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Antiproliferation and antioxidant properties of lipid extracts of the microalgae *Scenedesmus obliquus* grown under stress conditions

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ABSTRACT

Microalgae Scenedesmus obliquus was cultivated in nutrient medium either aerated with various levels of CO_2 (0.3%, 3%, 9% and 12%) or supplemented with various Fe^{+3} ions (2.5, 5, 10 and 20 mgL^{-1}) in order to accumulation of high amounts lipid and evaluated their chemical composition and bioactivity. Among all algae cultures, biomass yield, total lipid content and lipid productively were significantly differences ($P<0.05\%$), with various degrees of the treated CO_2 concentrations, cultures with 12% CO_2 exhibited higher lipid content (33.14% of dry weight) and lipid productivity (69.33 $mgL^{-1}d^{-1}$), with optimum content of fatty acid (FAs) compositions. The major FAs were found to be oleic (18:1, 32.19%), palmitic (16:0, 29.54%) and stearic (18:0, 12.26% of total FAs). Algal cells grown at 20 mg/L Fe^{3+} concentration had the highest lipid content (28.12%), lipid productivity (94.35 mg/L/d) and FAs profile were C16:0 (25.12%), C18:0 (16.58%) and C18:1 (34.44%).The main antioxidant compounds: total carotenoids, tocopherols and phenolic in algae cells grown at favorite condition (12% CO_2 or and 20 mg/L/d) were occurred in significant quantity. The oil obtained from S. obliquus grow at large scale (300 liter, in media aerated with12% CO_2 and supplemented with 20 Fe^{+3} mg/L), showed good antioxidant activity, compared with that of antioxidants standards (α-tocopherol and BHA) as assayed with five antioxidant methods (scavenging radicals of DPPH, ABTS, ·OH, superoxide and ferric reducing power). Their antioxidant effect in S. obliquus oil may be correlated with the percent of phenolic, tochopherols and carotenoids. Moreover, the oil showed high proliferation inhibitors action against human breast (MCF-7), hepatic (HepG2) and colon (HCT-116) cancer cell lines, with IC_{50} values ranged from 11.62 to 15.22 µg ml^{-1}. Thus, S. obliquus oil may serve as sources of natural antioxidants for health promotion and as chemopreventive strategies for various human cancers.

Key words: *Scenedesmus obliquus*, microalgae oil, anticancer, Human breast adenocarcinoma cells (MCF-7), hepatocellular carcinoma cells (HepG2), and colon carcinoma (HCT-116) and antioxidant activity

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INTRODUCTION

Marine sources are widely regarded as possessing interesting lipid compositions, which make them attractive as a source for lipid extraction. Lipids possess several functional activities including anti-inflammatory, antiviral antibacterial, anti-tumor and anticancer action [1]. The lipid products from microalgae include oil/steroids, fatty acids (e.g., saturated and unsaturated fatty acids), fat-soluble pigments (carotenoids), and fat-soluble vitamins (Vit. E, A, D) with potential applications in biodiesel, food, cosmetics, and pharmaceuticals [1,2]. Thus, microalgae lipids have gained attention in recent decades due to their application potential in many areas. Algae lipid class consists primarily of the high polyunsaturated fatty acids (PUFAs) that are well documented as essential for good human health [3]. For instance, it has been documented that ω -3 fatty acids play an important roles in the prevention of cardiovascular diseases [4]. Also, other lipophalic compounds such as carotenoids and tocopherols have been related to the prevention of several diseases including cancer, coronary heart diseases, inflammatory disorders, neurological

degeneration and aging [1]. Polyphenols like-lipid of microalgae exhibited markedly antioxidant activities and other physiological actions [5] including antibacterial [6], chemopreventive, UV- protective [7] and antiproliferative effects [8]. They are also known to act as detoxifying agents against heavy metals [9&10], and have myriad other bioactivities that could potentially be exploited for use in functional foods [11].

The growth of cells and lipid accumulation by microalgae is influenced by nutrient and environmental conditions [12]. To enhance the economic feasibility of microalgal based, biomass, lipid and fatty acids productivity are the key parameters to be optimized. The biomass yield and biochemical composition of microalgae can be easily modified by changing medium ingredients or culture conditions to obtain a higher yield of desirable biomolecules [1]. Previous studies proved that lipid content in some microalgae could be modified under various growth conditions such as nitrogen starvation [13,14], high salinity or CO_2 concentration [15], phosphorus limitation [16] or some metals stress such as ferric [13,17].

According to mentioned previously, nutrient concentration is a key factor in the microalgae as bio-system of lipid production. In this study, the effects of CO_2 and Fe^{3+} ions concentrations on the biomass yield, lipid accumulation, lipid productivity, fatty acid profile and the level of antioxidant compounds of *S. obliquus* cultures were evaluated. Antioxidant and antiproliferation activities of the oil obtained from microalgae grown at large scales in medium containing high Fe^{+3} ions and aerated with 12% CO_2 were assessed.

