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Antioxidant Activity of Algal Extracts on Lipid Peroxidation

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The antioxidant of seven algal extracts was evaluated by decrease the thiobarbituric acid reactive substance (TBARS) produced from lipid peroxidation of liver microsomes induced by Fe^{++}/ascorbate, Fe^{++}/H_2O_2 and CCl_4 model systems. All algal extracts had significant effect to prevent the production of TBARS in all oxidizing model systems and this phenomenon was increased by increasing their concentration. The values of inhibition % of TBARS generate from Fe^{++}/ascorbate, Fe^{++}/H_2O_2 and CCl_4 model system by *Dunaliella salina* were 100, 94.43 and 90.9% respectively, (at 15 min) at concentration level 200 ppm, while the inhibition values were 98.49, 89.8 and 88.8% at 100 ppm. This extract possesses higher antioxidant activity with average 2 times than the BHT at the same concentration level (100 ppm). On the other hand, mutant strains extract of *Scenedesmus dimorphus*, *Scenedesmus acutus* and *Chlorella ellipsoida* showed higher antioxidant activity than the normal cells extracts. The most of all algal extracts showed more potent as an antioxidant than the BHT which, is one of the powerful synthetic antioxidant agent. The antioxidant activity of algal extract and BHT against the induced lipid peroxidation in all model system were in the following descending order: *Dunaliella salina* > *Sc. dimorphus* mutant = *Chlorella* mutant > *Sc. dimorphus* normal > *Chlorella normal* > *Sc. acutus* (mutant) > *Sc. acutus* (normal) > BHT. The antioxidant activity of algal extracts on induced lipid peroxidation in different model system was dependent on the chemical composition of their extracts which containing mainly the carotenoids, tocopherol and vitamin C. These substances can protect lipid peroxidation in all model system by different mode of action. Therefore, micro algae extracts inhibited the lipid peroxidation products by scavenging reactive oxygen species ($^{\cdot}OH$, $^{\cdot}O^{-}$ and $^1O_2$) and chain reaction breaking consequently its protect bodies from harmful effect of their substances.

Key words: Antioxidant, vitamin-C, tocopherol, peroxidation, carotenoids, micro algae

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Introduction

Lipid peroxidation has attracted much attention in relation to oxidative damage of biological molecules, due to the formation of lipid hydroperoxides which in the presence of cellular iron containing compounds, can break down to yield oxygen radicals (Pryor, 1987). Unsaturated fatty acids and cholesterol are easily oxidized, particularly in biological systems. The lipid peroxidation chain reaction yields a variety of mutagenesis, promoters and carcinogens such as fatty acid hydroperoxides, cholesterol hydroperoxide, endoperoxides, cholesterol and fatty acids epxoids. The formation of these compounds in biological systems leads to a serious diseases such as ischemia-reperfusion injury, coronary arteriosclerosis and diabetes mellitus as well as being associated with aging and carcinogenesis (Gaziano, 1996; Gerber ***et al.***, 1996; Halliwell, 1997; Haraguchi ***et al.***, 1997 and Abd El-Baky ***et al.***, 2002). In addition, the lipid hydroperoxide can be decomposed to produce alkoxy ($LO^{\bullet}$) and peroxy radical ($LOO^{\bullet}$). They eventually yield numerous carbonyl products, which are responsible for DNA damage and generation of cancer and aging related diseases (Fraga ***et al.***, 1990 and Shinmoto ***et al.***, 1992).

Recently, there are some discussions about toxicological effect of hydroperoxidation. For example, De Zwart ***et al.*** (1998) mentioned that lipid peroxidation is associated with wide variety of harmful effects including decreased membrane fluidity function, impaired hepatic, mitochondrial and golgi apparatus function, inhibition of enzyme such as glucokinase, succinate dehydrogenase and synthetase and decomposed some SH - compounds such as CoA.

Patel and Gores (1997) reported that oxidation of unsaturated fatty acids in biological membranes leads to a disruption of membrane structure and function which cause lysis of cell as mechanism of cell death (necrosis). On the other hand, lipid peroxidation of some biological molecules can induce apopotosis against the cells which is a mode of cell death with morphological features quite distinct from those of necrosis.

