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Impact of Stress-Induced Shocks on Carotenoids and Phenolics Production from *Chlorella vulgaris* and *Anabaena torulosa*

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ABSTRACT

Chlorella vulgaris and *Anabeana torulosa* are microalgae containing many valuable bioactive compounds. This study aimed to investigate the impact of novel strategy by applying stress-induced shocks on the biomass and biological activities of the growing microalgae. The effects of stress-induced shocks at high light intensities, high and low temperatures, high salinities, excision and depletion of nitrate, and gamma radiation on biomass, extracts, carotenoids, and phenolics production of the two studied microalgae in relation to their antioxidant activity percentage (A.O%) were tested. Methanol extracts of *C. vulgaris* and *A. torulosa* grown on BG-11 were tested by using Fourier-transform infrared spectroscopy and gas chromatography mass spectrophotometer. The chosen optimal stress parameters were illumination shocks at 350µmol photons m^{-2} s^{-1} for 20min, temperature at 43ºC for 6h, and 0.5g/ L nitrate concentration for 6 days of incubation. Additionally, 100mM salinity concentration and 1kGy of gamma radiation dose for *A. torulosa*, while, 150mM and 1.5 kGy for *C. vulgaris.* 136.0 and 138.3mg/ g of total phenolics and 141.7 and 134.7mg/ g of total carotenoids for *C. vulgaris* and *A. torulosa*, respectively. The percentages of antioxidant activity produced by *C. vulgaris* and *A. torulosa* were 95.7 and 94.3%, respectively, recording the highest antioxidant activity percentage. Employing of abiotic stress-induced shocks on *C. vulgaris* and *A. torulosa* is considered as a promising economic technique for the production of carotenoids and phenolics. The produced carotenoids and phenolics from the studied algae were a protective agent in overcoming stress conditions and are therefore considered as powerful antioxidant and radioprotective agents.

INTRODUCTION

Carotenoids and phenolics are important and valuable bioactive compounds contributing to many industries, especially various interests of the human structure. Carotenoids are phytochemicals, pigments with lipophilicity mainly produced by plants and microorganisms **(Li *et al.*, 2022)**. Carotenoids structure is terpenoid consisting of a long chain which is conjugated. Tetraterpenes characterizes the remarkable carotenoids **(Gupta *et al.*, 2021)**. Carotenoids can be separated into two groups according to their structure, carbohydrate type and oxidized type. Carotenoids have important biological

activities in organisms **(Nwoba *et al.*, 2021)** and can act as a vital role in boosting anti-oxidation, immunity, fighting cancer, preventing tumor, and delaying aging **(Yuan *et al.*, 2021)**. Carotenoids also diminish the risk of cataracts, obesity, metabolic syndrome and chronic diseases like Age-rated macular degeneration (AMD), nutritional complements, and colorant of food. Carotenoids are vastly used in the industry that is related to health care, medicine, and food **(Brudzyńska *et al.*, 2021)**.

Phenolic compounds are secondary metabolites with a broad variety of chemical constructions with well-known health benefits as antioxidant properties **(Jerez-Martel *et al.*, 2017)**, mainly exist in aquatic macrophytes and terrestrial plants. Microalgae is also considered as a natural source, including flavonoids, and phenolic acids. Further, phenolic acids, such as caffeic, gallic, salicylic acid, and p-hydroxybenzoic are presented in various species of marine algae **(Cichoński *et al.*, 2022)**. Phenolic acid derivatives are present as μg levels in microalgae **(Joana Gil-Chávez *et al.*, 2013)** that are characterized as radical scavengers **(Kurutas, 2015)**. Epidemiological studies have approved that consuming food loaded with phenolic compounds may stop the dawn of degenerative diseases **(Rudrapal *et al.*, 2022)**. Purified phenolic compounds display many actions such as anti-radical, UV-protection, and antioxidant **(Suganya *et al.*, 2016)**. Whilst, the major bioactivity to phenolic compounds is antioxidant activity. It has been informed that phenolic compounds of some microalgae have been linked with anti-inflammatory effeectiveness, such as hesperidin, caffeic acid, epigallocatechin gallate, catechin, catechol, and rutin. These compounds have the potency to combat free radicals **(Herrero *et al.*, 2013; de Morais *et al.*, 2015)**. Phenolic compounds can be destinguished by their formula which have at minimal one phenol unit. According to their chemical formula, phenolic compounds can be sectioned into various subgroups, such as tannins, phenolic acids, quinones, flavonoids, stilbens, curcuminoids, coumarins, and lignans **(Gan *et al.*, 2019)**.