MATERIALS AND METHODS

Alga sources

Scenedesmus obliquus was isolated from Egyptian soil

Growth treatments

1-Effect of CO_2 concentration

Scenedesmus obliquus was cultivated in 4 Liter Erlenmeyer flask with 3 L working volume of N-9 medium under 25 ±3.0°C and illuminated with 10 florescent tubes. For the experiments on the effects of CO_2, the cultures were aerated with different flow rates of CO_2 mixed with ambient air to prepared CO_2 concentration of 0.03, 3, 9 and 12%. The initial pH of cultures was 7.0 and regularly determined with Hanna pH meter..

2- Effect of Fe^{3+} ions concentration

In this experimental, *Scenedesmus obliquus* was cultivated in 4 Liter Erlenmeyer flask with 3 L working volume of N-9 medium supplemented with various levels of Fe^{3+} 2.5, 5, 10 and 20 mg/L (in $FeCl_3$ form). The cultivation conditions were: temperature 25 ±3.0°C; pH 7.0; illuminated continuously with 10 florescent tubes and aerated with 0.3 % CO_2.

Growth analysis

The growth of *S. obliquus* culture was spectrophotometracally determined regularly every two days at 685 nm (OD_{685}). The cell dry weight (CDW) of *S. obliquus* biomass was obtained by filtering 20 ml aliquots of culture through a cellulose acetate filter membrane (0.45 µm pore size, 47 mm in diameter). Then, the loaded filter was dried at 70^{o}C in a vacuum oven until constant weight. The dry weight of the blank filter was subtracted from that of the loaded filter to obtain the CDW. The calibration curve was prepared by drawing the relationship between OD_{685} and CDW.

Cultivation of algal cells in large scale

In this experimental, *S. obliquus* was cultivated in an aquarium (300 L) with 250L working volume of N-9 medium supplemented with 20 mg/L Fe^{3+} and aerated with 12% CO_2 under optimum conditions as mentioned above, to obtain a large quantity of algal biomass and oil yield in order to use in biological assays.

Determination of lipid content:

Cells were harvested by centrifugation at 10,000 xg for 10 min and lyophilized. The total lipids were extracted with chloroform/methanol (2:1, v/v) and gravimetrically quantified [18].

Identification of fatty acids

Fatty acids were converted into methyl esters (FAMEs) by direct transmethylation of lipid extracts with sulphuric acid in methanol [19]. The FAMEs were analyzed by the Agilent Technologies 6890 N- GC-system (USA) equipped with a flame ionization detector (FID) and a HP-5% Phenyl Methyl Silixane capillary column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Nitrogen was used as a carrier gas at a flow rate of 1.2 ml/min. The oven temperature was 70^{o}C with a 2-min hold, raised to 230^{o}C at 8^{o}C min-1 and held at 230^{o}C for 20 min. Injector and

detector temperatures were 250 and 280^{o}C, respectively. FAMEs was identified by comparison their retention times with those of standard FAMEs (Sigma, purity > 99.9% by GC). Performance of GC-system was checked with blanks (methylation reagent in hexane) and standard FAMEs prior to analysis. All the tests were performed in triplicates.

Determination of total tocopherols:
Total tocopherols were spectrophotometrically determined as described by Wong et al., [20].

Determination of carotenoid contents:
The total catotenoids content in algae oil were spectrophotometrically determined as reported in AOCS [21] method.

Determination of total phenolic content:
Total phenolic content (TPC) was determined using Folin-Ciocalteu's phenol reagent with gallic acid as a standard [22].

Antioxidant activity
The antioxidant activity of *S. obliuuqs* oil (SOO) was measured by scavenging ability of hydroxyl, superoxide ABTS and DPPH radical and ferric reducing power methods. All tests were run in triplicates and averaged.

1- 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay
The ability of the SOO to scavenge DPPH radical was estimated based on the method of Tagashira and Ohtake [23]. In brief, 3 ml methanolic DPPH (0.15 mM) was added to 1 ml of algae lipid (containing 2 to 100 µg of lipid) and incubated for 30 min at room temperature. The absorbance of tested sample and standard antioxidants (BHA and α-tochopherol) were measured at 517 nm. The concentration providing 50% inhibition (IC_{50}) was calculated from a graph representing the inhibition percentage (I%) against S.O concentration.

2-Ferric reducing power (FRP)
The ferric reducing power (FRP) described by Chu et al. [24] was adopted. A 2.5 ml of 0.1 M potassium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution were mixed well with 1.0 ml of SOO of varying dilution sample. After incubation at 50^{o}C for 20 min, 2.5 ml of trichloroacetic acid (10%, w/v), purified water (2.5 ml) and $FeCl_3$ (0.5 ml 0.1%, w/v) were added to the reaction mixture. The mixture was incubated at 30^{o}C for 30 min and absorbance was measured at 700 nm.