Consequently, there is an urgent need for natural antioxidants to protect our bodies from harmful effect of lipid peroxidation products. The present work was conducted to study the ability of some vitamins, from algal extracts as antioxidant inhibition of induced lipid peroxidation on liver microsomes by different peroxidation model systems including Fe^{++}/H_2O_2, Fe^{++}/ascorbate and CCl_4.

Materials and Methods

Algal source

Dunaliella salina was obtained from the culture collection of Dr. W.H. Thomas, La Jolla, CA, U.S.A., ***Chlorella ellipsoidea*** was obtained from the algae culture collection of the University of Gottingen, Germany. ***Scenedesmus acutus*** 276-3a and ***Scenedesmus dimorphus*** were obtained from the Culture Center of Algae and Protozoa, Cambridge, U.K. Mutant strains of ***Chlorella ellipsoidea***, ***Scenedesmus acutus*** 276-3a and ***Scenedesmus dimorphus*** were generated from the parent strains as described by El-Baz ***et al.*** (2002).

Growth conditions

Algae cells were cultivated in specific growth medium and under stress environmental conditions as described by El-Baz ***et al.*** (2002).

Preparation of liver microsomes

Microsomes were isolated from females Swiss mice weighing 25±2 g and male Wister rats (120±5 g) as follows:

Animals were decapitated and allow exsanguinating. The liver was quickly excised and washed several times with ice cold saline solution (0.15 M KCl, pH 7.4), then homogenized in the same saline solution using a Teflon-glass homogenizer. The homogenate was filtered through a cold double layer of gauze and then centrifuged successively at 1600xg for 10 min the supernatants were followed by centrifugation at 20,000xg for another 10 min and the pellets were discarded. Microsomes were obtained from the 20,000xg supernatant by centrifugation at 105,000xg for 1 h at 4°C. The microsomal pellet was washed by suspension in cold ice Tris-buffer (pH 7.4), then frozen by liquid nitrogen then stored at -30°C (Kornbrast and Mavis, 1980).

Preparation of algal extracts model system

A known weight of ascorbic acid extracts (33.3 mg) was added to test tube (3 ml) contained carotenoide and tocopherol extracts (66.6 mg). Then (1 ml) Tris-buffer pH 7.4 was added. All contents were then agitated using a Julabo Ultrasonic bath (40 KHz) for 15 min. This system was stable for at least 4 h. The algal extracts were prepared freshly before each addition. The final concentration of their algal vitamins mixture extracts were 100 and 200 ppm in Tris-buffer pH 7.4.

Incubation condition for microsomal lipid peroxidation

Lipid peroxidation of microsomes has been shown to be absolutely depended on concentration of free ferrous iron added. The liver microsomes (10 μl), containing 100 μg protein were incubated at 37±1°C for the specified lengths time (15, 30 and 45 min) with iron (1 μM) combined with ascorbic acid (500 μM) or hydrogen peroxide (0.03 mM) in final volume 1.5 ml. Two concentrations of algae extracts were tested 100 and 200 ppm and butylated hydroxytoluene (BHT) was used as standard antioxidant (0.2 and 0.5%) in a final reaction mixture.

Determination of lipid peroxidation products

Lipid peroxidation products were measured by quantitation of thiobarbituric acid reactive substance (TBARS, including malondialdhyde) during incubation period, which would be produced by complete peroxidation of the oxidizable polyunsaturated fatty acids (PUFA) present in liver microsomal fraction. After incubation at different times the lipid peroxidation was determined by rapid addition of 2.5 ml TCA-TBA reagent (15% w/v trichloroacetic acid (TCA) and 0.375% thiobarbituric acid TBA) to the reaction mixture. The reaction mixture was heated for 20 min in

boiling water bath and after cooling, the flocculent precipitate was removed by centrifugation at 1000xg for 5 min. The colour absorbency of TBARS in the supernatant was recorded at 532 nm against a blank containing all the reagents expect liver microsomal as described by Haraguchi ***et al.*** (1997).

