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Isolating Some fatty Acids- Enriched Oils Used in Biofuels from alga Salinity Tolerant *Dunaliella* sp.

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ABSTRACT

This study aimed at isolating and purifying *Dunaliella* sp., which belongs to chlorophyte division, which reflects a high tolerance to salinity ranging from (0.1-5 M). Also, this study looked for isolating bio-active oils as materials for the alternative or clean energy associated with fossil fuel (biodiesel). *Dunaliella* sp. grown at Ben Amotz (BA) medium at batch culture under ideal laboratory circumstances. *Dunaliella* sp. exposed to a diversity of salinity concentrations, light sources and some nutrients were removed, individually or doubled. This study used GC/MS to analyze oils isolated from above algae. Chemical analyses have disclosed that it contains sixteen fatty acids, nine of which belongs to saturated fatty acids, at 56%, while the other seven are unsaturated, at 44%. It was noted that fatty acids of long carbon chains (C18) are associated with oil isolated from algae grown in cultural media supported with (+N) compared with (-N) media. Values, data and statements have showed that *Dunaliella* sp. (local isolation) is very suitable to produce Biodiesel, and it contains a high level of fatty acids along with its instant growth and ability to adapt to different diversified environments and circumstances.

Keywords: *Dunaliella sp., Biodiesel, Fatty acids, GC/MS Device.*

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INTRODUCTION

Biofuels is defined as a fluid, solid or gas fuel produced using a biomass. Biofuels is used for transportation and burn. It could be also produced from cultural crops such as field crops, deserts, decomposable part of the industrial and domestic waste (Dufey, 2006). Many research papers and essays described advantages of using algae to produce biodiesel in comparison with other available raw materials. From a scientific point of view, algae is suitable and easy for culture, does not require insecticides, and can grow in saline waters. Also, it can use human undrinkable water and easily get nutrients, thus, take part in bio-treatment for wastewater through eliminating nutrients example: NH_4, NO_3 and PO_4. (Dragone *et al*., 2010; Meta *et al*., 2010).

Algae grow by photosynthesis (taking CO_2 from gases emitted from chimneys of power stations, then curbing greenhouse gases emission) for transferring solar power to chemical power and carrying out a complete growth circle within a few days. Furthermore, it grows often or almost everywhere (Dragone *et al*., 2010; Meta *et al*., 2010).

Rates of growth and production increased compared with traditional deserts, agricultural crops and other aquatic plants. algae produce and accumulate huge amounts of oils which estimated (20-50%) of the dry weight of a biomass. Besides, some algae have a high content of unsaturated fatty acids which are necessary for nutrition. These acids make algae known as a product for many types of complicated fat and oil hydrocarbons such as (Phospho-&glycolipid, Tri-& diglyceriedes) (Niehaus *et al*., 2011; agwa *et al*., 2012).

Algae recently have attracted the attention due to its production of hydrocarbon compounds which include methane through anaerobic digestion of the alga biomass, and biodiesel is derived from an alga oil (Thomas, 2006). In addition, some algae accumulate sugars that fermented to bioethanol or butanol (Sivakumar *et al*., 2012).

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algae are considered a Cellular factory that obtains solar power and transfers aerial CO_2 to useful products such as oils, which are transferred to biodiesel of different forms. Production of oils could be increased through changing an environment of algae, which is known as biochemistry engineering, as well as, content of oils is affected by many environmental factors. There is a diversified group of methods to conduct this in the different types of algae including depriving from some nutrients, temperature, light, PH, CO_2 ventilation and osmotic pressure. Some salinity-tolerant types of algae Halotolerant can produce oils as dissolved substances to face high or variable salinity. The aims of current study were isolating and purifying *Dunaliella* sp. and isolating bio-active oils as materials for the alternative or clean energy associated with fossil fuel (biofuels).

Materials and Methods

Sample Collection

Aquatic samples were selectively collected during January 2015 from different locations out of saltwater marshes scattered in Basrah city/Iraq. The red-colored saltwater marshes were chosen and plastic bottles of (500) cm^3 tightly closed were used. Then, bottles were brought directly to laboratory for alga investigation and isolation.

Cultural Medium

The following cultural medium Ben Amotz medium (BA-medium), of pH 7.5 (Murthy, 2005) were used for growing of *Dunaliella* sp.

Obtaining and Purifying Uni-Alga Isolates

Streaking method was used for solid cultural medium, and dilution method was used for liquid medium (Stien, 1973) for the purpose of attaining isolations of unialga. The method described in (Baggessen, 2014) was used for obtaining axenic culture.

Estimating Growth Rate

Growth rate of an alga under study was measured using measuring optical density. The optical density was measured by using Science UV/Vis Spectrophotometer manufactured by (BECKMAN COULTER™) on the

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following wavelengths 750 nm (Tran *et al*., 2013; Vo and Tran, 2014a; Nguyen et al., 2014), 680 nm (Farhat *et al*. 2011; Arun & Singh, 2013), 687 nm (Fazeli *et al*., 2006 a) and 600 nm (Kusumaningrum *et al*., 2004).

Laboratory Experiments

Impact of Different Saline Concentrations on Salinity-Tolerant *Dunaliella* sp.

Dunaliella sp. was grown in a cultural medium (BA) containing different concentrations of Sodium chloride (NaCl) as stated in table (1), as well as the medium was treated by adding Nitrogen (KNO_3) which then called (+N). Other media prepared were (-N) for each concentration.

Table 1: Saline Concentrations for NaCl used in the present study.

Nitrogen treatment KNO_3	Saline concentrations (M) NaCl	references
+N -N	1.5	**(Fazeli *et al*.,2006 a ;Farhat *et al*.,2011; Sathasivam *et al*., 2014)**
+N -N	3	**(Fazeli *et al*.,2006 b; Ak *et al*., 2008; Rad *et al*.,2011)**
+N -N	4	**(Pisal &Lele,2005; Sathasivam *et al*., 2012; Vo &Tran,2014 b)**

Alga from stock culture injected by BA-medium with an amount of 10 cm^3/100 cm^3, which equals to (4.27) mg/m^3 Chlorophyll a.

Impact of Intermittent lighting on the Growth of the Saline-Tolerant *Dunaliella* sp.