3-ABTS scavenging radical assay
TBAS radical scavenging activity of the SOO was determined according to the Re, et al. [25] method. The reduction percentage of absorbance at 15 min compared to the initial value was determined.

4-Hydroxyl radical assay
Hydroxyl radical scavenging activity of the SOO was carried out according to the method of Muller [26].

5-Superoxide anion radical scavenging assay
The superoxide anion scavenging activity of was spectrophotometrically determined at 560 nm [27]. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percent inhibition (%) of superoxide anion generation was calculated as follows: %Inhibition = [(A_0, control – A, sample) / $A_{0,}$ control] $A_{0,}$ control X 100. The scavenging of superoxide anion as IC_{50} value (50% of inhibitory concentration in mg/ml) was calculated from inhibition curve.

Antiproliferation activity
1. Cell Culture
Human cancer cell lines MCF-7 (breast adenocarcinoma cells), Hep-G2 (hepatocellular carcinoma cells) and HCT-116 (colon carcinoma cells) were obtained from Cambrex BioScience (Copenhagen, Denmark) and used to evaluate the cytotoxic activity of moringa oil and etoposide (positive control, 25 µg/ml). The cells were cultured in DMEM (Dulbeco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum, L-glutamine (2 mmol/l), penicillin G sodium (100 U/ml), streptomycin sulphate (100 units/ml), amphotericin B (250 ng/ml) and maintained at 37ºC with 5% CO_2 in a humidified atmosphere. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested oil were dissolved in dimethylsulphoxide (DMSO), and then diluted thousand times in the assay. All experiments were repeated three times.

2. Antiproliferation assay

The cytotoxic activity of SOO on various cancer cells was measured by MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay. The assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form dark blue insoluble formazan crystals [28]. Cells ($0.5X10^5$ cells/ well), in serum-free media, were plated in 96-wells microplates, and treated with 20 µl of SOO solution at concentrations ranging from 4.0 – 100 µl/l, subsequently the cells were incubated for 48 h. Negative control was treated with DMSO. After incubation, media were removed and 40 µl MTT (5mg/ml of MTT in 0.9% NaCl) solution / well were added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 µl of acidified isopropanol (0.04 N HCl in absolute isopropanol) / well and plate was shacked at room temperature, followed by photometric determination at 570 nm. The data were obtained from three independent assays, using five well for each assay, and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability

Statistical analysis

All measurements were carried out in triplicate. Statistical analyses were performed using a one-way analysis of variance ANOVA, and the significance of the difference between means was determined by Duncan's multiple range tests. Differences at $P < 0.05$ were considered statistically significant. The results were presented as mean values ± SD (standard deviations).

RESULTS AND DISCUSSION

Recently, microalgae biomass appears to be the one of the promising feedstock's as source of functional food ingredient. The feasibility of use microalgae as source of lipids has been well documented [1,11]. The lipid content could be increased in microalgae when cultivated under define conditions, such as high iron concentration and nitrogen starvation [11,13]. In the present study, the CO_2 as carbon source and Fe^{3+} concentrations approaches were used to accumulate lipid content in *S. obliquus* cells.

Tabel (1): The livels of lipid production and growth rate of *Scenedesmus obliquus cultivated* cultivated in median supplemented with diferent CO_2 concentrations.

CO_2 Aeation %	Biomass (Cells dry weight $mg^{-1}L$)	Ratio	Total lipid Productivity ($mgL^{-1}d^{-1}$)	Lipid content %
0.3	512 ±210[a]	1.0	25.1	4.21
3.0	891 ± 263[a]	1.74	45.32	8.24
9.0	1651 ±262[b]	3.22	51.96	20.63
12.0	411 ± 105[a]	0.80	69.23	33.14

Each value represents the mean of three replicates and based on dry weight

All values are significant at (P< 0.5)

Effect of CO_2 concentrations on *S. obliquus* growth and total lipids

The *S. obliquus* was grown on N-9 medium, gases with four different CO_2-concentrations of 0.3% (ambient air level), 3%, 9% and 12% in air at 23 ±3°C as only one carbon source, for 10 days (Table 1). The biomass yield (BY, dry weight d.w), total lipid productivity and total lipid contents in *S. obliquus* cultures are shown in Table 1. The significant differences (P>0.5%) in these parameters among all cultures were observed, depend on CO_2-concentrations. The highest BY were recorded (1651 ±262 mg L^{-1}) in 9% CO_2 culture, followed by (891 ±210 g L^{-1}) in 3% CO_2 and (512± 263 mg L^{-1}) in 0.3% CO_2 cultures. The lowest BY (411 ± 105 mg L^{-1}) was obtained in 12% CO_2 culture. Thus, *S. obliquus* culture in 9% CO_2 had higher BY than that recorded in other cultures. The results is in agreement with the finding of Fulka et al. [29], who reported high biomass was found in *Chlorella* sp. cultivating with 3% CO_2. On other hand, the result is similar to those reported for several green algae in the literature, showing that an excess concentration of CO_2 above 15% leading to a decline in the algal growth rate [30, 31, 32]. Chiu et al. [30] reported that *Chlorella* sp. *D. terticlecta* and *N. oculata* microalgae had optimal growth potential with 5 % CO_2,

while the growth rate was decreased with increasing of CO_2 (up to 15%) levels. Tang et al. [33] demonstrated that *S. obliquus* SJTU-3 strain grown with 5% and 10% CO_2 concentration had relatively high biomass yield and high biomass productivity than that obtained in 2% CO_2 culture. De-Morais and Costa [31] found that microalgae like *S. obliquus* can grow beyond 6% and reach the maximum biomass at 18% CO_2.