Chlorella vulgaris and *Anabeana torulosa* are two remarkable microorganisms, which contain high concentration of photosynthetic pigments and many carotenoids as well as phenolics that mainly work as free radical scavengers **(Widyaningrum & Prianto, 2021; De Rosa *et al.*, 2024)**. The concentration of carotenoids and phenolics within *Chlorella vulgaris* and *Anabeana torulosa* differ based on the habitat, season, and factors as salinity, light, nutrient availability, and UV rays **(Cichoński & Chrzanowsk, 2022)**. Extremist environmental conditions, such as concentrations of high salt, the lack of nutrition, low temperature, and exposure of UV radiation, could stimulate the gathering of high concentrations of phenolic compounds and carotenoids **(Fawzy & Alharthi, 2021)**. The stress response enables organisms to adapt to and interact with their surroundings, allowing them to survive under severe conditions **(Barkia *et al.*, 2019)**.

In general, to fix the struggle between microalgal cell growth and their production of worthy bioactive molecules, two-stage cultivation designing was carried out, devoting

the primary stage with optimal growth conditions to earn the ultimate production of biomass, followed by inserting the stress at the second stage of cultivation for the over-production and accumulation of secondary metabolites as carotenoids and phenolics, for instance iron or salt concentration, intensity of light, depletion of nitrogen, and temperature **(Sun *et al.*, 2018)**. In one path, red LEDs (660nm) was used in the growing of microalgae in the first phase to gain the ultimate biomass production, and stressed using green LEDs (520nm) in the second phase to spur secondary metabolites accumulation **(Jung *et al.*, 2019)**. In parallel, a two-stage culture strategy was performed to raise *Isochrysis galbana* biomass under nutrients sufficient, appended by low-salt stress condition, which maximize the lipid content from 24 to 47% **(Liyanaarachchi *et al.*, 2021)**. Another type of stress effect called continuous stress strategy was applied by growing the microalgal cells with a stress response **(Song *et al.*, 2022)**.

This study aimed to create a new stress effect and to enhance bioactive compounds production specifically carotenoids and phenolics; *Chlorella vulgaris* and *Anabaena torulosa* by influencing stress-induced shocks strategy including combined multiple stresses on different growth parameters, such as temperature, light intensity, nitrogen concentration, and exposure to gamma radiation.

MATERIALS AND METHODS

Cultivation conditions

Chlorella vulgaris Beijerinck and *Anabaena torulosa* Lagerheim ex Bornet & Flahault obtained from microbiology lab in Egyptian Atomic Energy Authority (EAEA) were separately sub-cultured by inoculating 15ml of microalgal suspension into 100ml conical flasks containing 35ml sterile BG-11 broth medium **(Stanier *et al.*, 1971)** at an intensity of 100µmol photons m^{-2} s^{-1} with a photoperiod of 12:12 (L:D) **(Serra-Maia *et al.*, 2016)**, at a temperature of 25 ± 2°C. Microscopic examination was carried out to check the purity of each isolate according to **Prescott (1982)**. The cultures were incubated for 15 days then, the growth was harvested by centrifugation at 4000rpm for 15min, weighed, and then displayed to oven drying at 40°C, then, the dry weight was determined.

