INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2007/09–2–220–225
http://www.fspublishers.org

Antioxidant and Antibiotic Activities of Some Seaweeds (Egyptian Isolates)

SANAA M.M. SHANAB[1]
Botany Department, Faculty of Science, Cairo University, Egypt
E-mail: sanaashanab1@yahoo.com

ABSTRACT

Antioxidant activity was determined, by free radical scavenging (DPPH-decolorization method) and inhibition of lipid peroxidation (Fe^{2+}/Ascorbate), in three species of seaweeds [*Sargassum dentifolium*, *Laurencia papillosa* & *Jania corniculata* (Egyptian isolates)]. The three species of seaweeds were collected from Defressoar (Suez Canal, Egypt) during spring 2004. Silymarin was used as a reference control in both assays. Algae were cleaned from epiphytes, washed, air dried and powdered. Extraction of each algal species was carried out by aqueous ethanol or dichloromethane and different concentrations were prepared. Dichloromethane extract of each algal species demonstrated greater antioxidant activities than the ethanol extract using both bioassays. Maximum free radical scavenging activity was exhibited by higher concentration of dichloromethane extract of *S. dentifolium* followed by *L. papillosa* and *J. corniculata*. Also, higher concentration of dichloromethane extract of *L. papillosa* had the maximum anti-lipid peroxidation activity followed by *S. dentifolium* and *J. corniculata*. All extracts showed antibiotic activity against four bacterial and two fungal species. Spectrophotometric and chromatographic determination of the active compounds revealed that the antioxidant and antibiotic activities might be attributed to algal content of chlorophylls, carotenoids and free phenols as well as fatty acids.

Key Words: Antioxidant; Antibiotic; Free radical; Lipid peroxidation; Seaweeds

INTRODUCTION

Membrane lipid peroxidation is induced by free radicals or reactive oxygen species, which are formed as by-products of many biochemical reactions as well as in electron transport chain (Ewing *et al.*, 1989). Reactive oxygen species have the potential to cause several cellular disorders if not scavenged properly (Halliwell & Gutteridge, 1986). Peroxidation of polyunsaturated fatty acids can proceed through processes that are enzymatically catalyzed or through non-enzymatic auto-oxidative pathways (Slater, 1984). Lipid peroxidation leads to the destruction of cellular membrane, proteins and nucleic acids and eventually cell death. It is commonly recognized that antioxidants can scavenge the harmful active free radicals in the cells and reduce the potential mutations.

Another field strongly affected by lipid peroxidation is the food sector, where the free radical peroxidation of lipids, is the predominant cause of food decay, destruction of vitamins and rancidity during storage and transformation (St Angelo, 1992). Therefore, many products with antioxidant properties, mainly of synthetic origins, are widely used to increase the shelf life of foodstuffs. These compounds are phenol derivatives such as, butylhydroxy toluene (BHT) and butyl hydroxyanisol (BHA). They have recently been suspected to their toxicity and cause lipid alterations as well as carcinogenic effects (Grillo & Dulout, 1995; Safer & Al-Nughamish, 1999). Therefore, attention is focusing on the development of new, safe and cheap antioxidants of natural origin (Larson, 1988; Lewis, 1989). Higher plants are continuously being investigated for their potential antioxidant effectiveness as α-tocopherol, flavonoids and β-carotene, which are incorporated in food industries to inhibit lipid peroxidation (Tsuchihashi *et al.*, 1995; Cook & Samman, 1996).

Several studies have investigated the antioxidant activity of natural products in marine and freshwater algae (Fujimoto & Kaneda, 1984; Matsukawa *et al.*, 1997; Lim *et al.*, 2002; Xue *et al.*, 2004). Marine algae, like all photosynthesizing plants are exposed to a combination of light and high oxygen concentrations, which lead to the formation of free radicals and other strong oxidizing agents. The elements of the photosynthetic apparatus are vulnerable to photodynamic damage, because polyunsaturated fatty acids are important structural components of the thylakoid membrane (Sukenik *et al.*, 1993). The absence of such damage in seaweeds, in spite of the proximity of the photosynthetically produced oxygen and suitable target within the photosynthetic apparatus suggests that these cells have protective antioxidative mechanism and compounds (Matsukawa *et al.*, 1997; Lim *et al.*, 2002). This work was, therefore, conducted to screen the antioxidant activities of some seaweeds inhabiting Suez Canal (Red Sea, Egypt). The behavior of the Egyptian isolates was also compared with that of the worldwide investigated species.

MATERIALS AND METHODS

Algal materials and extraction. Three seaweeds were collected from Suez Canal (Egypt) at Defressoar during Spring 2004. Algae were identified as Egyptian isolates of the following species: *Sargassum dentifolium* (Turner) C.