The extinction coefficient of TBARS was taken as 1.56×10^5 (Joseph ***et al.***, 1988) to convert the absorbance values into concentration (M). The TBARS concentration in liver microsomes was normalized with protein concentration.

Results and Discussion

Antioxidant activity of vitamin algal extracts on Fe^{++}/ascorbic acid model system

This model system contained mice liver microsomes and Fe^{++}/ascorbic acid as oxidizing agent to initiate the lipid peroxidation. Free ferrous iron (1 μM) produced a small amount of thiobarbituric acid reactive substances (TBARS) after TBA addition in liver microsomes, whereas much greater amounts of TBARS products were produced by the same iron concentration in the presence of ascorbate. The enhancement of microsomal lipid peroxidation in presence of ascorbate has been attributed to their ability to directly promote reduction of iron (Kornbrust and Mavis, 1980).

The inhibition (%) of TBARS produced from system containing mice liver microsomes and Fe^{++}/ascorbic acid catalyzed by vitamin extracts (Table 1) obtained from different algae strains or BHT (100 and 200 ppm). All algal extracts in model systems showed a potent inhibition of mice liver microsomal lipid peroxidation. All model systems catalyzed by different algal extracts were always higher than the control model system (BHT). Therefore, vitamin extracts of algae had markedly antioxidant activities.

Vitamins extract of ***Dunaliella*** showed more potent inhibition against microsomal lipid peroxidation among vitamin extracts of other algae strains, which exhibited almost complete inhibition (100%) at concentration level 200 ppm. Vitamin extract of mutant ***Sc. dimorphus*** showed higher inhibitory effect against lipid peroxidation than the parent ***Sc. dimorphus*** cells extract. Similar trend was observed by mutant strains, ***Chlorella*** and ***Sc. acutus***, which always had higher antioxidant activity than the native strains.

In lipid peroxidation model system catalyzed with BHT (100 ppm), the inhibition % values of TBARS were 69.73, 65.91 and 44.52% at concentration levels of 100 ppm, when incubated at 15, 30 and 45 min, respectively. All vitamin algal extracts have more potent inhibitor for mice liver microsomal lipid peroxidation, compared with BHT, common synthetic antioxidant (positive control).

The results showed the effectiveness of various vitamin extracts of algae strains and BHT on inhibition of mice microsomal lipid peroxidation induced by Fe^{++}/ascorbic acid were as the following descending order:

Table 1: Effect of algal extracts on inhibition of mice liver microsome lipid peroxidation induced by Fe^{++} and ascorbic acid system

	Incubation period (min)						
	15		30		45		Mean of
Treatments	TBARS[a]	Inh.(%)[b]	TBARS[a]	Inh.(%)[b]	TBARS[a]	Inh.(%)[b]	Inh[c]. %
$FeSO_4$ (1 µM)	0.101		0.231		0.345		
Control ($FeSO_4$ ascorbic 0.5mM)	4.690	0.0	7.890	0.00	9.840	0.0	0.0
BHT (40 ppm)	3.430	26.90	5.330	32.45	6.270	36.30	31.88
BHT (100ppm)	1.420	69.73	2.690	65.91	5.460	44.52	60.05
Ext. of *Sc. ac.* (100 ppm)	1.180	74.85	3.420	56.70	5.260	46.55	59.36
Ext. of *Sc. acutus* (200 ppm)	0.859	81.69	2.970	65.40	4.690	52.34	66.47
Ext. of *Sc.acutus* mut. (100 ppm)	0.858	78.70	3.010	61.86	4.830	50.90	65.99
Ext. of *Sc. acutus* mut. (200 ppm)	0.756	83.89	2.550	67.70	4.360	55.70	69.09
Ext. of *Chlorella* (100 ppm)	1.110	76.34	3.000	61.98	3.420	65.25	67.85
Ext. of *Chlorella* (200 ppm)	0.993	78.83	2.690	65.90	3.130	68.20	70.97
Ext. of *Chlorella* mut. (100 ppm)	0.850	81.88	2.250	71.50	2.920	70.33	74.57
Ext. of *Chlorella* mut. (200 ppm)	0.580	87.64	1.920	75.67	2.630	73.28	78.86
Ext. of *Sc. dimorphus* (100 ppm)	0.780	83.37	2.130	74.00	3.210	67.40	74.92
Ext. of *Sc. dimorphus* (200 ppm)	0.690	85.30	1.620	79.50	2.920	70.30	78.36
Ext. of *Sc. dimorphus* mut. (100 ppm)	0.650	86.20	1.270	83.91	2.250	77.14	82.40
Ext. of *Sc. dimorphus* mut. (200 ppm)	0.520	88.90	0.782	90.09	1.620	83.50	87.46
Ext. of *Dunaliella* (100 ppm)	0.071	98.49	0.530	93.29	0.650	99.93	97.23
Ext. of *Dunaliella* (200 ppm)	0.000	100.00	0.211	98.90	0.480	99.95	99.60