Table (2): Fatty acid profile extracted oils from *S. obliqus* cultivated on nutrient media with medium with the feeding of 12% CO_2 and 20 mg^{-1} L Iron.

Fatty acids[a]	Relative content %[b]	
	12% CO_2	20 mg^{-1} L Iron
Lauric acid (C12:0)	4.6	5.32
Myristic acid (C14:0)	5.45	3.45
Palmitic acid (C16:0)	29.54	25.12
Heptadecanoic acid (C17:0)	5.73	4.85
Stearic acid (C18:0)	12.26	16.58
Oletic acid (C18:1)	32.19	34.44
Linoletic acid (C18:2)	7.43	5.68
Linolenic acid (C18:3)	1.37	2.44
Arachidic (C20:0)	1.43	1.85

[a:] Fatty acid was identified based on the total known fatty acids(Retention time, min)

[b.]: The amount of the fatty acid was evaluted throwth the peak area.

Tabel (3):Levels of lipid production and growth rate of *Scenedesmus obliquus* cultivated on nutrient media with different Iron concentrations

Iron Concentrations mg^{-1} L	Biomass (Cells dry weight $mg^{-1}L$)	Total lipid Productivity ($mgL^{-1}d^{-1}$)	Lipid content %
0	0.891 ±0.210[a]	20.1	5.75
2.5	0.912 ± 0.263[a]	33.24	9.21
5	1.311 ± 0.110[b]	58.34	13.52
10	2.450 ±0.151[c]	75.69	15.34
20	1.250 ± 0.85[b]	95.35	28.12

Each value represents the mean of three replicates and based on dry weight
All values are significant at (P< 0.5)

Table 1, shown the total lipid contents (TL, w/w) and total lipid productivity (TLP, mg L^{-1} d^{-1}) in *S. obliquus* cultures with CO_2 (0.3- 12%) concentrations. Among all cultures, the total lipids content (4.21-33.14%, w/dw.) in algal cells was increased as results to increase CO_2 (0.03% - 12%) concentration. The TL contents in 3%, 9% and 12% CO_2 cultures were 8.24%, 20.63 % and 33.14% (d.w), respectively and it was found to be 4.21% in 0.03% CO_2 (ambient air) culture. Therefore, accumulation of TL was enhanced as resulted to increase CO_2 concentration. The total lipid productivity (TLP, mg $L^{-1}d^{-1}$) of *S. obliquus* showed increasing trend with increase of CO_2 concentration in the following order: 25.1 (0.03% CO_2) < 45.32 (3% CO_2) < 51.96 (9% CO_2) < 69.23 mg $L^{-1}d^{-1}$ (12% CO_2). Thus, among all CO_2 cultures, the TL content was significantly positive correlated with increase of TLP in *S. obliquus*. The high TLP obtained at high CO_2, possibly due to a high rate of photosynthesis process, which algae

require CO_2 as one of the main nutrients for their growth. Also, the results is agrees with the finding of Velea et al. [34], who reported that 1 g of micro-algal biomass could consume 1.5 g/L of CO_2, that lead to increase lipid content and lipid productivity. However, CO_2 ranged 5–20% could be considering as optimal carbon source for benefit of microalgae growth and synthesis more bio-molecules. Similar results were reported by Tang et al. [33], that *S. obliquus* SJTU-3 showed great abilities of CO_2 fixation under high CO_2 levels (ranges 5 to 20%) and performed the better growth potential at 10% CO_2 level.