Microalgal extracts

Known weights of the dried biomasses of both microalgal isolates were completely homogenized separately and extracted using 96% Methanol for 4h, followed by re-extraction for 3h. The process was repeated thrice and the clear greenish supernatant was pooled together and left to dry. The dried extract was stored at 4°C for further analysis **(Salem *et al.*, 2014; Jayshree *et al.*, 2016)**.

Total carotenoids (TC)

The quantification of total carotenoids (TC) within both algal methanolic extracts were estimated according to **Dere *et al.* (1998)**, the absorbance (A) was recorded at 653, 666, and 470nm, then calculating the the concentration using the following equations:

Chlorophyll a (Chl $_a$) = 15.65 (A_{666}) – 7.34 (A_{653})

Chlorophyll b (Chl $_b$) = 27.05 (A_{653}) – 11.2 (A_{666})

Total carotenoids = (1000 (A_{470}) – 2.86(Chl $_a$) – 129.2 (Chl $_b$)/ 245

Total phenolics (TP) compounds

The total phenolics (TP) of the methanolic extract of both isolates was determined by using Ciocalteu's reagent **(Abd El-Aziz *et al.*, 2024)**. Extra specifically, 100µl of methanolic extract was combined with sterile distilled water to a final volume of 8ml. Subsequently, the addition of 0.5ml folin reagent was performed. After 3min, 1.5ml of 1.89 M Na_2CO_3 solution was added to the mixture. The volume was finally set to be 10ml. The mixtures were halted temporarily in dark for 2h. Every sample was measured spectrophotometrically at 760nm by T60 UV-Visible spectrophotometer. Gallic acid was used as a standard for the total phenolics stanardization and are crossed as mg gallic acid equivalent (G.A.E.) g^{-1} biomass.

Antioxidant activity analysis

In order to define the antioxidant activity for both isolates methanolic extracts, methanolic extracts scavenging activity on 2,2-diphenyl-1 picrylhydrazyl (DPPH) free radicals were assayed. Aliquot of 2ml of concentration 100µg/ ml (the tested samples and the control standard) was added separately to 0.1-mM DPPH fluxed in 96% methanol. The blend was set for 30min at room temperature, subsequently estimating the absorbance at 517nm **(Shaheen *et al.*, 2023)**. A minimal absorbance symbolizes an elevation of DPPH. The positive control was performed by ascorbic acid (AA, 100µg/ ml). The percentage of antioxidant activity (A.O%) was acrossed, as shown in the following equation:

$$A.O\% = \left| \left(\frac{A_{control} - A_{sample}}{A_{control}} x100 \right) \right|$$

GC-MS spectrophotometry

Gas Chromatography-Mass Spectrophotometry (GC-MS) profiling of methanoilc extracts of both tested microalgae was carried out by adding 300µL aliquot of *N,O-Bis(trimethylsilyl)trifuoroacetamide* (BSTFA) with *trimethyl chlorosilane* (as a silylation

agent) to the methanolic extracts of *Chlorella vulgaris* and *Anabaena torulosa*. Each blend was placed at 80ºC water bath into two hours to be ready for injection into GC-MS at the Faculty of Science, Ain Shams University, Cairo, Egypt. The GC-MS analysis was thoroughed using Agilent Technologies 7890B GC systems combined with 5977A Mass Selective Detector. The used capillary column was HP-5MS capillary; 30.0m x 0.25mm ID x 0.25µm film and the carrier gas at a pressure of 8.2 psi was helium with 1µm injection. Defining the constituents was made by mass fragmentations with the NIST mass spectral search program for the NIST/EPA/NIH mass spectral library version 2.2 (June 2014).

Fourier-transform infrared spectroscopy characterization

Fourier-transform infrared spectroscopy (FTIR) is the farthest influential strategy to reveal the chemical functional groups of extracts in less than a few seconds. FTIR spectrum was pressed in the range of 400 and 4000cm^{-1}. The functional groups of the dried methanol crude extracts were detected by FTIR-Vertex 70 spectrometer, Bruker-Germany, at the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Egypt **(Abd El-Aziz *et al.*, 2024)**.