(TBARS): Thiobarbturic acid reactive substance a: TBARS formation was expressed as µM

b: Inhibition (%) was expressed as the reduction of TBARS formation from sample compared to control

c: Mean of inhibition (%) at different time intervals (15, 30, 45 min) *: All values are means of 3 replicates

Dunaliela salina > ***Sc. dimorphus*** mutant > ***Chlorella*** mutant > ***Sc. dimorphus*** normal > ***Sc. acutus*** (mutant) > ***Chlorella normal*** > ***Sc. acutus*** (normal) > BHT (Table 1).

It was also, clear that the effect of vitamin extracts showed the highest inhibitory effect after 15 min of incubation.

Antioxidant activity of vitamin- algal extracts on Fe^{++}/H_2O_2 model system

In second lipid peroxidation model system, the antioxidant activity of vitamin algal extracts were examined by challenges oxidation model system containing Fe^{++}/H_2O_2. The hydroxyl radical (strong oxidizing agent) is readily generated from H_2O_2 to initiate multitude of possible free radical reactions via Fenton reaction and radical peroxidation (Namiki, 1990 and Shinmoto ***et al.***, 1992) as follows:

$$Fe^{++} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{+}$$

$$LH + {}^{\bullet}OH \rightarrow L^{\bullet} + H_2O$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$$

A Fe^{2+} and lipid hydroperoxide -dependent pathway also occurs.

$$Fe^{2+} + LOOH \rightarrow LO^{\bullet} + OH^{-} + Fe^{3+}$$
$$LO^{\bullet} + LH \rightarrow LOH + L^{\bullet}$$

The values of thiobarbituric acid reactive substances generated from Fe^{++}/H_2O_2 induced peroxidation in mice liver microsomes model system were lower vitamin extracts than values obtained from control. In other words, the vitamin algal extracts did not enhance the production of TBARS products (Table 2). The data showed that the inhibition (%) of TBARS generated from oxidizing model systems catalyzed by all algal vitamin extracts exhibited antioxidant activity. Since all these model systems differ in their inhibition (%) values, but their values were gradually increased with higher levels of algal vitamin extracts (100 and 200 ppm).
other algal vitamin extracts.

Sc. dimorphus and ***Chlorella*** mutant vitamin algal extracts have similar antioxidant activities towards the oxidation of mice liver microsomes. Also, vitamin extract of both normal cells of ***Chlorella*** and ***Sc. acutus*** have approximately similar antioxidant activity.

In order to compare the antioxidant activity against lipid peroxidation induced by Fe^{++}/H_2O_2 model system, the system was catalyzed with BHT [positive control (100 ppm)]. The inhibition (%) values (at 15, 30 and 45 min) of this system were 57.76, 66.15 and 51.09%, respectively. The inhibition values were less than the inhibition caused by vitamin extracts of all algal strains at similar concentration (100 ppm) under the same conditions. From the above mentioned data, the effectiveness of the vitamin algal extracts against lipid peroxidation induced by Fe^{++}/H_2O_2 was in the following descending order: ***Dunaliella salina*** > ***Sc. dimorphus*** (mutant) > ***Sc. dimorphus*** (normal) = ***Chlorella*** (mutant) > ***Sc. acutus*** (mutant) > ***Chlorella*** (normal) > ***Sc. acutus*** (normal) > BHT.