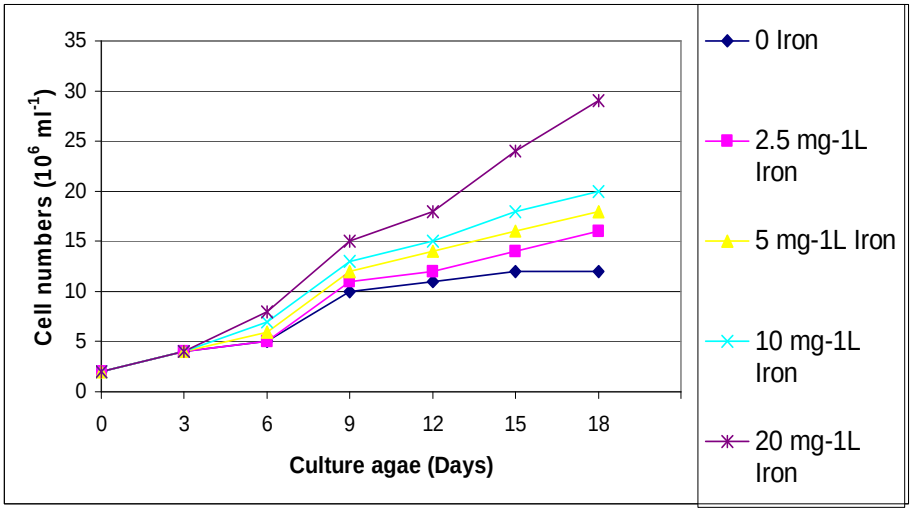

Fig.(1): Effect of iron concentrations on growth curve of *Scenedesmus obliquus*

Table (4): Antioxidant compounds of oils extracted from Scenedesmus obliquus cultivated on nutrient media with medium with the feeding of 12% CO_2 and 20 mg^{-1} L Iron.

Antioxidant compounds	Content % (DW)	
	12% CO_2	20 mg^{-1} L Iron
Carotenoids	2.36	3.64
α–tocopherols	1.65	2.31
total phenolic	0.65	0.59

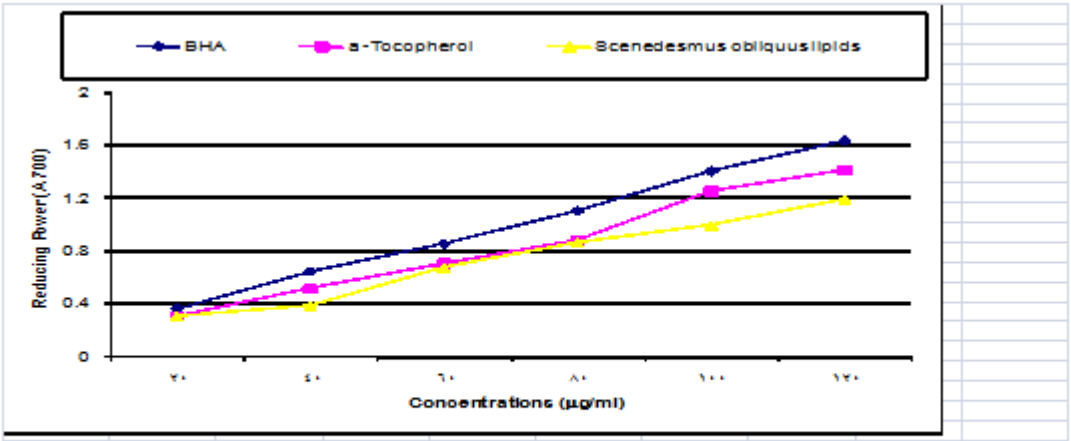

Fig. (2): Ferric reducing power at different concentrations of *Scenedesmus obliquus oil*, BHA and α-Tocopherol
Each reaction was performed in 3 ml of reaction mixture.
The control reaction was performed in a reaction mixture without the extract

Table (5): Antioxidant activity of *Scenedesmus obliquus* oil,, BHA and α –Tocopherol

Assay	IC_{50} (mg ml^{-1})			LSD at level
	BHA	α -Tocopherol	*S. obliquus oil*	(P< 0.05)
DPPH· (% Scavenging DPPH· radical)	14.67 ±1.12	17.34 ±1.11	35.76 ±1.54	**2.86**
ABTS (% scavenging ABTS radical)	9.67 ±0.87	12.98 ±0.89	25.54 ±2.67	2.52
OH·(% scavenging OH· radical)	13.44 ±0.88	19.23 ±1.32	51.73 ±2.84	2.78
Superoxide (%scavenging O^{-}_{2} radical)	15.65 ±0.98	20.54 ±1.34	39.45 ±2.39	3.95

The target oxidative substances values represent the % of scavenging activity.
Each value represent the mean of three replicates (n=3)

Table (6): Anti-proliferation activity of *Scenedesmus obliquus* oil against three models human cancer cell lines breast MCF-7, HepG2, HCT-116 and Paclitaxel as anticancer drug

Human cancer cell lines	IC_{50} g/ml	
	Scenedesmus obliquus oil	Paclitaxel
MCF-7	2.92	0.45
HepG2)	9.40	0.52
HCT-116	9.48	0.35

Each value represent the mean of three replicates (n=3)