To study the impact of the Intermittent lighting on the Growth of *Dunaliella* sp., alga cultures were incubated in a growth room at temperature of 25 ^oC +/- 2, at a lighting intensity (200 Lux), and was exposed to a cycle of lighting/darkness for a period of (8/16) hours consecutively using fluorescent lights.

Impact of Continuous Lighting on the Salinity-Tolerant *Dunaliella* sp.

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Alga cultures were incubated in a growth room and were exposed to continuous lighting using fluorescent lights.

Preparation of Biomass for the Study

Algae were collected at the beginning of stationary phase for each concentration using centrifuge 6000 rotations/ a minute, type Table Top Centrifuge PLC-05, manufactured by Gemmy Taiwan /Industrial Crop. Then the biomass would be dried by freeze drier, type LABCONCO (18), after that samples were kept in tightly closed glass containers at (4 ^{o}C) for further study.

Impact of Ultra Violet (UV) on the Growth of Salinity-Tolerant *Dunaliella* sp.

The optimum saline concentration for producing substances (oils) was used and a culture medium was prepared with the selected saline concentration of the two forms (+N) and (-N). After being injected with a stock alga culture, the cultures then were incubated on the growth room. Cultures were exposed to (8/16) hours Intermittent lighting using fluorescent lights for two weeks, then, the above cultures were exposed to Ultra Violet (UV) for four hours daily (Abd El-Baky *et al*., 2004a).

Impact of Phosphorous and Nitrogen Removal on the Growth of Salinity-Tolerant *Dunaliella* sp.

The optimum saline concentration was used, and culture media of Nitrogen and Phosphorous as the following forms (+N-P), (+N+P), (-N+P) and (-N-P) were prepared and incubated on same circumstances.

Isolating of Oils

One gram of the dried samples of *Dunaliella* sp. was taken from each of the above experiments, and mixed with 250 ml of Hexane and continuous extraction was carried out using Soxhlet device for (8) hours. The solution was concentrated in a Rotary evaporator at 40^{o}C. A viscous yellow liquid was obtained (Zarzuelo *et al*., 1991), then weights of isolated oils were taken by using electronic balance, type ABS 120-4, manufactured in Germany/KERN & Sohn GmbH.

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Chemical Diagnosis for Isolated Oils

Determination of isolated oils components using TLC technology

The method described by Peeler (1989) was followed in determining components of oils isolated from *Dunaliella* sp. Acetic acid, diethyl ether, petroleum ether was used at percentage of (1, 30, 70) respectively. Isolated spots were made clear using UV torch at a length of 254 nm, iodine vapor and naked eyes.

Diagnosis by Gas Chromatography-Mass Spectrometry (GC/MS)

Technology of GC/MS using a device called QP2010 Ultra, manufactured in Japan, Shimadzu, was adopted at Basra University, College of Agriculture/ Unit of Food Research and Consumer Protection.

Results

Alga Description

The current study isolated a single alga belonging to *Dunaliella* sp., and has taken the following description (Borowitzka and Siva, 2007):

Kingdom: Plantae
Division: Chlorophyta
Class: Chlorophyceae
Order: Dunaliellales
Family: Dunaliellaceae
Genus: *Dunaliella*

An alga is a unicellular, moving by a same-length flagellum and having a single chloroplast, a goblet shape, amyloid body, one side eye-spot, as well as, absence of a cell wall and compensated with periplast, image 1.

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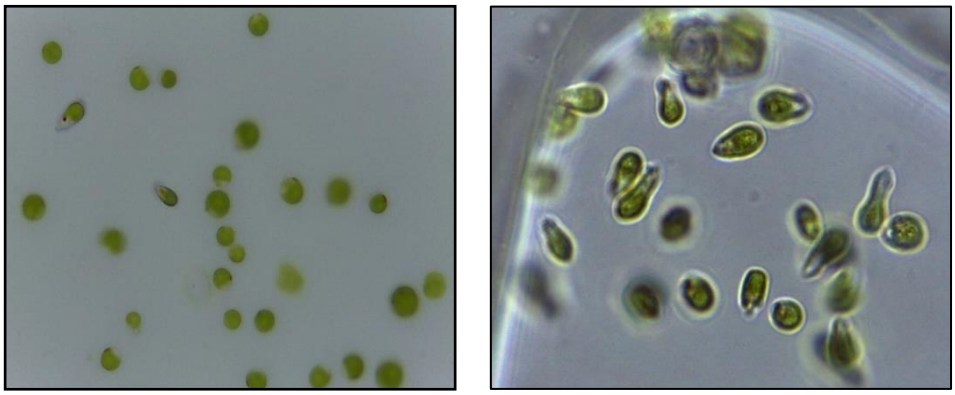

Image (1): *Dunaliella* sp. isolated from saline water marshes scattered in Basrah governorate (zoom power x100)

Growth Rate

Growth rate of the *Dunaliella* sp., which was grown in the culture medium Ben Amotz medium (BA-medium) is measured by an optical density method using different wavelengths, as indicated in table (2) and figure (1), the Lag phase has begun on the first four days, whereas the Exponential phase has begun since the fourth day with a steady increase till the sixteenth day, followed by Stationary phase which has gone till the thirty seventh day. It is considered the beginning of a Decline phase. No significant differences occurred at P>0.05 among the different wavelengths.

Table (2): Phases of Growth Measured by Days for *Dunaliella* sp.

Method of Measuring Growth	Phases by Days			
	Lag	Exponential	Stationary	Decline
Optical Density	0-4	4-16	16-37	-37

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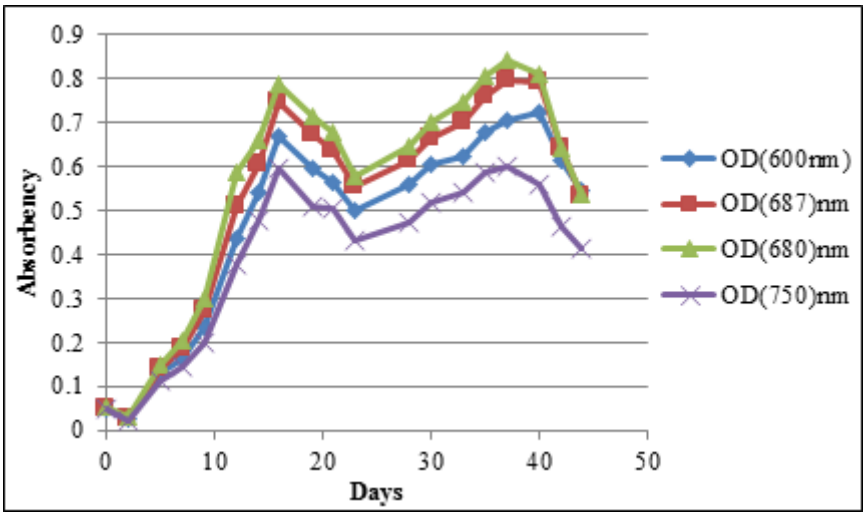

Figure (1): Growth Curve of *Dunaliella* sp. Grown on Ben Amotz medium (BA) by the Optical Density Using Different wavelengths

Laboratory Tests

Impact of Different Saline Concentrations on the Growth of Saline-Tolerant *Dunaliella* sp. Impact of Intermittent lighting on the Growth of the Saline-Tolerant *Dunaliella* sp.