Antioxidant activity of vitamin- algal extracts on CCl_4 model system

Carbon tetrachloride (CCl_4) was added to rat liver microsomal model system to induce the peroxidation by mechanism, which does not require free iron (Fe^{+++}) like other peroxidation model systems. CCl_4 is reductively bioactivated by cytochrome 450 E_1 into trichloromethyl free radical ($^{\bullet}CCl_3$), which in presence of oxygen is subsequently converted into peroxy radical ($^{\bullet}OOCCl_3$). These reactive free radical metabolites can contently bind to macromolecules and also initiate lipid peroxidation (Goeptare ***et al.***, 1995; De Zwart ***et al.***, 1998).

Table 3 indicate the values of inhibition (%) of TBARS formation for the systems consisting of rat liver microsomes, lipid peroxidation induced by CCl_4 and catalyzed by vitamin algal extracts (100 and 200 ppm), BHT (40 and 100 ppm) in comparison with control.

Table 2: Effect of algal extracts on inhibition of mice liver microsome lipid peroxidation induced by Fe^{++} and H_2O_2 system

	Incubation period (min)						
	15		30		45		Mean
Treatments	TBARS[a]	Inh.(%)[b]	TBARS[a]	Inh.(%)[b]	TBARS[a]	Inh.(%)[b]	of Inh[c]. %
$FeSO_4$ (1 µM)	0.101		0.231		0.345		
Control ($FeSO_4$ ascorbic 0.5 mM)	5.990	00.00	9.81	00.00	12.30	00.00	00.00
BHT (40 ppm)	4.012	33.02	6.23	36.49	8.23	33.30	34.27
BHT (100 ppm)	2.530	57.76	3.32	66.15	6.035	51.09	58.33
Ext. of *Sc. ac.* (100 ppm)	1.776	70.35	4.06	58.60	5.45	56.23	61.72
Ext. of *Sc. acutus* (200 ppm)	1.50	74.95	3.61	63.20	5.01	59.40	65.85
Ext. of *Sc. acutus* mut. (100 ppm)	1.62	72.95	3.66	62.69	5.33	56.80	64.00
Ext. of *Sc. acutus* mut. (200 ppm)	1.46	75.62	3.19	67.48	4.49	63.60	68.90
Ext. of *Chlorella* (100 ppm)	1.85	69.11	3.42	65.13	3.98	67.74	67.32
Ext. of *Chlorella* (200 ppm)	1.51	74.79	3.13	68.09	3.66	70.34	71.07
Ext. of *Chlorella* mut. (100 ppm)	1.44	75.95	2.92	70.20	3.42	72.20	72.78
Ext. of *Chlorella* mut. (200 ppm)	1.16	80.63	2.63	73.19	3.06	75.20	76.34
Ext. of *Sc. dimorphus* (100 ppm)	1.28	78.60	2.89	70.54	3.72	69.80	72.95
Ext. of *Sc. dimorphus* (200 ppm)	1.27	78.79	2.56	73.90	2.66	78.40	77.03
Ext. of *Sc. dimorphus* mut. (100 ppm)	0.89	85.14	2.77	71.76	2.55	79.33	78.74
Ext. of *Sc. dimorphus* mut. (200 ppm)	0.66	88.90	2.26	77.57	1.30	89.40	85.29
Ext. of *Dunaliella* (100 ppm)	0.61	89.80	1.16	88.17	1.36	88.97	88.98
Ext. of *Dunaliella* (200 ppm)	0.34	94.43	0.852	91.31	1.19	90.35	90.35

The effect of algal vitamin extracts has shown a feature of an antioxidant activity. All algal vitamin extracts caused significant increases in inhibition (%) of TBARS generated from the CCl_4 model system. The antioxidant efficiency of all vitamin algal extracts (100 and 200 ppm) were superior than that of BHT a common synthetic antioxidant (positive control) (Table 3).