Impact of induced stress on certain biological aspects of *Chlorella vulgaris* and *Anabaena torulosa*

Both microalgal organisms *Chlorella vulgaris* and *Anabaena torulosa* grown for two weeks under these controlled conditions, temperature at 25 ± 2ºC and intensity of 100µmol photons m^{-2} s^{-1} with a photoperiod of 12:12 (L:D), were exposed to different stress shocks and were then harvested after incubation period and then carotenoids, phenolic compounds as well as antimicrobial activities were detected in known weights of their methanolic extracts.

Effect of different durations high light intensity shocks

According to the above-mentioned normal cultivation conditions, after the 7^{th} day of incubation, both microalgal isolates were exposed separately to different high light intensity shocks: 100, 150, 250, and 350 µmol photons m^{-2} s^{-1} for different time intervals and 10, 20, and 30min within the light period of cultivation. The best results were chosen for further experiments.

Effect of different temperature shocks

After 12 days of incubation, both microalgae were subjected to temperature shocks either lower or higher than the normal growth temperature **(Barten *et al.*, 2021)**

17 ± 2 ºC, 25 ± 2ºC, and 43 ± 2ºC for different periods; 3, 6, and 9 hours separately during the dark period of incubation.

Effect of different nitrogen concentration for different incubation time

Three distinct concentrations (0.5, 1.5, and 2.5g/ L) of nitrogen source (NaNO3) were applied separately in the BG-11 growth media before 2, 4, and 6 days of ending the incubation period **(Abd El Fatah *et al.*, 2020)**.

Effect of salinity stress

Both isolates were exposed to different salinities: 50, 100, 150, and 200mM of NaCl along their incubation period on BG-11 **(Maltsev *et al.*, 2021)**.

Effect of gamma irradiation

The impact of gamma radiation several doses on the microalgal strains was studied as a stress parameter. Both microalgal isolates were irradiated separately by various doses of gamma radiation (0, 0.5, 1.0, 1.5 and 2.0 kGy) at National Center of Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Nasr City, Cairo, Egypt. ^{60}Co Gamma chamber (Canadian) was used with a dose rate average of 0.856 kGy/h. Then, irradiated microalgal isolates were incubated for two weeks under the studied cultivation conditions **(Abd El Fatah *et al.*, 2020)**.

Statistical analysis

All tests were destined in triplicates and the results were crossed as mean ± standard error (SE). The statistical tests were achieved by one-way ANOVA at $P< 0.05$ and registered as least significant difference (LSD) and Duncan's multiple range analysis utilizing the SPSS (IBM) software (V25).

RESULTS

1. Determination of total carotenoids (TC), total phenolics (TP), and antioxidant activity of isolates

Carotenoids were determined in the form of TC. Based on Table (1), TC were estimated as 77.0 ±0.5mg/ g for *Chlorella vulgaris* and 58.7±0.3mg/ g for *Anabaena torulosa*.

As shown in Table (1), TP constituents of both microalgal extracts that dissolved in methanol were quantified as 62.8±0.1mg/ g for *Chlorella vulgaris* and 77.0±0.5mg/ g for *Anabaena torulosa*.

The percentage of A.O. activity of the crude methanolic extracts was determined to be 66.8±0.1mg/ g and 65.5±0.3mg/ g for *Chlorella vulgaris* and *Anabaena torulosa*, respectively (Table 1).