The impact of Intermittent lighting on the Growth of the Saline-Tolerant *Dunaliella* sp. under study has been studied, measured by Chlorophyll **a** and **b** and the total Chlorophyll at saline concentrations (1.5, 3 and 4) molar. Results showed at figure (2). The highest value of Chlorophyll a was registered (206.8) micrograms/cm^3 at the saline concentration 1.5 molar, and below was (132.5) micrograms/cm^3 at the saline concentration 3 molar in the culture mediums treated by Nitrate (+N) figure (2- A). As to the percentage of Chlorophyll b, the highest value reached at (241.0) micrograms/cm^3 at the saline concentration 1.5 molar, followed by (158.7) micrograms/cm^3 at concentration of 4 molars, thus the total Chlorophyll's values was the highest at the saline concentration 1.5 molars, and lowest at the concentration 4 molars. As to the culture medium, which is free from Nitrate (-N), the results

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were as follows: value of Chlorophyll b registered an increase at the saline concentration 1.5 molar compared to other saline concentrations (3 and 4) molars, where it reached at 109.8 micrograms/cm^3, figure (2- B), whereas values of Chlorophyll b were close at the two saline concentrations (1.5 and 3) molars, as they reached at (38.9) and (40.6) micrograms/cm^3 respectively. Its lowest value reached at (28.2) micrograms/cm^3 at the saline concentration 4 molars. Therefore, the total Chlorophyll's values were the highest at the saline concentration (1.5) molars, (148.7) micrograms/cm^3, and the lowest was (98.8) micrograms/cm^3 at concentration (4) molars.

Statistical results showed significant differences at $P<0.05$ at values of the concentration of Chlorophyll (a) and (b) and concentration of the total Chlorophyll at different saline concentrations, whereas, no significant differences were appeared at $P>0.05$ at values of Chlorophyll (b) concentration for the saline concentrations 1.5 and 3 molars at mediums free from Nitrate or exposed to Intermittent lighting.

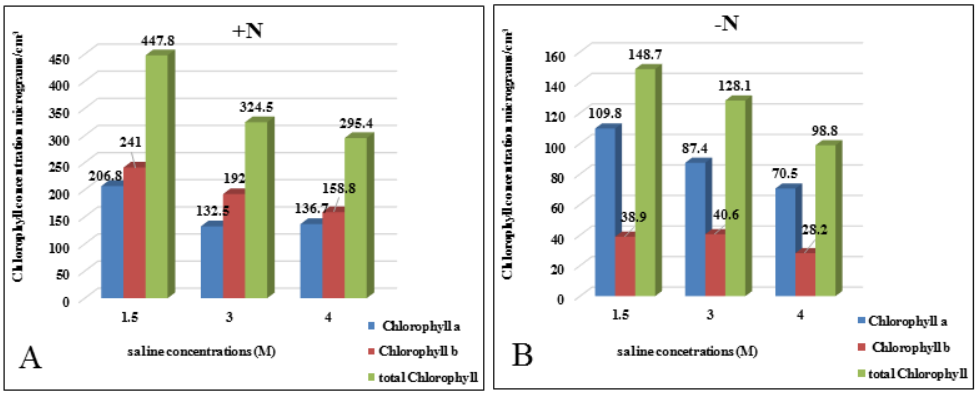

Figure (2): Chlorophyll (a, b & total) at culture mediums. A: Treated with Nitrate (+N), and B; Not treated with Nitrate (-N) and at saline concentrations (1.5, 3 & 4) molars NaCl under Intermittent lighting, Lighting/Darkness Period (8/16).

Impact of Continuous Lighting on Salinity-Tolerant *Dunaliella* sp.

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Cultures treated with Nitrate (+N) and exposed to continuous lighting recorded (199.7) micrograms/cm^3 for chlorophyll (a), while chlorophyll (b) recorded (251.6) micrograms/cm^3. Thus, the value of the total chlorophyll was (451.3) micrograms/cm^3, figure (3- A). At the other hand, cultures not treated with Nitrate (-N), exposed to continuous lighting, the chlorophyll (a) reached (86.7) micrograms/cm^3, and the chlorophyll (b) reached (106) micrograms/cm^3, figure (3- B). statistical analysis showed significant differences at $P<0.05$.

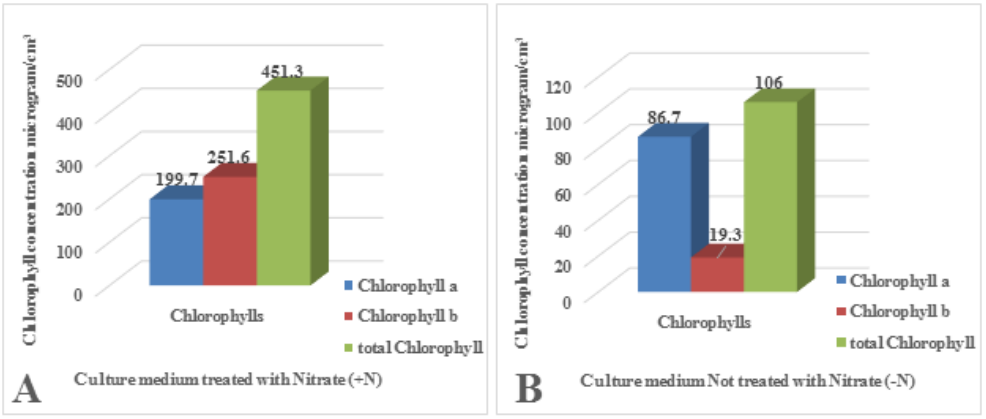

Figure (3): Concentration of chlorophyll (a), (b) and (total) extracted from *Dunaliella* sp., grown on A: Culture medium treated with Nitrate (+N), and B: Not treated with Nitrate (-N), and at a saline concentration (1.5) molars NaCl under the impact of continuous lighting.