Effect of CO_2 concentration on fatty acid composition

Fatty acid (FAs) profile of *S. obliquus* culture (with at 12% CO_2) characterized by high lipid content and lipid productivity are shown in Table 2. In general, FAs mainly contains the saturated (SFA) and monounsaturated (MUFA) carbon chain length from C16-C18 and lower amount of polyunsaturated fatty acids (PUFA). The most abundant (>10%) FAs were oleic (18:1, 32.19%), palmitic (16:0, 29.54%) and stearic (18:0, 12.26% of total FAs). Also, minor quantity (<10%) of FAs were detected as follows: 7.43% linoleic (18:2), 5.73% heptadecanoic (C17:0), 5.45% myristic (14:0), 4.60% lauric (12:0). Thus, *S. obliquus* had a high content of palmatic, oleic acid, steric and other saturated FAs (12:0, 14:0 and 17:0) which accounted about 91 % of total fatty acids (TFAs). Ho et al. (2010) found that *S. obliquus* cultivated at 10% CO_2 has a relatively simple fatty acid profile with C16:0 (15.06 %), C18:0 (17.16 %), C18:1 (15.55%) and C18:2 (13.39%) as the major fatty acids. However, low CO_2 level (3-12%) in cultures, was favorable condition for the accumulation of saturated fatty acids in algae cells, while at high CO_2 (< 20%) was the favorable for accumulation of polyunsaturated fatty acids [35]. It has been also reported that high CO_2 levels (> 20%) were favorable for the accumulation of total lipids and polyunsaturated fatty acids [33]. Over all, the results in this study showed that *S. obliquus* had similar fatty acid profile like that found in *Dunaliella*, *Chlorella*, and *Spirulina* species.

Effect of Fe^{3+} concentration on growth and total lipids

Table 3 and Fig. 1 shown the biomass yield (BY) of *S. obliquus* cultivated in medium supplemented with Fe^{3+} ions at concentration ranged from 2.5 to 20 mgL^{-1}. The BY in algae cultivated at 2.5, 5.0, 10.0 and 20 mgL^{-1} Fe^{3+} concentration were 0.912 ± 0.263, 1.311 ±0.110, 3.735 ± 0.238.4 and 1.25 ±0.085 g L^{-1}, respectively. Thus, the highest final BY was obtained in cultures with 10 mgL^{-1} Fe^{3+}. Also, the BY obtained at 2.5 mg/L Fe^{3+} did not significantly ($P < 0.05$) differences that in cultures without added of Fe_3^{+}. Therefore, no trend was observed between increases of biomass and increased of Fe^{3+} concentration in medium. Total lipid productivity (LP) and total lipid contents (TL) in *S. obliquus* cultures showed increasing trend with increasing Fe^{3+} concentration in nutrient medium (Table 3). The maximum TL (%) and LP (in parentheses, mg $L^{-1}d^{-1}$) of 28.12 (95.35) was obtained at 20 mg L^{-1} Fe^{3+}, followed by 15.34 % (75.69) at 10 mg L^{-1} Fe^{3+} and 13.52% (58.34 mg $L^{-1}d^{-1}$). In general, the levels of TL and LP in algal culture supplemented with various Fe^{3+} concentrations ranged 2.5 – 20 mg L^{-1} significantly ($P<0.05$) higher than that obtained in cultures without Fe^{3+} addition. However, a possible reason for this could be that Fe^{3+} ions are essential for the cell growth and induce synthesis of lipid due to predominant lipid metabolic pathway. Liu et al. [36] reported that high iron level could induce lipid accumulation in *Chlorella* species. However, microalgae could increase the lipid biosynthesis under nutrient limitation when energy source (light) and CO_2 are available and when the cellular mechanisms for the photosynthesis are active [37]. Previous studies had displayed that lipid content in some microalgae could be modified as results to changes the growth condition such as nitrogen deprivation and high salinity [13]. Generally, the relatively high Fe^{+2} levels could be inducing the considerable lipid accumulation due to changes the metabolic pathways to the lipid synthesis [36]. Cao et al. [15] stated that the lipid accumulation and lipid productivity improved in *C. minutissima* with the increase in Fe^{3+} concentration. In our

previous research, with the increase of Fe^{3+} levels, the accumulation of total lipid and total lipid productivity in *S. obliquus* cultures was increased [17].

Effect of Fe^{3+} concentration on fatty acid composition

As shown in Table 2, the main FAs in *S. obliquus* cultures supplemented with Fe^{3+} 20 mgL^{-1} were C16:0 (25.12%), C18:0 (16.58%) and C18:1 (34.44%), which accounted about 76% of the total fatty acids (TFAs). This results revealed that the major fatty acid compositions of *S. obliquus* cultivated in a nutrient rich medium with either 12% CO_2 or 20 mg/L Fe^{3+} were palmitic, stearic acid and oleic acids. Lauric (12:0), linoleic (18:2) and linolenic (18:3) acids were also present in minor quantities. Many reports have shown that composition of nutrients medium can aid lipid accumulation and change in fatty acid composition in most several microalgal species [13,17,38].

Finally, the major fatty acids in *S. obliquus* were C16:0, C18:0, C18:1, C18:2 and C18:3. The palmitic acids, stearic acid, oleic acid and linoleic acid are well known for their various applications in pharmaceutical and cosmetic and food industries. On other hand oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) are a source of healthy food fats. Also, the profile of fatty acid has a composition approximately similar to olive-oil, which contains a higher percentage of oleic acids. Oleic acid has a good balance of oxidative stability for longer storage and as function food ingredient [17].