Table 1. Phenolic and carotenoid contents as well as antioxidant activities of *Chlorella vulgaris* and *Anabaena torulosa* methanol extracts

	TP mg/g	TC mg/g	A.O%
Chlorella vulgaris	63.7±0.1	77.0±0.5	66.8±0.1
Anabaena torulosa	62.8±0.6	58.7±0.3	65.5±0.3

TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage

2. GC-MS analysis of methanol extract

In present study, the GC-MS spectra of *Chlorella vulgaris* and *Anabaena torulosa* methanol extract that were grown at controlled normal conditions revealed the presence of 3 distinct peaks at 12.26, 12.58, and 13.04min for *Chlorella vulgaris* and 12.26, 13.08, and 13.16min for *Anabaena torulosa* (Figs. 1, 2). The most abundant compounds in methanol extract according to Tables (2, 3) were 13-Octadecenoic acid, (E)- TMS derivative, methyl ester of 7-Octadecenoic acid, and Hexadecanoic acid, and TMS derivative (11-Octadecenoic acid, (E)-, Palmitic Acid, and Stearic acid) for *C. vulgaris* and *A. torulosa*, respectively. The lack of abundant phenolic compounds was noticeable.

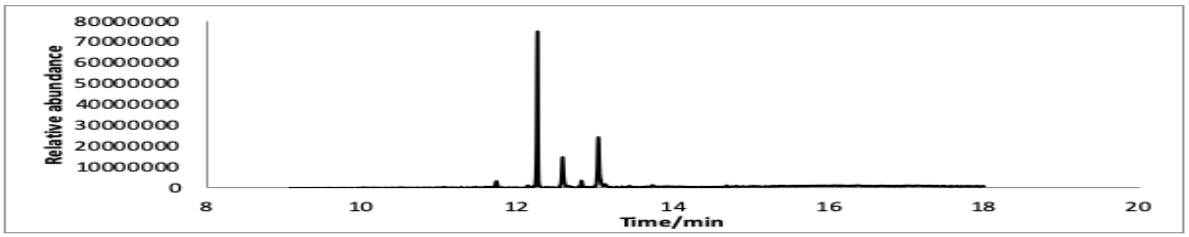

Fig. 1. GC-MS profile of the major constituents of *Chlorella vulgaris* methanol extract

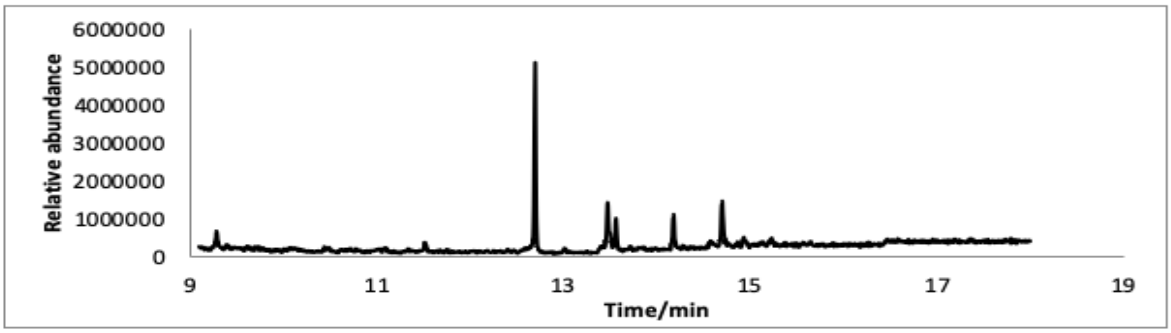

Fig. 2. GC-MS profile of the major constituents of *Anabaena torulosa* methanol extract