Impact of Ultra Violet (UV) on the Growth Salinity-Tolerant *Dunaliella* sp.

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Figure (4- A) shows that the value of chlorophyll (a) is less compared to the value of chlorophyll (b) in a culture medium treated with Nitrate (+N) and exposed to UV along with normal lighting. While, culture mediums free from Nitrate (-N), exposed to UV recorded the highest value (71) micrograms/cm^3 for chlorophyll (a) and lowest value (22.9) micrograms/cm^3 for chlorophyll (b). The total chlorophyll was (93.9) micrograms/cm^3, figure (4- B). The statistical analysis showed significant differences at $P<0.05$.

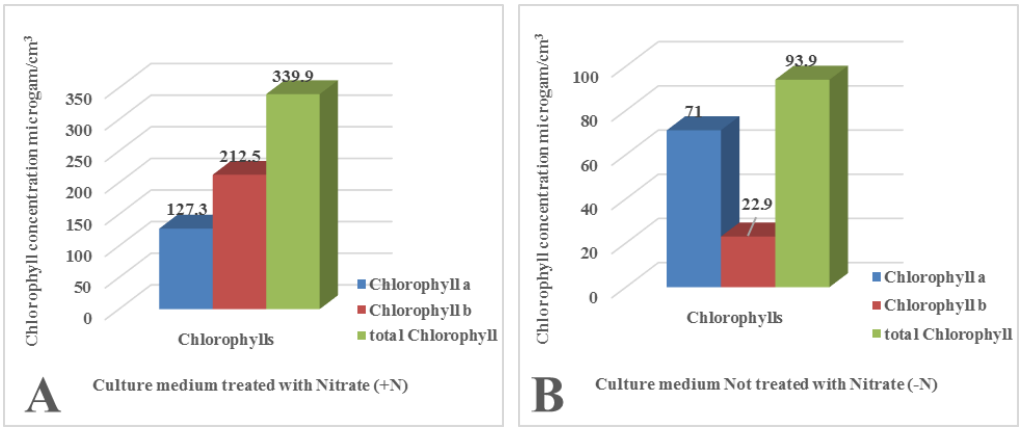

Figure (4): Concentration of chlorophyll (a), (b) and (total) extracted from *Dunaliella* sp., grown on A: Culture medium treated with Nitrate (+N), and B: Not treated with Nitrate (-N), and at a saline concentration (1.5) molars NaCl under the impact of Ultra Violet (UV).

Impact of Nutrients' Removal from a Culture Medium on Growth of Saline-Tolerant *Dunaliella* sp.

Values of chlorophyll (a) and (b) appeared clear differences among the four treatments (-N-P), (-N+P), (+N-P) and (+N+P). the experiment showed the rise in the value of chlorophyll (a) as it reached (206.8) micrograms/cm^3 at treatment (+N+P), whereas the lowest value (109.8) micrograms/cm^3 recorded at treatment (-N+P), figure 5. the value of chlorophyll (b) declined to (38.9) micrograms/cm^3 at (-N+P) treatment compared to other treatments.

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While, the highest value was (241) micrograms/cm^3 at (+N-P). treatment. The total chlorophyll values recorded a clear fluctuation among the four treatments, and values increased in mediums treated with Nitrate, and the highest reached (447.8) micrograms/cm^3 at (+N+P) treatment while the lowest value was (148.7) micrograms/cm^3 at (-N+P) treatment. statistical analysis showed significant differences for the four treatments at P >0.05.

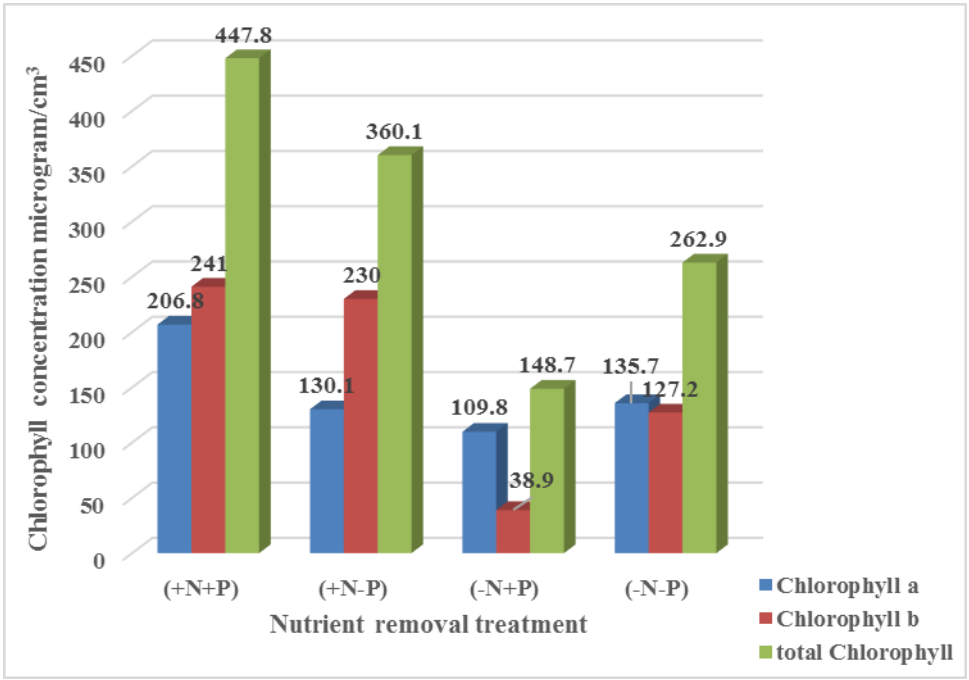

Figure 5: Chlorophyll (a), (b) and (total) extracted from *Dunaliella* sp., grown at a saline concentration of 1.5 molars NaCl under treatment of nutrients' removal from a culture medium.

Oils Isolation

Impact of Saline Concentrations on Quantity of Oils Isolated from Saline Tolerant *Dunaliella* sp.

