoglu NU, Ozdemir G, Horzum Z (2006). Antimicrobial activity of volatile component and various extracts of *Enteromorpha linza* (Linnaeus) J. Agardh from the coast of Izmir, Turkey. *Ann. Microbiol.*, 56(3): 275-279.
- Taylor WS (1985). Marine algae of the eastern tropical and subtropical coasts of Americas. ANN Arbor the university of Michigan press.
- Trono JGC (1999). Diversity of the seaweed flora of the Philippines and its utilization. *Hydrobiol.*, 1-6: 398-399.
- Tüney I, Cadirci BH, Unal D, Sukatar A (2006). Antimicrobial activities of the Extracts of marine algae from the coast of Urla (Izmir, Turkey). *Turk. J. Biol.*, 30: 171-175.
- Vlachos V, Critchley AT, Von HA (1996). Establishment of a protocol for testing antimicrobial activity in southern African macroalgae. *Microbios.*, 88: 115-123.
- Wefky S, Ghobrial M (2008). Studies on the bioactivity of different solvents extracts of selected marine macroalgae against fish pathogens. *Res. J. Microbiol.*, 3: 673-682.
- WHO (2004). The world health report. Changing history. Statistical annex. Death by cause, sex and mortality stratum in WHO regions, estimates for 2002. Geneva, Switzerland, pp. 120-121.