Antioxidant compounds in algal cells

The quantitative values (% of dry weight) of the antioxidant compounds: total carotenoids, tocopherols and phenolic in algae cultures aerated with 12% CO_2 or supplemented with 20 Fe^{+3}mg/L (in parenthesis) are shown in Table 4. The results revealed that these antioxidants were found in high quantity with values of 2.36% (3.64%), 1.65% (2.31%) and 0.65% (0.59%), respectively. These results are in agreement with previous studies [39]. The characteristics of these antioxidant compounds in algae cultures may offer interesting prospects for food function products. It is known that phenolic and carotenoids concentration had high correlation with significant antiproliferation and antioxidant properties [2,3,12,17].

The aforementioned results revealed that *S. obliquus* had a great ability to accumulate high lipid content with favorite's fatty acid composition and antioxidant compounds. Therefore, it was cultured at large scales in define medium aerated with 12% CO_2 coupled with supplemented with 20 mg/L, for accumulation high lipid content in order to use the oil produce as function food ingredients or for their application potential in many areas.

Antioxidant capacity of the oil obtained from *S. obliquus* cells (SOO) grown at large scales were assessed with five methods (scavenging of DPPH, ABTS, superoxide ($^{\cdot-}O_2$), $^{\cdot}OH$ radicals and ferric reducing power techniques). The SOO exhibited good antioxidant activity, which may be expected mainly due to the presence of high quantity of lipophilic compounds (tocopherols, carotenoids and phenolic-like lipid, Table 5).

Radical $^{\cdot-}O_2$ and $^{\cdot}OH$ scavenging activity

The both of Superoxide anion and hydroxyl radicals are a precursor to active free radicals that have potential of reacting with biomacro-molecules (DNA, protein, and lipid) and there by inducing tissue damage. As shown in Table 5, SOO exhibited the scavenging activity against superoxide anion ($^{\cdot-}O_2$) and $^{\cdot}OH$ radicals generated from non-enzymatic systems, with concentration dependent manner. The IC_{50} values were 39.45±2.39 and 51.73±2.84 µg /ml, respectively. This scavenging effect was found to be comparable with that of standard antioxidants (BHA and TOC),

ABTS and DPPH scavenging activity

Data in Table 4 showed that SOO had a good scavenging ability against ABTS and DPPH radicals, with concentration dependent manner. The IC_{50} values were 25.54±2.39 and 35.76 ±1.54 µg /mL, respectively. According to these values, SOO exhibited a high radical scavenging activity towered ABTS radical than that the DPPH radicals. These differences in scavenging activity of SOO toward both radicals are due to different mechanisms, in DPPH assay, the antioxidant effect may be dependent on their ability to pair with the unpaired electron of a radical. Whereas, antioxidant effect towered ABTS may be due to scavenging of proton radicals induced through donation of electrons [30].

Ferric reducing power (FRP)

The FRP assay measures the ability of an antioxidant compound to reduce a ferric oxidant (Fe^{3+}) to a ferrous form (Fe^{2+}) by electron-transfer; this indicates the capacity of the compound to reduce reactive species [40]. The data in Fig. 2 revealed that the FRP values were increased with increasing of SOO concentrations. For example, these values were 0.36, 0.75 and 1.27 at concentration of 20, 60 and 100 µg/ml, respectively. These values were closed to those values of standard antioxidants (α-tecophrol and BHA). Thus, this activity may be attributed to its hydrogen-

donating ability compared with that for standard antioxidants. It is known well that BHT and BHA had a high RP effect, due to its ability to donate a hydrogen atom to a free radical, that terminating free radical reaction [1].