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Figure (6) indicated that the highest production of oils isolated from *Dunaliella* sp. was (0.07) grams at the saline concentration (3) molars in culture medium treated with Nitrate (+N), and lowest production was (0.01) grams at the saline concentration (4) molars in culture medium not treated with Nitrate (-N), whereas the saline concentration (1.5) molars, treated with Nitrate (+N) and no Nitrate treatment (-N) recorded the same quantity of oils which produced (0.03) grams. The statistical analyses for oils isolated from *Dunaliella* sp. showed significant differences at $P<0.05$ between saline concentrations and for both Nitrate-supported medium and zero-Nitrate medium.

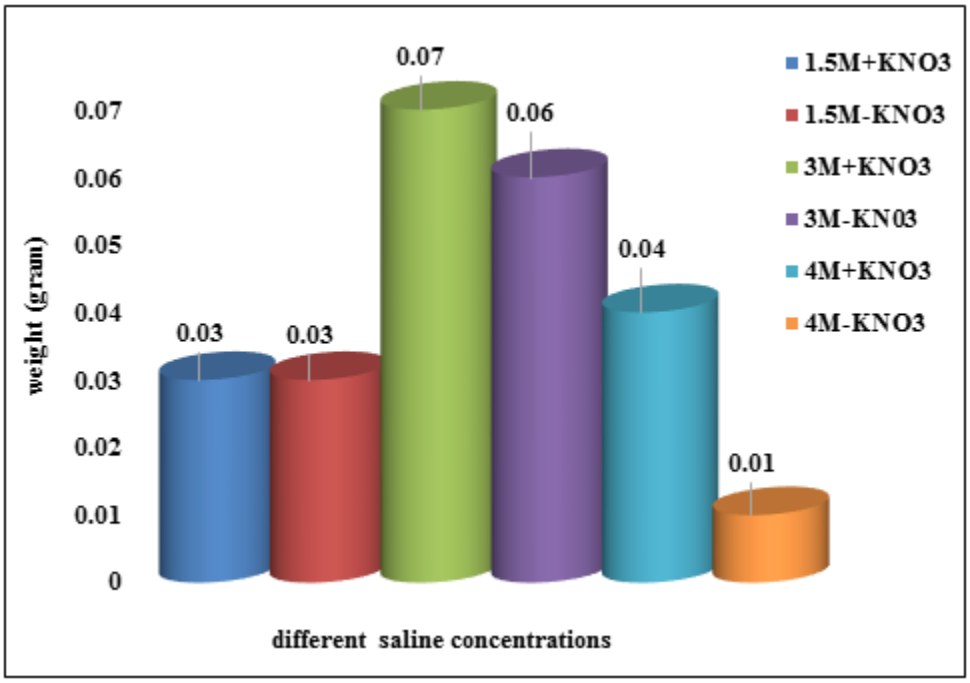

Figure (6): Impact of Saline Concentrations on Quantity of Oils Isolated from *Dunaliella* sp.

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Impact of Quality of Lighting on Quantity of Oils Isolated from Saline-Tolerant *Dunaliella* sp.

Figure (7) clarifies the largest quantity of oils (0.2) grams was obtained when exposing an alga culture to Ultra Violet (UV) at a zero-Nitrate medium (-N) compared with the lowest production obtained when exposing an alga culture to continuous lighting at a medium treated with Nitrate (+N) at a saline concentration of (3) molars.

The statistical analyses for oils isolated from *Dunaliella* sp. showed no significant differences at P>0.05 for the impact of lighting quality at a medium treated with Nitrate and Zero-Nitrate medium.

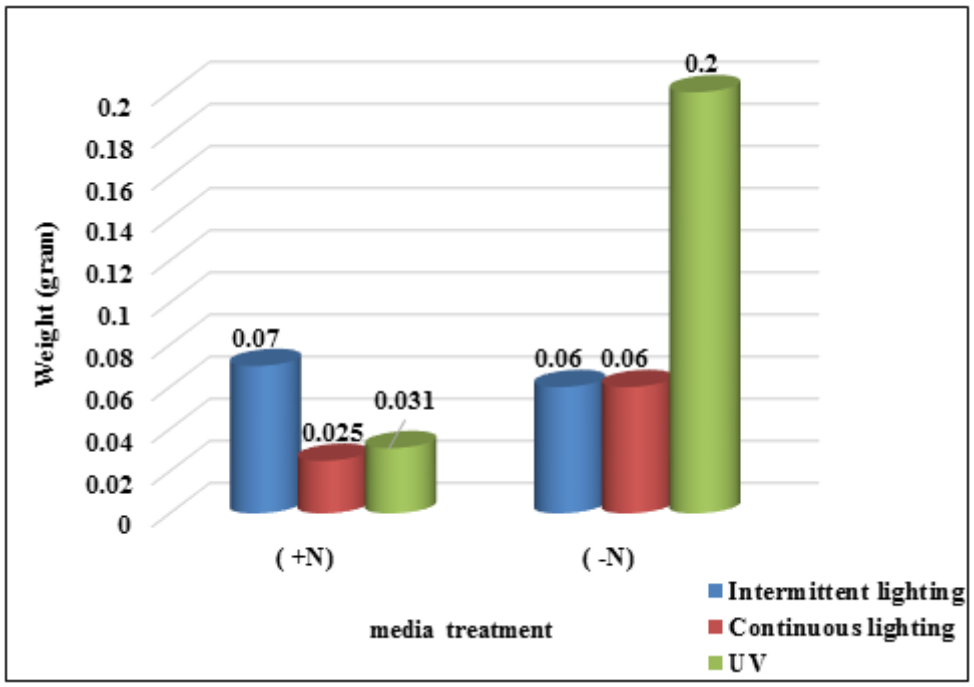

Figure (7): Impact of lighting quality on *Dunaliella* sp. isolated oils' quantity, grown on a medium treated with nitrate and a zero-Nitrate medium.

Impact of Nutrients' Removal on Quantity of Oils Isolated from Saline-Tolerant *Dunaliella* sp.

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Figure (8) discloses an inverse proportion between quantity of oils isolated from *Dunaliella* sp. and a treatment of phosphate removal from a culture medium. Removal of phosphate from the culture medium resulted in an increase in the quantity of oils isolated from *Dunaliella* sp. for both treatments: (+N) and (-N). The statistical analyses showed significant differences at $P<0.05$ among treatments of nutrients' removal.