Agardh, *Laurencia papillosa* (C. Agardh) Greville and *Jania corniculata* (Linnaeus) Lamouroux. Seaweeds were carefully cleaned from epiphytes and washed several times with tap and distilled water then air dried and powdered. Hundred grams of each algal species were extracted three times by aqueous ethanol (70%) or dichloromethane. Extracts were lyophilized and their weights were determined. Two methods were used for the investigation of antioxidant activity:

1.-Free radical scavenging activity (DPPH - decolorization assay). Free radical scavenging activity of different algal extract concentrations (10, 50 & 100 µg mL^{-1}) were evaluated spectrophotometrically (at 517 nm) against the absorbance of the indicator 2, 2 diphenyl-1-picrylhydrazyl (DPPH) solution (20 mg L^{-1}). All reactions were carried out in triplicates and the degree of decolorization indicates the free radical scavenging activities of the algal extracts (Viturro *et al.*, 1999). Silymarin was used as reference free radical scavenger and percentage of DPPH – decolorization was calculated using the following equation:

$$\text{Free radical scavenging \%} = 1 - (Ac - As)/Ac \times 100$$

Where

Ac = Absorbance of control and As = Absorbance of algal sample.

2.-Inhibition of lipid peroxidation (Fe^{2+}/Ascorbate assay). Mitochondria were isolated from liver of decapitated rates according to method descried by Kimora *et al.* (1984) and the protein content was determined by the method of Lowry *et al.* (1951). In this assay the method described by Haraguchi *et al.* (2002) was used. A mixture of 0.5 mL of liver mitochondrial suspension (containing 5 mg protein) with 0.1 mL of each of the following: Hepes buffer (pH 7.4), 20 mM KCl, 10 µM Fe SO_4, 0.2 mM ascorbate, algal extract (concentrations of 0.25, 0.5 & 1 mg mL^{-1}) in a final volume of 1 mL, were incubated at 37°C for 30 min. The marker of lipid peroxidation products (malondialdhyde) was quantitatively determined according to Burits and Bucar (2000) by the rapid addition of 2.5 mL TCA-TBA reagent [15% w/v trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA) to the mixture. The color absorbancy of TBARS (thiobarbituric acid reactive substance) was measured at 532 nm. Experiments were carried out in triplicates and Silymarin was used as reference control. Percentage inhibition of lipid peroxidation was calculated as:

$$I\% = 100 \times (A_1 - A_2/A_1)$$

Where

A_1 = absorbance of control reaction, A_2 = absorbance in the presence of the inhibitor (algal extract).

Determination of antioxidant active compounds. Total chlorophylls, carotenoids were determined spectrophotometrically (Jenway 6300 Spectrophotometer, U.K) according to method of Holden (1965). Total free phenols were determined by Folin-Ciocalteu method as described by Van Alstyne (1995).

Chromatographic identification. Methylation or Silylation of extracts were performed before injection in gas chromatography combined with mass spectrometry (GC-MS) in food-processing department, Faculty of Agriculture, Cairo University. Total ion chromatograms and active compounds were analyzed.

Antimicrobial activity of algal extracts. Screening for the antimicrobial activity of all seaweed extracts under investigation was performed using paper disc diffusion method against four bacterial species: gram positive; *Bacillus subtilis*, *Staphylococcus albus*, *Streptococcus faecalis*; gram negative, *Escherichia coli* and two fungal species: *Candida albicans* and *Aspergillus flavus* according to the method of Muanza *et al.* (1994). After incubation at favorable conditions for bacterial and fungal growth, results were recorded with digital camera and diameter of inhibition zones.

Statistics. Data obtained were statistically analyzed using the least significant difference test (L.S.D.) at 1 and 5% levels of probability.

RESULTS AND DISCUSSION

Screening the antioxidant activity by free radical scavenging assay showed that extracts of the three seaweeds in both solvents used (ethanol or dichloromethane) at low extract concentration (10 µg mL^{-1}) had comparable free radical scavenging activities (≃ 48%) (Table I). The scavenging activity was increased with increasing the extract concentrations (50 & 100 µg mL^{-1}), the maximum value was obtained by *S. dentifolium* (82 & 86% in ethanol & dichloromethane, respectively) followed by *J. corniculata* (81 & 77.9%) and *L. papillosa* (77 & 79%) (Fig. 1A). The scavenging activity at high extract concentration was compared with that of Silymarin, used as reference control, which scavenged 92% of the free radicals (Table II).

