

Antioxidant and Antibiotic Activities of Some Seaweeds (Egyptian Isolates)

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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 (Suknik *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 $\mu\text{g mL}^{-1}$) 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:

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

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 rats according to method described 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 (malondialdehyde) 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:

$$\text{I\%} = 100 \times (\text{A}_1 - \text{A}_2/\text{A}_1)$$

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 $\mu\text{g mL}^{-1}$) had comparable free radical scavenging activities ($\approx 48\%$) (Table I). The scavenging activity was increased with increasing the extract concentrations (50 & 100 $\mu\text{g mL}^{-1}$), 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
<i>Sargassum dentifolium</i>	48.38 ± 1.91 ^a	33.03 ± 2.01 ^b	48.68 ± 2.11 ^a	52.68 ± 3.21 ^b
<i>Laurencia papillosa</i>	48.83 ± 2.27 ^a	45.53 ± 2.75 ^a	47.21 ± 3.01 ^a	66.07 ± 3.56 ^a
<i>Jania corniculata</i>	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 silymarin 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)
<i>Silymarin</i>	92.00 ± 3.67 ^a	96.50 ± 3.67 ^a	92.00 ± 3.67 ^a	96.5 ± 3.67 ^a
<i>Sargassum dentifolium</i>	82.17 ± 6.32 ^b	68.50 ± 3.11 ^b	86.18 ± 4.15 ^{ab}	83.44 ± 3.51 ^b
<i>Laurencia papillosa</i>	77.45 ± 4.01 ^b	72.23 ± 5.41 ^b	79.38 ± 3.58 ^{bc}	87.15 ± 3.57 ^b
<i>Jania corniculata</i>	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	
	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Staphylococcus albus</i>	<i>Streptococcus faecalis</i>	<i>Candida albicans</i>	<i>Aspergillus flavus</i>
Dichloromethane	0.0	0.0	0.0	0.0	0.0	0.0
<i>S. dentifolium</i>	12	11	11	12	11	0.0
<i>L. papillosa</i>	12	11	11	12	12	0.0
<i>J. corniculata</i>	13	12	12	13	13	0.0
Ethanol (70%)	0.0	0.0	0.0	0.0	0.0	0.0
<i>S. dentifolium</i>	10	11	11	11	11	0.0
<i>L. papillosa</i>	13	13	12	12	13	0.0
<i>J. corniculata</i>	14	15	12	12	14	0.0

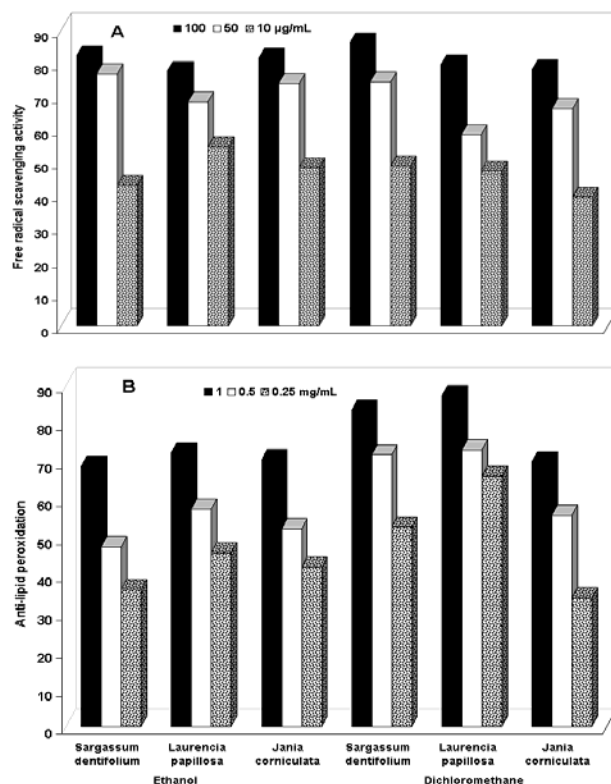
anti-lipid peroxidation efficiency (87%) comparing with those of silymarin (92 & 96%, respectively), which was used as reference control.

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 peroxy 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 critaeifolium*, *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 $\pm$ SD of 5 replicates

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

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

Separated compounds	<i>Sargassum dentifolium</i>		<i>Laurencia papillosa</i>		<i>Jania corniculata</i>	
	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 (Stearic, 18:0)	30.625	6.78	35.4	4.86	30.642	3.2
6-Octadecenoic acid (Petroselinic, 18:1)	34.083	2.06	—	—	35.533	3.7
cis-9-octadecenol (Oleic acid, 18:1)	—	—	—	—	34.108	1.15
cis-9-Octadecenoic acid (Oleic acid, 18:1)	—	—	35.058	17.08	40.783	0.63
			35.158	6.54	35.167	2.53
					35.2	4.51
					35.275	5.28
Morpholine derivatives	—	—	35.25	0.77	—	—
Tetradecanoic acid (Myristic, 14:0)	—	—	41.883	1.38	—	—
15-Tetradecenoic 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).

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