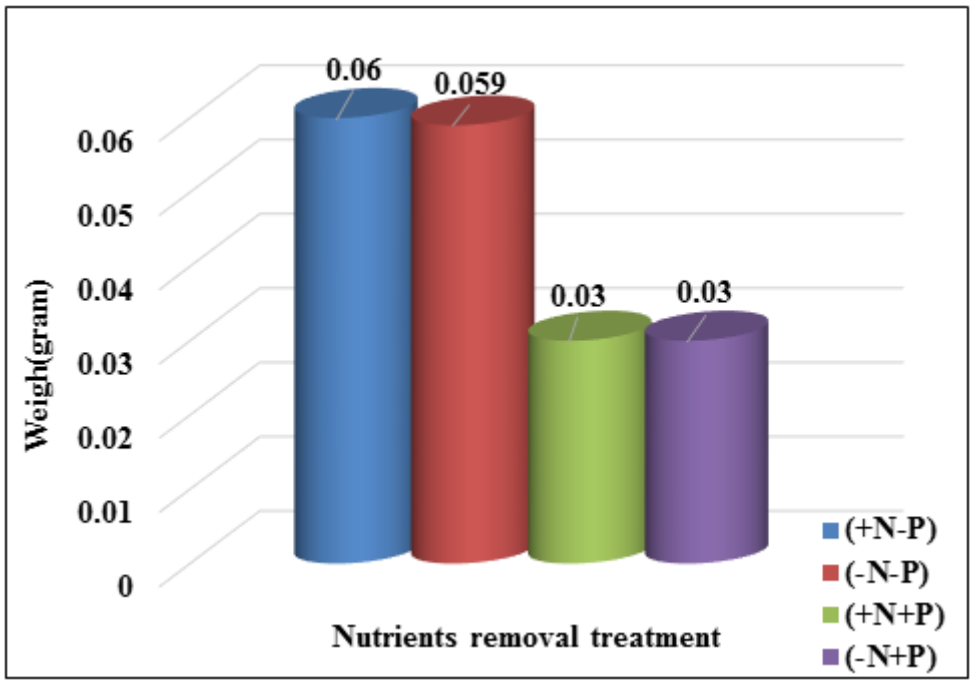

Figure (8): Impact of Removal of Nutrients-Nitrate and Phosphate from *Dunaliella* sp., grown on a culture medium of a saline concentration (3) molars.

Determining Components of Oils Isolated from Salinity-Tolerant *Dunaliella* sp. by Thin Layer Chromatography TLC

Results of TLC showed that the oil isolated from *Dunaliella* sp. under study contains (12) components of Relative Flow (Rf), as listed in table (4) and image (4).

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Table (4): Values of Relative Flow (Rf) for Components of Oils Isolated from *Dunaliella* sp. by Thin Layer Chromatography TLC and acetic acid: diethyl ether: petroleum ether in a ratio of 1:30:70.

Rf Value	Spot Number
0.0705	1
0.123	2
0.18	3
0.22	4
0.24	5
0.28	6
0.32	7
0.37	8
0.45	9
0.55	10
0.705	11
0.75	12

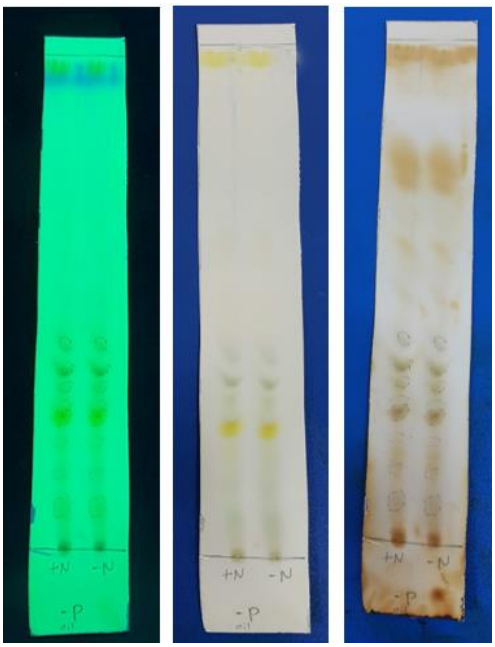

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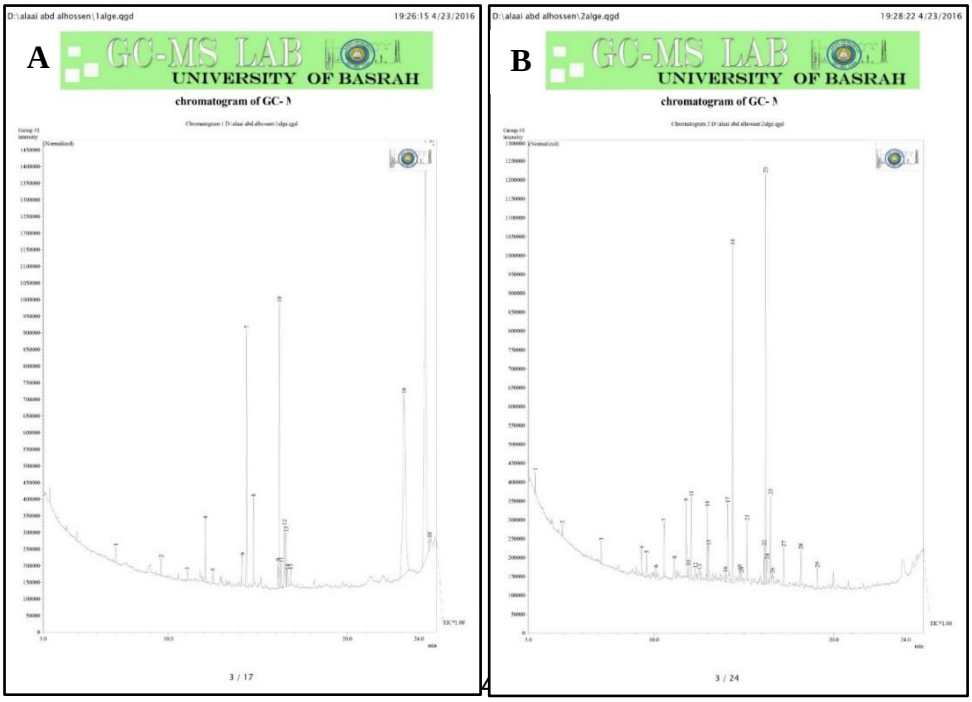
Image (4) Determining Components of Oils Isolated from *Dunaliella* sp., grown on culture medium free from Phosphate by TLC, which made clear by (a) UV, (b) naked eyes and (c) Iodine.