Testing the antioxidant activity by inhibition of lipid peroxidation, the lower ethanolic extract concentration (0.25 mg mL^{-1}) of *L. papillosa* and *J. corniculata* had similar activity values (45%), whereas *S. dentifolium* had activity value of 33%. However, the same concentration of dichloromethane extract gave greater activity values: 66% in *L. papillosa,* 52% in *S. dentifolium* and 33% in *J. corniculata*. Higher extract concentrations (0.5 & 1.0 mg mL^{-1}) increased the inhibition of lipid peroxidation especially for dichloromethane extracts of most algal species under investigation than for ethanol extracts (Table II, Fig. 1B). *Sargassum dentifolium* and *L. papillosa* recorded 83.44% and 87.15% suppression of lipid peroxidation, while both extracts of *J. corniculata* showed comparable activities (70.33% & 69.82%) (Table II).

Our results indicated that dichloromethane extracts of *S. dentifolium* had the greatest free radical scavenging activity (86%) and that of *L. papillosa* showed the greatest

Table I. Effect of *Sargassum dentifolium*, *Laurencia papillosa* and *Jania corniculata*, in ethanol or dichloromethane extract, at 10 µg/mL on free radical scavenging activity and 0.25 mg/mL on anti-lipid peroxidation. Each value is the mean ± SD of 5 replicates. Means in the same column with the same letter are not significant at P < 0.05

Samples	Ethanol extract		Dichloromethane extract	
	Free radical scavenging activity (DPPH radical assay)	Anti-lipid peroxidation (Fe^{2+}/ Ascorbate assay)	Free radical scavenging activity (DPPH radical assay)	Anti-lipid peroxidation (Fe^{2+}/ Ascorbate assay)
Control	0.00 ± 0.00 [b]	0.00 ± 0.00 [c]	0.00 ± 0.00 [b]	0.00 ± 0.00 [d]
Sargassum dentifolium	48.38 ± 1.91 [a]	33.03 ± 2.01 [b]	48.68 ± 2.11 [a]	52.68 ± 3.21 [b]
Laurencia papillosa	48.83 ± 2.27 [a]	45.53 ± 2.75 [a]	47.21 ± 3.01 [a]	66.07 ± 3.56 [a]
Jania corniculata	48.16 ± 1.83 [a]	45.43 ± 1.97 [a]	46.18 ± 2.81 [a]	33.93 ± 1.61 [c]
L.S.D. (0.01)	4.775	5.390	6.338	6.927
(0.05)	3.281	3.704	4.356	4.761

Table II. Effect of *Sargassum dentifolium*, *Laurencia papillosa* and *Jania corniculata*, in ethanol or dichloromethane extract, at 100 µg/mL on free radical scavenging activity and 1.0 mg/mL on anti-lipid peroxidation, compared with silymain as reference control. Each value is the mean ± SD of 5 replicates. Means in the same column with the same letter are not significant at P < 0.05

Samples	Ethanol extract		Dichloromethane extract	
	Free radical scavenging activity (DPPH radical assay)	Anti-lipid peroxidation (Fe^{2+}/ Ascorbate assay)	Free radical scavenging activity (DPPH radical assay)	Anti-lipid peroxidation (Fe^{2+}/Ascorbate assay)
Silymarin	92.00 ± 3.67 [a]	96.50 ± 3.67 [a]	92.00 ± 3.67 [a]	96.5 ± 3.67 [a]
Sargassum dentifolium	82.17 ± 6.32 [b]	68.50 ± 3.11 [b]	86.18 ± 4.15 [ab]	83.44 ± 3.51 [b]
Laurencia papillosa	77.45 ± 4.01 [b]	72.23 ± 5.41 [b]	79.38 ± 3.58 [bc]	87.15 ± 3.57 [b]
Jania corniculata	81.44 ± 5.37 [b]	70.33 ± 2.72 [b]	77.93 ± 3.16 [c]	69.82 ± 2.52 [c]
L.S.D. (0.01)	13.583	10.594	10.019	9.177
(0.05)	9.335	7.281	6.886	6.307

Table III. Antimicrobial activity of dichloromethane or aqueous ethanol extract of *Sargassum dentifolium*, *Laurencia papillosa* and *Jania corniculata* using paper disk diffusion method