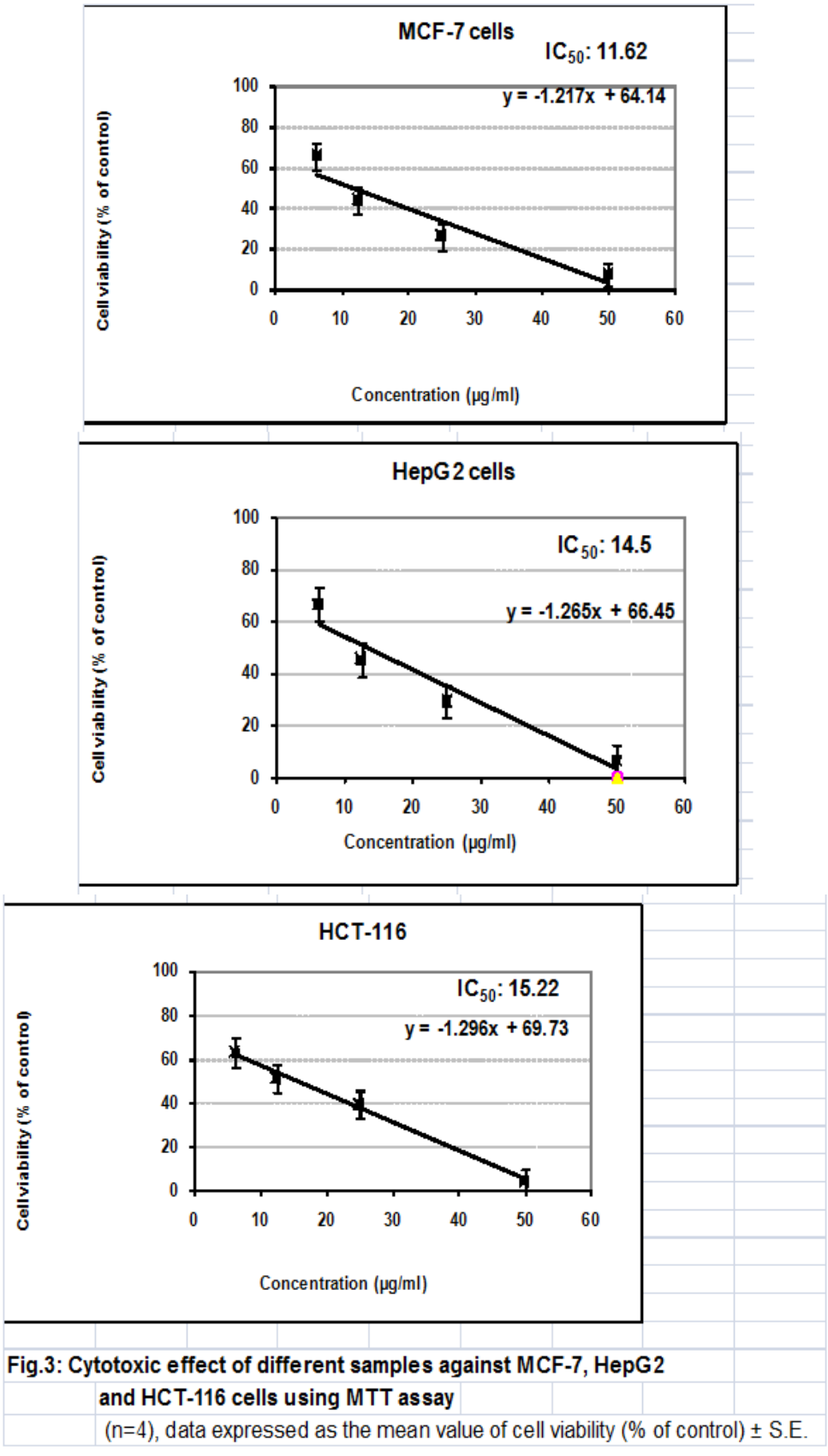

Fig.3: Cytotoxic effect of different samples against MCF-7, HepG2 and HCT-116 cells using MTT assay

(n=4), data expressed as the mean value of cell viability (% of control) ± S.E.

In general, a relationship was existed between concentration of SO and its reducing power and scavenging activities against DPPH, ABTS, $^{\cdot}OH$ and $^{\cdot-}O_2$ radicals. In previous reports demonstrated that there was a positive correlation between antioxidant properties of algal oils in various oxidative systems and content of antioxidant compounds include: tocopherol, carotenoides, phenolic compounds [2,12]. It is known well that lipid-soluble antioxidant compounds protect cellular membranes damage from lipid peroxidation induced by free radicals and reactive oxygen species by (i) suppressing their formation; (ii) acting as scavengers and (iii) acting as their substrate mechanisms.

Antiproliferation activities (APA)

Antiproliferation activities (APA) of *S. obliquus* oil (SOO) were assessed against three human cancer cell lines (MCF-7, Hep-G2 and HCT-116) at doses ranged from 0.20 to 20.0 µg/ml for 48 h (the cell viability was compared with both negative and positive controls). As shown in Table 6 and Fig. 4, SOO exhibited a good APA effect against tested cell lines, in a dose depended manner. Among all cell lines tested, MCF7 was the most sensitive cell to SOO followed by Hep-G2 and HCT-116, with IC_{50} values of 11.62, 14.5 and 15.22 µg/ml, respectively. The APA of SOO could be correlated to present of fatty acids (saturated and unsaturated), carotenoids and phenolic compounds in the oil. These compounds are display very effective anti-proliferation affect against cancer cell lines through apoptosis action [42]. Also, the APA action of fatty acids could be related to their ability to induction of lipid oxidative process in tumor cells. The lipid peroxide product could be lead to damage of the bio-molecules such as variety of enzymes, proteins, DNA and deplete ATP levels in cancer cells as well as causes apoptosis action [43,44].

In conclusions, the results showed that S. *obliquus* accumulate high amount of oil characterize by significant amounts of oleic and linoleic acids and natural antioxidant compounds. Also, oil exhibit good antioxidants and antiproliferation action, which may be help for health promotion and disease prevention and could be use as function ingredient. Further research should be conducted to identified the most active compound have biological activity in the S. *obliquus* microalgae oil.

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