Diagnosing Using Gas Chromatography-mass spectrometry (GC/MS)

Figure (9) and table (5) showed the oils component isolated by *Dunaliella* sp., both saturated and unsaturated fatty acids. 8-Octadecoenoic acid methyl ester and Hexadecanoic acid methyl ester formed the highest concentrations among other fatty acids, figure (10). Fatty acids of the number of carbon atoms (18) were associated with oils isolated from an alga grown on a medium supported with Nitrate (+N), while fatty acids of low weight were



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associated with an alga grown in a free-Nitrate medium.

Figure (9): Gas Chromatography-mass spectrometry of oils isolated from *Dunaliella* sp. at (A): Culture mediums treated with Nitrate (+N), (B): Culture mediums not treated with Nitrate (-N).

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Chemical Name	Symbol	Nitrate Treatment	Common Name	Molecular Formula	References
Saturated Fatty Acids					
Hexanoic acid methyl ester	C6:0	-N	Caproic acid methyl ester methyl caproate	$C_7H_{14}O_2$	Rasoul-Amini *et al*.,2009.
Octanoic acid methyl ester	C8:0	**-N**	Caprylic acid methyl ester methyl methyl caprylate	$C_9H_{18}O_2$	Abd El Baky *et al*., 2004b;Rasoul-Amini *et al*.,2009;Fakhary & El-Maghraby, 2013
Decanoic acid methyl ester	C10:0	+N, -N	Copric acid methyl ester methyl caprate	$C_{11}H_{22}O_2$	Abd El Baky *et al*., 2004b;Rasoul-Amini *et al*.,2009;Fakhry & El-Maghraby, 2013.
Dodecanoic acid methyl ester	C12:0	+N, -N	Lauric acid methyl ester methyl Laurate	$C_{13}H_{26}O_2$	Abd El Baky *et al*., 2004b; Rasoul-Amini *et al*.,2009; Fakhry & El-Maghraby,2013;Rasoul-Amin *et al*.,2014; Cakmak *et al*.,2014;Sohio & Eghdam, 2014.
Methyl tetradecanoate	C14:0	+N,-N	Methyl myristate myristic acid methyl ester	$C_{15}H_{30}O_2$	Al-Hasan *et al*.,1987;Rasoul-Amini *et al*.,2009;Fakhry & El-Maghraby,2013;Cakmak *et al*., 2014.
Tetradecanoic acid	C14:0	+N	Myristic acid	$C_{14}H_{28}O_2$	Herrero *et al*.,2006;Rasoul-Amini *et al*., 2009;Cakmak *et al*.,2014;Zonouzi *et al*., 2016.
Hexadecanoic acid methyl ester	C16:0	+N, -N	Palmitic acid methyl ester methyl palmitate	$C_{17}H_{34}O_2$	Mendoza *et al*.,1999;Herrero *et al*.,2006; Rasoul-Amini *et al*.,2009;Fakhary & El-Maghraby,2013;Talebi *et al*.,2014;Cakmak *et al*.,2014;sohi & Eghdam,2014;Rasoul-Amini *et al*.,2014;casadiego *et al*.,2016;Zonouzi *et al*.,2016.
Octadecanoic acid methyl ester	C18:0	+N, -N	Stearic acid methyl ester methyl stearate	$C_{19}H_{38}O_2$	Herrero et al.,2006; Rasoul-Amini *et al*., 2009; Fakhary & El-Maghraby, 2013;Cakmak *et al*.,2014;sohi & Eghdam, 2014; Talebi *et al*., 2014;Casadiego *et al*.,2016;Kalantaryan *et al*.,2016;Zonouzi *et al*.,2016.
Octadecanoic acid	C18:0	+N	Stearic acid Steric acid	$C_{18}H_{36}O_2$	Rasoul-Amini *et al*.,2009;Rasoul-Amini *et al*.,2014;Zonouzi *et al*.,2016
Unsaturated Fatty Acids					
Methyl myristoleate	C14:1	-N	Methyl myristoleate	$C_{15}H_{28}O_2$	Abd El Baky *et al*.,2004b;Cakmak *et al*., 2014;Kalantaryan *et al*.,2016.
9- Hexadecanoic acid methyl ester (z)-	C16:1	+N	Palmitoleic acid methyl ester methyl palmitoleate	$C_{17}H_{32}O_2$	Al-Hasan *et al*.,1987;Mendoz *et al*.,1999;Herrero *et al*.,2006;Rasoul-Amini *et al*., 2009;Fakhary & El-Maghraby,2013;Talebi *et al*.,2014;Cakmak *et al*.,2014;Kalantaryan *et al*., 2016;Zonouzi *et al*.,2016.
8- Octadecenoic acid methyl ester	C18:1	+N, -N	Oleic acid methyl ester methyl oleate	$C_{19}H_{36}O_2$	Mendoza *et al*.,1999;Fakhary & El-Maghraby,2013;Cakmak *et al*., 2014;Talebi *et al*.,2014;Kalantaryan *et al*.,2016;Zonouzi *et al*.,2016.
6- Octadecenoic acid (Z)-	C18:1	+N	Oleic acid methyl ester	$C_{18}H_{34}O_2$	Rasoul-Amini *et al*.,2009;Zonouzi *et al*., 2016.