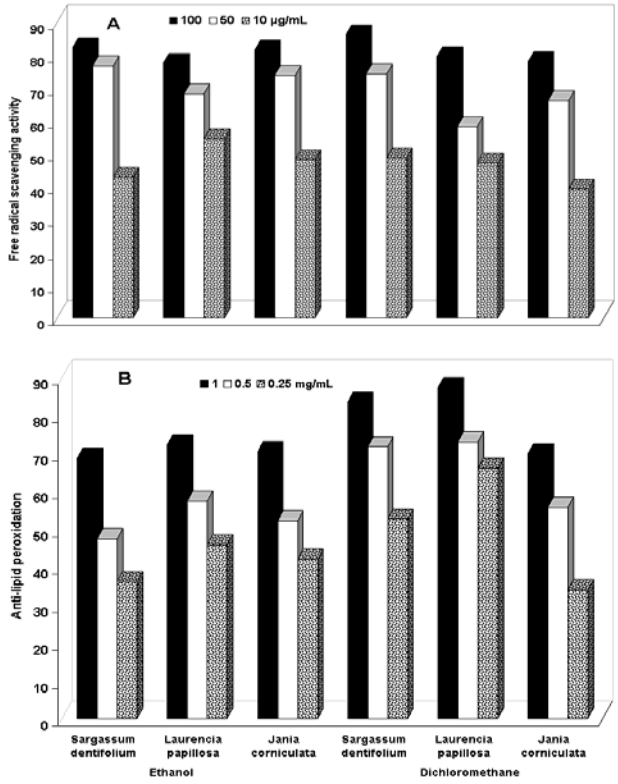
Algal species (Seaweeds)	Diameter of the inhibition zone (mm)					
	Bacterial species				Fungal species	
	Bacillus subtilis	Escherichia coli	Staphylococcus albus	Streptococcus fuecalis	Candida albicans	Aspergillus flavus
Dichloromethane	0.0	0.0	0.0	0.0	0.0	0.0
S. dentifolium	12	11	11	12	11	0.0
L. papillosa	12	11	11	12	12	0.0
J. corniculata	13	12	12	13	13	0.0
Ethanol (70%)	0.0	0.0	0.0	0.0	0.0	0.0
S. dentifolium	10	11	11	11	11	0.0
L. papillosa	13	13	12	12	13	0.0
J. corniculata	14	15	12	12	14	0.0

anti-lipid peroxidation efficiency (87%) comparing with those of silymarin (92 & 96%, respectively), which was used as reference control.

The obtained results of the current investigation are in agreement with those of Le Tutour (1990) and Le Tutour *et al.* (1998), who investigated the antioxidant activities of different Seaweeds. In their studies, the brown algae, *Laminaria digitata* and *Himanthalia elongata* exhibited the most valuable antioxidant activities compared with those of vitamin E and Butylhydroxyl Toluene (BHT). Extracts of *L. digitata* and *H. elongata* synergistically enhanced the antioxidant activity of vitamin E and this synergism was found only in algae collected in summer season. The previous authors recommend the use of these algal extracts with vitamin E for the preservation of sunflower oil.

The maximum antioxidant activities obtained by *S. dentifolium* and *L. papillosa* in the present study are consistent with the results of Anggadiredja *et al.* (1997), who reported greater antioxidant activity of the polar *Sargassum polycystum* and the non-polar *Laurencia obtusa* extracts. Also, Matsukawa *et al*. (1997) screened the antioxidant activity in methanol extract of three *Sargassum* species (*S. horneri*, *S. macrocarpum* & *S. siliquastrum*), which exhibited the greatest antioxidant activities. The great antioxidant activity manifested by *S. siliquastrum* reported by Lim *et al*. (2002) was also in accordance with the higher activity produced by *S. dentifolium* in the present investigation. The free radical scavenging activity of the brown *S. kjillmanianum* and *Chorda filum* (Yan *et al.*, 1998) and that of the brown seaweeds *Hizikia fusiformis*, *Undaria pinnatifida* and *S. fulvellum* supports our results. The potent anti-lipid peroxidation activity obtained by *L. papillosa* and the higher free radical scavenging activity of *S. dentifolium* in the present work parallel the potent antioxidant activity reported by the red alga *Grateloupia filicia* (Athukorata *et al.*, 2003) and the brown alga *Hizikia fusiformis*

Fig. 1. Effect of *Sargassum dentifolium, Laurencia papillosa* and *Jania corniculata* ethanol or dichloromethane extract at 10, 50 and 100 µg/mL on free radical scavenging (A) and 0.25, 0.5 and 1 mg/mL on anti-lipid peroxidation activities (B)



(Siriwardhana *et al.*, 2004).

Screening for antimicrobial activity of the three seaweeds, both extracts (ethanol & dichloromethane) exhibited more or less similar antimicrobial activities. Red algal species (*J. corniculata* & *L. papillosa*) demonstrated greater activity than the brown alga *S. dentifolium* (Table III).

Searching for the active substances in algal extracts, which exhibited either the antioxidant or the antimicrobial activities, total chlorophylls, total carotenoids, total free phenols and saturated/un-saturated fatty acids of both extracts of each Seaweed species were determined.