The values of inhibition % of TBARS generated from CCl_4 - model system catalyzed by ***D. salina*** vitamin extracts were higher than the values obtained from other catalyzed system. The inhibition % (at 15 min) values were 88.8 and 90.9% at the level 100 and 200 ppm, respectively. Therefore, the vitamin mixture extract of ***D. salina*** was the most potent one as an antioxidant. The antioxidant activity was 3.9 and 2.1 times higher than the BHT at concentration levels of 40 and 100 ppm (model system), respectively. The vitamin extracts obtained from mutant strain of ***Sc. dimorphus*** at 100 and 200 ppm (model system) inhibited the production of TBARS with 83.5 and 88.8% (at 15 min), respectively. While the vitamin extract of ***Sc. acutus*** including parent and mutant cells caused inhibition of 58.3-62 and 50.9-71.1% at both concentration levels of 100 and 200 ppm, respectively after 15 min of incubation period. On the other hand, the antioxidant activity of vitamin extract of ***Scenedesmus acutus*** was relatively lower than vitamins extract of other strains.

Table 3: Effect of algal extracts on inhibition of rats liver microsome lipid peroxidation induced by CCl_4 system

	Incubation period (min)						
	15		30		45		Mean
Treatments	TBARS[a]	Inh.(%)[b]	TBARS[a]	Inh.(%)[b]	TBARS[a]	Inh.(%)[b]	of Inh[c]. %
Control (CCl_4)	6.93	00.0	10.09	00.0	12.40	00.0	00.00
BHT (40 ppm)	5.33	23.0	7.83	22.3	9.35	24.5	23.26
BHT (100ppm)	3.93	43.3	6.55	35.0	6.34	49.0	42.40
Ext. of *Sc. ac.* (100 ppm)	3.40	58.3	4.62	54.2	5.04	59.0	57.16
Ext. of *Sc. acutus* (200 ppm)	2.89	50.9	5.40	46.5	5.92	52.0	49.80
Ext. of *Sc.acutus* mut. (100 ppm)	2.63	62.0	4.65	54.0	5.47	56.0	57.33
Ext. of *Sc. acutus* mut. (200 ppm)	2.00	71.1	3.93	61.0	4.49	64.0	65.36
Ext. of *Chlorella* (100 ppm)	2.64	62.0	4.47	55.7	5.02	59.5	59.06
Ext. of *Chlorella* (200 ppm)	1.93	72.1	3.33	66.9	3.98	68.0	69.00
Ext. of *Chlorella* mut. (100 ppm)	2.46	64.5	3.83	73.0	4.07	67.0	68.16
Ext. of *Chlorella* mut. (200 ppm)	1.78	74.3	2.71	73.0	3.15	75.0	74.10
Ext. of *Sc. dimorphus* (100 ppm)	2.08	70.0	2.76	73.0	3.15	75.0	72.66
Ext. of *Sc. dimorphus* (200 ppm)	1.38	80.0	2.13	79.0	2.45	80.0	79.60
Ext. of *Sc. dimorphus* mut. (100 ppm)	1.14	83.5	1.93	81.0	2.38	81.0	81.80
Ext. of *Sc. dimorphus* mut. (200 ppm)	0.77	88.8	1.62	84.0	1.80	85.0	85.90
Ext. of *Dunaliella* (100 ppm)	0.77	88.8	1.38	86.3	1.60	87.0	87.36
Ext. of *Dunaliella* (200 ppm)	0.63	90.9	1.22	88.0	1.28	90.0	89.60

(TBARS): Thiobarbturic acid reactive substance, a: TBARS formation was expressed as μM

b: Inhibition (%) was expressed as the reduction of TBARS formation from sample compared to control

c: Mean of inhibition (%) at different time intervals (15, 30 and 45 min) *: All values are means of 3 replicates