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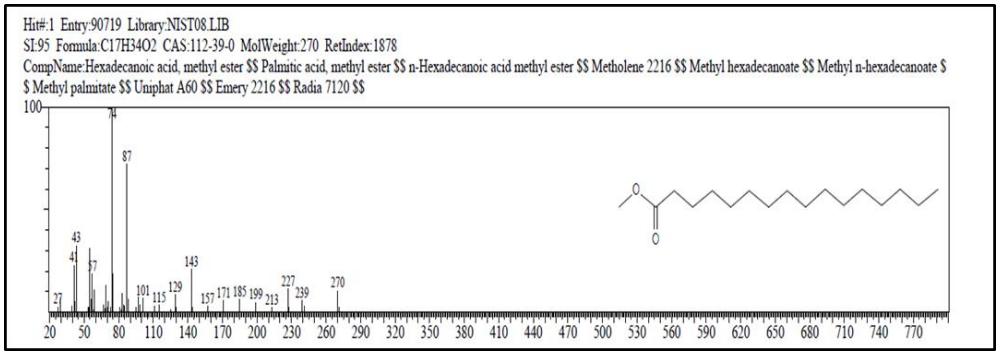
			methyl oleate		
9,12- Octadecadienoic acid (Z,Z)methyl ester	C18: 2	+N	Linoleic acid methyl ester methyl linoleate	$C_{19}H_{34}O_2$	Mendoza *et al*.,1999;Herrero *et al*.,2006; Rasoul-Amini *et al*.,2009;Fakhary & El-Maghraby,2013;Talebi *et al*., 2014;Kalantaryan *et al*.,2016;Zonouzi *et al*.,2016.
10, 13 Octadecadienoic acid methyl ester	C18: 2	+N	Linoleic acid methyl ester methyl linoteate	$C_{19}H_{34}O_2$	Mendoza *et al*.,1999;Herrero *et al*.,2006; Fakhary & El-Maghraby, 2013; Talebi *et al*., 2014; Kalantaryan *et al*.,2016;Zonouzi *et al*.,2016.
8,11 Octadecadienoic acid methyl ester	C18: 2	-N	Linoleic acid methyl ester methyl	$C_{19}H_{34}O_2$	Mendoza *et al*.,1999 ;Fakhary & oEl-Maghraby,2013;Talebi *et al*.,2014; Kalantaryan *et al*.,2016.

Table (5): Diagnosed Fatty Acids of the *Dunaliella* sp. Studied by the Technology of GC/MS



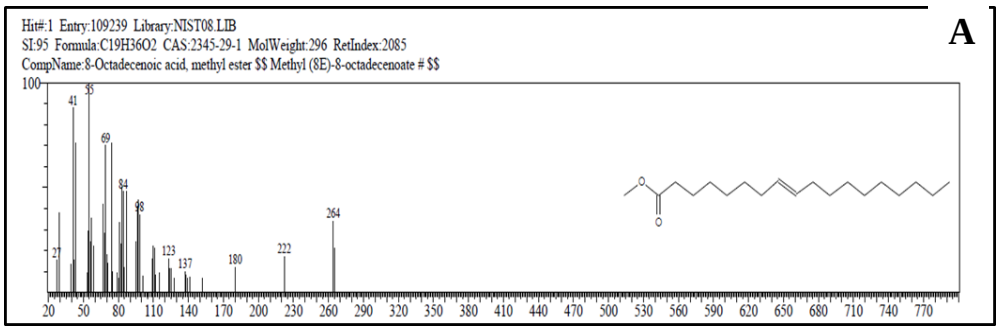


Figure (10): GC/MS and Chemical Structure of the Two Fatty Acids, (**B** Hexadecanoic acid methyl ester, and (B): 8-Octadecoenoic acid methyl ester.

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Discussion

Laboratory Study

Growth Rate

Growth rate is considered one of the instant growth measures, which refers to multiplication of the biomass during a certain time unit, which constitutes a growth rate on batch cultures, type sigmoid growth curve (Stein, 1973). In the current study, the growth rate was measured using the method of optical density for determination of the best wavelength suitable for the growth of *Dunaliella* sp. on batch cultures. It was noticed from results made available at figure (1) and table (2) that the optical density method was the best in expressing the growth rate of an alga under study. An increase in the Turbidity of an alga suspension refers to a growth density or a rise in the number of cells during a time. The period lies between the end of an exponential phase and start of stationary phase has lasted for (16) days, which is a compatibility period for an alga harvest. These results are consistent with Tran *et al*. (2014), in which they found that *Dunaliella* sp. had started adapting and growing after (3) days through measuring a cell density, and arrived at a stationary phase after (2) weeks. Then a death phase started due to shortage of nutrients or release of inhabiting metabolic products from algae cultures.

Oils Isolating

Results of the current study disclosed that a saline concentration (3) molars of NaCl had a prominent impact on the increase of quantity of oils isolated from *Dunaliella* sp. compared with other saline concentrations, figure (6). This was in line with a study conducted by (Hounslow, 2010), which evidenced that an ideal saline concentration to produce the largest quantity of oils at type *D. tertiolecta* was (3.5) molars. Therefore, the saline concentration (3) molars was used in the following experiments. Also, exposure to Ultra Violet and removal of nutrients (phosphate) from a culture medium, figures (7 & 8) resulted in an increase in the quantity of oils isolated from *Dunaliella* sp. This was consistent with a study conducted by

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Lv *et al*. (2016) which reflected that cells exposed to deprivation of phosphate were rich in oils which accumulated in huge amounts. Besides, Sharma *et al*. (2012) stated that exposure to UV caused an increase in mono unsaturated fatty acids and saturated ones alike. However, removal of nitrates from a culture medium resulted in accumulation of carbohydrate instead of oils. This elaborates the mechanism of nitrates removal from a culture medium is useless for oils accumulation (Srirangan, 2015). This was consistent with the present study in which the removal of nitrates (-N) from a culture medium resulted in a decline in isolated oils, figure (8).

Chemical Diagnosis of Components Isolated from Saline-Tolerant *Dunaliella* sp.

Results of Thin Layer Chromatography TLC, image (4) showed the isolated oils from *Dunaliella* sp. under study contained (12) components. Figure (9) and Table (5) represented GC/MS for isolated oils and showed that fatty acids diagnosed by the above-mentioned technique were (16) fatty acids (9) of it belong to saturated fatty acids and (7) belong to unsaturated fatty acids which constituted 44% and 56% respectively. Long-chain fatty acids (C18) were accompanied with oils isolated from an alga grown on a culture medium supported with (+N), while short-chain fatty acids (C8, C6) were accompanied with oils isolated from an alga grown on a medium free from nitrate (-N). It's well known that quantity and quality of oils is affected by many factors such as conditions of the growth and culture mediums used and supported by nutrients or removed from it. Also, it is affected by a temperature, quality and intensity of lighting, which makes it hard to determine any of those factors has an effect on quantity or quality of isolated oils (Fakhry & Maghraby, 2013). Marine green algae are used to produce quantity and quality of fatty acids such as arachidonic acid, an important acid in manufacturing an animal hormone called prostaglandin (Stewart, 1974).

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