Table IV revealed that both extracts of *S. dentifolium* and *J. corniculata* had more or less similar total chlorophylls as well as chlorophyll a contents, while the dichloromethane extract of *L. papillosa* had higher values than those of the ethanol extract. Higher values of total carotenoids were recorded in the non-polar dichloromethane extract of all the algal species than the ethanolic ones (two folds increase in *S. dentifolium*, twenty folds increase in *L. papillosa* & six folds increase in *J. corniculata*). On the contrary, the polar ethanol extract of all algal species, showed higher values of total free phenols (about nine fold increase in all algal species) than those obtained by dichloromethane extract. Chlorophylls especially chlorophyll a and related compounds with porphyrin ring exhibited some antioxidant activity in the dark (Endo *et al.*, 1985a, b). Chlorophyll a, connect to the negatively charged peroxyl radical, act as potent synergist of vitamin E (Le Tutour *et al.*, 1996, 98; Mendiola *et al.*, 2005) and enhance the antioxidant activity of α-tocopherol (Cahyana *et al.*, 1993). Carotenoids (carotenes & xanthophylls) are known to quench the excited sensitized molecules and singlet oxygen (Bondarev, 1997) and may act as antioxidant under conditions, where singlet oxygen is not formed (Tsuchihashi *et al.*, 1995; Murthy *et al.*, 2005).

The higher antioxidant activity demonstrated by *S. dentifolium* and *L. papillosa* of non-polar (dichloromethane) extracts might be attributed to the high content of the lipid soluble total chlorophylls especially Chl-a and related compounds (Le Tutour *et al*., 1998; Stajner *et al.*, 1999; Nakayama *et al.*, 1999), to the high content of total carotenoids (Krinsky, 1989; Yan *et al.*, 1999; Rivero *et al.*, 2003).

The polar ethanolic extracts contained higher content of phenolic compounds, which are more abundant in red alga (*J. corniculata*) than the brown algae (*S. dentifolium*) and the other red alga (*L. papillosa*) (Table IV). Phenolic compounds inhibited TBARS production during lipid peroxidation as well as scavenged DPPH free radicals and thus they exhibited antioxidant activity (Rice-Evans *et al.*, 1997; Xi *et al.*, 2003; Sook *et al.*, 2004; Yuan *et al.*, 2005).

The antimicrobial activity of red Seaweeds (*L. papillosa* & *J. corniculata*) might be attributed to the presence of fatty acids either saturated [Tetradecanoic (14:0), Hexadecanoic (16:0), Octadecanoic (18:0)] or un-saturated [Hexadecenoic (16:1) 9-octadecenoic (18:1) and Tetracosenoic (24:1)] acids in higher percentage in *L. papillosa* and *J. corniculata* (Table V). These results are in agreement with those obtained by Daoud and Foster (1993), Fei *et al.* (2002) and Kimura and Yokota (2004). They reported that both types of fatty acids exhibited antimicrobial activity against different species of bacteria and fungi. Previous studies report that the red algae *Laurencia obtuse*, *L. rigida* and *L. chondrioides* (contain sesquiterpenoids) have an antimicrobial activity against human pathogenic bacteria, yeast and fungi (Konig & Wright, 1997; Bansemir *et al.*, 2004) are in agreement with the antimicrobial activity found in the present study by *L. papillosa* and *J. corniculata* against four bacteria and two fungi. Also, phlorotannins, phenolic compounds and diterpenediol (crinitol) are reported to be produced by brown algae *Sargassum critaefolium*, *S. tortile*, *Ecklonia kurome*, *E. bicyclis* and *Cystoseira crinita* and exhibited antibacterial activity (Kubo *et al.*, 1992; Alam *et al.*, 1994; Nagayama *et al.*, 2002) and antifungal activity (Bhargava *et al.*, 1998; Vairappan *et al.*, 2004). The previous reports are consistent with the antimicrobial activity manifested by *S. dentifolium* in our study, which may be partly attributed to the presence of saturated (Stearic 18:0, Nondecyclic 19:0, Arachidic 20:0) and un-saturated (Petroselinic 18:1 &

Table IV. Spectrophotometric determination of chlorophylls, carotenoids and total free phenols of *Sargassum dentifolium*, *Laurencia papillosa* and *Jania corniculata*. Each value is the mean ± SD of 5 replicates

Extract	Ethanol extract			Dichloromethane extract		
Analysis	Sargassum dentifolium	Laurenci. papillosa	Jania corniculata	Sargassum dentifolium	Laurenci papillosa	Jania corniculata
Total chlorophylls	5.58 ± 0.21	2.21± 0.17	6.6 ± 0.31	5.53 ± 0.32	6.13±0.42	6.59 ± 0.34
Chlorophyll a	4.13 ± 0.19	1.49± 0.07	4.34 ± 0.2	3.75 ± 0.27	3.01±0.15	3.16 ± 0.15
Other chlorophylls	1.44 ± 0.06	0.71± 0.01	2.23 ± 0.04	1.78 ± 0.09	3.12±0.09	3.44 ± 0.08
Total carotenoids	0.59 ± 0.01	0.16±0.001	0.49 ± 0.01	1.06 ± 0.07	3.28±0.13	3.47 ± 0.21
Total free phenols	157.5±2.34	117.3±3.04	232.5±8.07	16.95±0.81	11.83±0.58	24.5 ± 1.71