Table 4: Antioxidant vitamin contents of algal strains

	Vitamins content			
Algal strains	Carotenoids	Vitamin E	Vitamin C	Total %
Dunaliella salina	13.14	1.23	2.50	16.87
Scendesmus dimorphus mutant	4.75	2.93	5.00	12.68
Chlorella ellippsoida mutant	4.69	2.62	5.12	12.43
Scendesmus dimorphus normal	3.95	2.00	3.33	9.28
Chlorella ellippsoida normal	3.51	1.69	2.81	8.01
Scendesmus acutus mutant	4.75	3.75	5.00	13.50
Scendesmus acutus normal	3.20	1.89	2.93	8.02

*: All values are means of 3 replicates

In general, the vitamin algal extracts had a significant effect to prevent the production of TBARS in CCl_4 oxidizing model system. The inhibitory effect of vitamin extracts obtained from different algae strains and BHT against the formation of TBARS products from CCl_4 oxidizing model system was in the following descending order: ***Dunaliella salina*** > ***Sc. dimorphus*** (mutant extract) > ***Sc. dimorphus*** (normal) > ***Chlorella*** (mutant) > ***Chlorella*** (normal) > ***Sc. acutus*** (mutant) > ***Sc. acutus*** (normal) > BHT.

In order to compare an antioxidant activity of different algal extracts on the model system under study, the mean (average) of the inhibition % of TBARS production was calculated for each treatment at different time intervals (15, 30 and 45 min) (Table 1, 2, 3). The antioxidant activity of ***Dunaliella salina*** and ***Sc. dimorphus*** (mutant) were greatly higher than all other strains. For instance Dunaliella salina inhibits TBRS products induced by Fe^{++}/ascorbate, Fe^{++}/H_2O_2 and CCl_4 model systems by 99.6, 90.35 and 89.6%, respectively. The extract of ***Sc. acutus*** (mutant) and ***Sc. acutus*** (normal) had the lowest values for reduction of TBARS among all algal extract. The results also showed that the antioxidant activity was concentration dependant because the reduction values of TBARS products by algal extracts at 200 ppm were higher than the values obtained by 100 ppm concentration.

According to the obtained results, the effectiveness of algal extracts as an antioxidant agents i.e reduce the production of TBAS inducing with Fe^{++}/ascorbate, Fe^{++}/H_2O_2 and CCl_4 as system was in the following order: ***Dunaliella salina*** > ***Sc. dimorphus*** (mutant) > ***Chlorella*** (mutant) = ***Sc. dimorphus*** (normal) > ***Chlorella*** (normal) > ***Sc. acutus*** (mutant) > ***Sc. acutus*** (normal) > BHT.

Algal extracts proved highly powerful effects for inhibition of microsomal lipid peroxidation. The algal vitamin extracts showed more potent for inhibition of liver microsomal lipid peroxidation than the BHT, which is one of the most powerful synthetic antioxidant (Haraguchi ***et al.***, 1997). The algal extracts under study having three major antioxidant substances, carotenoide, vitamin E and vitamin C, which differ in their reduction power (Table 4). These agents are known to act as an antioxidant.

Lipid peroxidation, which can cause extensive damage to subcellular organelles and biomembranes has been demonstrated to occur in isolated mitochondria, lysosomes, microsomes and nuceli (Tirmenstein and Reed, 1988). The destruction of unsaturated fatty acids which occurs in lipid peroxidation has been linked with altered membrane structure and enzyme inactivation (Reed, 1993). In addition to lipid hydroperoxides and lipid radicals, lipid peroxidation generates activated oxygen species, such as hydroxyl radicals ($^{\bullet}OH$) and superoxide anions ($O_2^{\bullet -}$). Also, decomposition of peroxidized polyunsaturated fatty acid generates reactive carbonyl compounds, sometimes toxic like hydroxylalkenals, alkenals and aldehydes (Cadenas, 1995; Zimniak ***et al.***, 1997; De zwart, 1998). Therefore, vitamin algal extract can prevent most of the harmful effects, which may be induced from lipid peroxidation in human. Hence, inhibition of lipid peroxidation ***in vitro*** by vitamin of algae is considered as an approach to what occurs ***in vivo*** (Hurtado ***et al.***, 1997).