Table V. Chromatographic separation (by GC/MS) of antimicrobial active compounds (%) in dichloromethane extract and their specific retension time (R.T.) of *Sargassum dentifolium, Laurencia papillosa* and *Jania corniculata*

Separated compounds	Sargassum dentifolium		Laurencia papillosa		Jania corniculata	
	R.T.	%	R.T.	%	R.T.	%
9-Hexadecenoic acid (Palmitoleic, 16:1)	—	—	—	—	30.217	2.86
Nonadecanoic acid (Nondecyclic, 19:0)	30.533	0.51	—	—	—	—
n-Hexadecanoic acid (Palmitic, 16:0)	—	—	31.475	1.31	—	—
Octadecanoic acid	30.625	6.78	35.4	4.86	30.642	3.2
(Stearic, 18:0)					35.533	3.7
6-Octadecenoic acid (Petroselinic, 18:1)	34.083	2.06	—	—	—	—
cis-9-octadecenol	—	—	—	—	34.108	1.15
(Oleyl alcohol)					40.783	0.63
cis-9-Octadecenoic acid	—	—	35.058	17.08	35.167	2.53
(Oleic acid, 18:1)			35.158	6.54	35.2	4.51
					35.275	5.28
Morpholine derivatives	—	—	35.25	0.77	—	—
Tetradecanoic acid(Myristic, 14:0)	—		41.883	1.38	—	—
15-Tetracosenoic acid (Nervonic, 24:1)	41.892	0.43	34.758	12.08	—	—
Ethyl Oleate	—	—	44.667	1.56	—	—
Eicosanoic acid (Arachidic, 20:0)	49.908	0.22	—	—	—	—

Nervonic 24:1) fatty acids (Table V).

Aknowledgements. The author is grateful to Dr. M.M. Farag, Professor of Biochemistry, Faculty of Agriculture, Cairo University for his scientific support.

REFERENCES

Alam, K, F. Agua, H. Maven, R. Taie, K.S. Rao, I. Burrows, M.E. Huber and T. Rali, 1994. Preliminary screening of seaweeds, seagrass and lemongrass oil from papua. New Guinea for antimicrobial and antifungal activity. *Int. J. Pharmacog.,* 32: 396–9

Anggadiredja, J., R. Andyani, Hayati and H. Muawanah, 1997. Antioxidant activity of *Sargassum polycystum* (Phaeophyta) and *Laurancia obtusa* (Rhodophyta) from Seribu Islands. *J. Appl. Phycol.,* 9: 477–9

Athukorata, Y., Lee-Kiwan, Song-ChoomBok, Ahn-ChangBum, Shin-TaiSun, Cha-YongJun, E. Shahidi and Jeon-Youjin, 2003. Potential antioxidant activity of marine red alga *Grateloupia filicina* extracts. *J. Fd. Lipids,* 10: 251–65

Bansemir, A., N. Just, M. Michalik, U. Lindequist and M. Lalk, 2004. Extracts and sesquiterpene derivatives from the red alga *Laurencia chondrioides* with antibacterial activity against fish and human pathogenic bacteria. *Chem. Biodiv.,* 1: 463–7

Bhargava, A., A. Srivastava and V. Kumbhare, 1998. Antifungal activity of polyphenolic complex of *Acacia nilotica* bank. *Ind. Forester,* 124: 292–8

Bondarev, S.L., 1997. Photophysics of β-carotene and related compounds. *J. Appl. Spectrose*, 64: 1–5

Burits, M. and E. Bucar, 2000. Antioxidant activity of *Nitella sativa* essential oil. *Phytother. Res.,* 14: 323–8

Cahyana, A., Y. Shuto and Y. Kinoshita, 1993. Synergistic antioxidative effects of prophyrin derivatives with α-tocopherol and ascorbic acid. *Biosci. Biotech. Biochem.,* 57: 1753

Cook, N.C. and S. Samman, 1996. Flavonoids chemistry, metabolism, cardioprotective effects and dietary sources. *J. Nutr. Biochem.,* 7: 66–76

Daoud, N.N. and H.A. Foster, 1993. Antifungal activity of Myxococcuss species, 1-production, physiochemical and biological properties of antibiotics from *Myxococcus fulvus* S110 (Myxobacterales). *Microbios.,* 173–84

Endo, Y., R. Usuki and T. Kaneda, 1985a. Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. I. Comparison of the inhibitory effects. *J. American Oil Chem. Soc.,* 62: 1375–8

Endo, Y., R. Usuki and T. Kaneda, 1985b. Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. II. The mechanism of antioxidative action of chlorophyll. *J. American Oil Chem. Soc.,* 62: 1387–90

Ewing, J.C., J.P. Cosgrove, D.H. Giamalva, D.F. Church and W.A. Pryor, 1989. Autoxidation of methyl linoleate initiated by the ozonide of allylbenzene. *Lipids,* 24: 609–15

Fei, T.D., W.R. Jing, L. Ming and L.Y. Xin, 2002. Studies on chemical compositions and antimicrobial activity of volatile oil of *Dictyophora echinovolvata*. *Mycosystema*, 21: 228–33

Fujimoto, K. and T. Kaneda, 1984. Separation of antioxygenic (antioxidant) compounds from marine algae. *Hydrobiologia,* 116: 111–3

Grillo, C.A. and F.N. Dulout, 1995. Cytogenetic evaluation of butylated hydroxytoluene. *Mutat. Res.,* 345: 73–8

Halliwell, B. and J.M.C. Gutteridge, 1986. Iron and free radicals: two aspects of antioxidant protection. *Trends Biochem. Sci.,* 11: 372–5

Haraguchi, H., J. Inoue, Y. Tamura and K. Mizutani, 2002. Antioxidative compounds of *Psoralea corylifolia* (Leguminosae). *Phytother. Res.,* 16: 539–44

Holden, M., 1965. Chlorophyll. *In*: Goodwin, T.W. (Ed.), *Chemistry and Biochemistry of Plant Pigments,* Pp: 462–88. Academic press, London

Kimora, Y., Y. Okuda, T. Okuda, T. Hatano, I. Aganta and S. Arichi, 1984. Studies on the activities of tannins and related compounds. V. Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. *Pl. Med.,* 61: 473–7

Kimura, H. and K. Yokota, 2004. Characterization of metabolic pathway of linoleic acid 9-hydroperoxide in cytosolic fraction of potato tubers and identification of reaction products. *Appl. Bioch. Biotech.,* 118: 115–32

Konig, G.M. and A.D. Wright, 1997. Laurencia rigida, chemical investigations of its antifouling dichloromethane extract. *J. Nat. Prod.,* 60: 967–70

Krinsky, N.I., 1989. Antioxidant functions of carotenoids. *Free Radical Biol. Med.,* 7: 617–35

Kubo, I., M. Himejima, K. Tsujimoto, H. Muroi and N. Ichikawa, 1992. Antimicrobial activity of crinitol and its potentiation. *J. Nat. Prod.,* 55: 780–5

Larson, R.A., 1988. The antioxidants from higher plants. *Phytochem.,* 27: 969–78

Le Tutour, B., 1990. Antioxidative activities of algal extracts, synergistic effect with vitamin E. *Phytochem.,* 29: 3759–65

Le Tutour, B., C. Brunet and F. Quemeneur, 1996. Effect de synergie de la chlorophyll a sur les propriétés antioxydantes de la vitamine E. *New J. Chem.,* 20: 707–21

Le Tutour, B., F. Benslimane, M.P. Gouleau, J.P. Gouygou, B. Saadan and F. Quemeneur, 1998. Antioxidant and pro-oxidant activities of the brown algae, *Laminaria digitata, Himanthalia elongate, Fucus vesiculosus, Fucus serratus* and *Ascophyllum nodosum*. *J. Appl. Phycol.*, 10: 121–9

Lewis, A.D., 1989. *Anti-inflammatory Drugs from Plant and Marine Souce,* Pp: 95–283. Birkäuser-Verlag, Basel

Lim, S.N., P.C.K Cheung, V.E.C. Ooi and P.O. Ang, 2002. Evaluation of antioxidant activity of extracts from a brown seaweed, *Sargassum siliquastrum. J. Agric. Food Chem.,* 50: 3862–6

Lowry, O.H., N.J. Rsebrough, A.C. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* 193: 265–75

Matsukawa, R., Z. Dubinsky, E. Kishimoto, K. Masaki, Y. Masuda, T. Takeuchi, M. Chihara, Y. Yamamoto, E. Niki and I. Karube, 1997. A comparison of screening methods for antioxidant activity in seaweeds. *J. Appl. Phycol*., 9: 29–35

Mendiola, J.A., F.R. Marin, S.F. Hernandez, B.O. Arredondo, F.J. Senorans, E. Ibanez and G. Reglero. 2005. Characterization via liquid chromatography coupled to diode array detector and tandem mass spectrometry of supercritical fluid antioxidant extracts of *Spirulina platensis* microalga. *J. Separation Sci.,* 28: 1031–38

Muanza, D.N., B.W. Kim, K.L. Euler and L. Williams, 1994. Antibacterial and antifungal activity of nine medical plants from Zaire. *Int. J. Pharmacog.,* 32: 337–45