The stimulation of peroxidation by Fe^{++}/ascorbic, Fe^{++}/H_2O_2 and CCl_4 are believed to be dependent on generated of Fe^{++}, hydroxy radical ($^{\bullet}OH$), singlet oxygen ($O^{\bullet}$), peroxy radical ($^{\bullet}OOCCl_3$) and trichloromethyl free radical ($^{\bullet}CCl_3$), respectively (Kornbrast and Mavis, 1980; Packer and Fuchs, 1993; Goeptar ***et al.***, 1995).

The algal vitamin extracts (which contained tocopherols, vitamin C and carotenoids) act as an antioxidant by different mode of actions (Larson, 1988; Ito and Hori,1989; Tutour, 1998). For example, ascorbic acid was demonstrated in many qualitative studies to possess significant

antioxidant activity. ***In vitro*** study, ascorbic acid was shown to inhibit the peroxy radical initiated peroxidation of fatty acids. Also, vitamin C was shown to act as a chain breaking scavenger for peroxy radicals and also to act as a synergist with vitamin E. The vitamin C can donate a hydrogen atom to vitamin E derives phenolate radical, thus regenerating its activity (Larson, 1988; Miura ***et al.***, 1995).

Ascorbic acid (AH^-) + Radical ($^{\cdot}OH$)	→	H_2O + Monodehydro ascorbic acid ($A^{\cdot -}$)
$AH^- + O_2^- + H^+$	→	$H_2O_2 + A^{\cdot -}$
$AH^- + R^{\cdot}$	→	$RH + A^{\cdot -}$
$AH^- + H_2O_2 + H^+$	→	$2\ H_2O$ + Dehydro ascorbic acid (A)

Vitamin algal extracts contained tocopherols, which are the best antioxidant in biological system. The biological activity of vitamin E is generally due to its antioxidant action, specifically inhibition of lipid peroxidation in biological membrane (Qureshi and Qureshi, 1993). Tocopherols possess a 40-60 times higher antioxidant activity against Fe^{++}/ascorbate and Fe^{++}/NADPH induced lipid peroxidation in rat liver microsomal membrane and 6.5 times greater protection of cytochrome P_{450} against oxidative damage (Qureshi and Qureshi, 1993). The normal mechanism of action for vitamin E as antioxidant is due to inactivation of two equivalents of chain carrying peroxy radicals and termination of two potential radicals, reaction per molecule of inhibition. Also, the potential exist for regeneration of vitamin E through reaction with ascorbic acid as a reducing agent (Larson, 1988).

Also, vitamins mixture of algal extracts contained carotenoids which have a protective function against oxidative damage and have very powerful agent for quenching excited sensitized molecules and singlet oxygen (Gaby ***et al.***, 1991; Bondarev, 1997). In addition to this protection, they may also act as antioxidant under conditions where singlet oxygen is not formed (El- Qualja ***et al.***, 1995). Carotenoids (CAR) act as trap to peroxyl radicals $RO._2$ to form resonance-stabilized carbon -centered radicals ROO-CAR$^{\cdot}$ which undergo a reversible reaction with oxygen (Burton and Ingold, 1984; Tutour ***et al.***, 1998). Therefore, carotenoids protect the biological lipid of membranes from reactions of O_2 leading to peroxidation (Larson, 1988).

In conclusion, vitamins mixture can protect the lipid peroxidation by different mode of action, it appears antioxidant activity in all model system. In addition, algal vitamin extracts are containing tocopherol, carotenoids and ascorbic acid. The antioxidant effectiveness of vitamins mixture is far more effective than the other when used alone due to the synergistically action between them (Cahyana ***et al.***, 1993). One would recommend the use of vitamin algal extracts as non-conventional source of vitamin to cover the requirement as vitamins source and also as an natural antioxidant to protect body from harmful effect of reactive oxygen species ($^{\cdot}OH$ and $O_2^{\cdot -}$) and lipid peroxidation products, which are involved in the pathophysiology of many human diseases, in addition the vitamin algal extracts do not exhibit side effects on human health (Takenaka ***et al.***, 1996).

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