Murthy, K.N.C., A. Vanitha, J. Rajesha, M.M. Swamy, P.R. Sowmya and G.A. Ravishankar, 2005. *In vitro* antioxidant activity of carotenoids from *Dunaliella salina*-a green microalga. *Life Sci.,* 76: 1381–90

Nakayama, R., Y. Tamura, H. Kikuzaki and N. Nakatani, 1999. Antioxidant effect of the constituents of Susabinori (*Porphyra yezoensis*). *J. American Oil Chem. Soc.,* 76: 644–53

Nagayama, K., Y. Iwamura, T. Shibata, I. Hirayama and T. Nakamura, 2002. Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. *J. Antimicrobial Chemother.,* 50: 889–93

Rice-Evans, C.A., N.J. Miller and G. Paganga, 1997. Antioxidant properties of phenolic compounds. *Trends Pl. Sci.,* 2: 152–9

Rivero, F., A. Fallarero, O. Castaneda, F. Dajas, E. Manta, F. Areces, J. Mancini, Filho and A. Vidal, 2003. Antioxidant activity *in vivo* and *in vitro* of Halimeda incrassate aqueous extracts. *Ciencia-e-Tecnologia-de-Alimentos*, 23: 256–63

Safer, A.M. and A.L. Nughamish, 1999. Hepatotoxicity induced by the antioxidant food additive butylated hydroxytoluene (BHT) in rats: An electron microscopical study. *Histol. Histopathol.,* 14: 391–406

Siriwardhana, N., K.W. Lee, S.H. Kim, J.H. Ha, G.T. Park and Y.J. Jeon, 2004. Lipid peroxidation inhibitory of Hizikia fusiformis methanolic extract on fish oil and Limoleic. *Fd. Sci. Technol. Int.,* 10: 65–72

Slater, A., 1984. Overview of methods used for detecting lipid peroxidation. *In*: Pocker, L. (ed.), *Methods in Enzymology*, Vol. 105, Pp: 283–93. Academic press, N.Y

Sook, H.H., C.H. Young, K.J. Young, S.B. Who, J.H. Ahand and C.J. Sue, 2004. Inhibitory phlorotannins from the edible brown alga *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation. *Arch. Pharmacol. Res.,* 27: 194–8

Stajner, D., N. Milic and J. Canadanovic-Brunet, 1999. An investigation into the antioxidant activity of *Allium nutans* L. *Phytother. Res.,* 13: 333–6

St Angelo, A.J., 1992. *Lipid Oxidation in Food*. ACS Symposium series 500, Washington D.C

Sukenik, A., O. Zmora and Y. Carmeli, 1993. Biochemical quality of marine unicellular algae with special emphasis lipid composition: II. *Nannochloropsis* sp. *Aquaculture*, 117: 313–26

Tsuchihashi, H., M. Kigoshi, M. Iwatsuki and E. Niki, 1995. Action of β-carotene as antioxidant against lipid peroxidation. *Arch. Biochem. Biophys*., 323: 137–47

Vairappan, C.S., T. Kawamoto, H. Miwa and M. Suzuki, 2004. Potent antibacterial activity of halogenated compounds against antibiotic resistant bacteria. *Pl. Med.,* 70: 1087–90

Van Alstyne, K.L., 1995. Comparison of three methods for quantifying brown algal polyphenolic compounds. *J. Chem. Ecol.,* 21: 45–58

Viturro, C., A. Molina and G. Schmeda-Hirchmann, 1999. Free radical scavengers from *Mutisia friesiana* (Asteraceae) and *Sanicula graveolens* (Apiaceae). *Phytother. Res.,* 13: 422–4

Xi, W.Y., L.Z. En, H.Y. Fen and X.Z. Hong, 2003. Inhibition of mouse liver lipid peroxidation by high molecular weight phlorotannis from *Sargassum kjellmanianum*. *J. Appl. Phycol.,* 15: 507–11

Xue, Z., C.H. Xue, Z.J. Li, Y.P. Cai, H.Y. Liu and H.T. Qi, 2004. Antioxidant and hepatoprotective activities of low molecular weight sulfated polysaccharide from *Laminaria japonica*. *J. Appl. Phycol.,* 16: 111–5

Yan, X., T. Nagata, X. Fan, X.J. Yan and F. Xiao, 1998. Antioxidative activities in some common seaweeds. *Pl. Fds Human Nutrit.,* 52: 253–62

Yan, X., Y. Chuda, M. Suzuki, T. Nagata and X.J. Yan, 1999. Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Biosci. Biotechnol. Biochem.*, 63: 605–7

Yuan, Y.V., D.E. Bone and M.F. Carrington, 2005. Antioxidant activity of dulse (*Palmaria palmata*) extract evaluated *in vitro*. *Fd. Chem.,* 91: 485–94

(Received 05 December 2006; Accepted 01 January 2007)