Table 2. GC-MS profiling of methanol extract of *C. vulgaris*

ID	**Compounds**	**Retention time (min)**	**Area %**
1	Propanoic acid, TMS derivative	11.0496	0.65
2	Hexadecanoic acid, methyl ester	11.6277	3.35
3	Palmitic acid, TMS derivative	12.2627	41.23
4	7-Octadecenoic acid, methyl ester	12.5831	13.37
5	Cyclopentanol, TMS derivative	12.8292	2.74
6	Phenol, 3-amino-2-chloro-	12.8343	0.09
7	Quinoline, 4-methyl-	12.8566	0.12
8	13-Octadecenoic acid, (E)- TMS derivative	13.0466	23.2
9	2-Hydroxyisocaproic acid, TMS derivative	13.1324	1.78
10	Ribitol, 5TMS derivative	13.8993	3.44
11	Catechol, 2TBDMS derivative	13.687	0.15
12	Monoamidoethylmalonic acid, O,O,O'-tris(trimethylsilyl)-	14.6661	0.42
13	Propanal, 2,3-bis[(trimethylsilyl)oxy]-, O-methyloxime, (S)-	14.8948	0.72
14	Dodecanoic acid, 2,3-bis(acetyloxy)propyl	15.2955	1.11
15	5.alpha.-Dihydrotestosterone, TMS derivative	16.3596	1.09
16	.alpha.-Ketoisovaleric acid, TMS derivative	17.1664	1.16

Table 3. GC-MS analysis of methanol extract of *A. torulosa*

ID	**Compounds**	**Retention time (min)**	**Area %**
1	2-Bromosebacic acid, 2TMS derivative	11.5419	0.04
2	Benzene, (2,2-dimethoxyethyl)-	11.5424	0.06
3	Valproic acid, TMS	11.7193	0.08
4	Palmitic Acid, TMS derivative	12.2686	26.3
5	Benzoic acid, p-(9-acridinylamino)-	12.2697	0.15
6	Arachidonic acid, TMS derivative	12.398	0.94
7	11-Octadecenoic acid, (E)-, TMS derivative	13.0868	50.2
8	Oleic Acid, (Z)-, TMS derivative	13.087	0.35
9	Benzene, 1-ethyl-4-(4-propylcyclohexyl)-, trans-	13.1418	0.19
10	Stearic acid, TMS derivative	13.1612	18.9
11	6-Chlorohexanoic acid, TMS derivative	13.5389	0.06
12	Suberic acid, 2TBDMS derivative	14.2255	0.03
13	Camphoric acid, 2TBDMS derivative	14.512	0.09
14	Codeine, TMS derivative	14.5116	0.10
15	Myristic acid, TMS derivative	14.6089	0.13
16	13-Docosenoic acid, (Z)-, TMS derivative	14.6890	0.17
17	Acetin, bis-1,3-trimethylsilyl ether	14.8664	0.06
18	Propanal, 2,3-bis[(trimethylsilyl)oxy]-, O-methyloxime, (S)-	15.141	0.25
19	Catechol, 2TBDMS derivative	15.4039	1.96
20	Silane, 1-(3-cyclodecen-1-yloxy)trimethyl-, (Z)-	15.4042	0.29
21	cyclohexanecarboxylic acid, 2-[[(trimethylsilyl)oxy]methyl]-, trimethylsilyl ester	15.5358	0.10
22	16-Hydroxydehydroandrosterone, (3.beta.,16.alpha.)-, 2TMS derivative	16.1366	0.41
23	17-Octadecynoic acid, TBDMS derivative	16.1767	0.61

3. FTIR analysis of methanol extract

Figs. (3, 4) reveal the FTIR transmittance spectra of *C. vulgaris* and *A. torulosa* methanol extracts of both microalgae grown for two weeks at normal cultivation conditions; light intensity of 100µmol photons m^{-2} s^{-1} with a photoperiod of 12:12 (L:D), temperature at 25 ± 2°C, by showing characteristic peaks which represents at 543.16cm^{-1}, 827.27cm^{-1}, 995.95cm^{-1}, 1047.66cm^{-1}, 1124.92cm^{-1}, 1270.86cm^{-1}, 1359.62cm^{-1}, 1655.01cm^{-1}, 2923.19cm^{-1}, and 3350.94cm^{-1} for *C. vulgaris* and at 542.72cm^{-1}, 833.36cm^{-1}, 990.18cm^{-1}, 1033.87cm^{-1}, 1266.94cm^{-1}, 1352.75cm^{-1}, 1652.16cm^{-1}, 2940.36cm^{-1}, 3005.64cm^{-1}, and 3330.85cm^{-1} for *A.* torulosa.

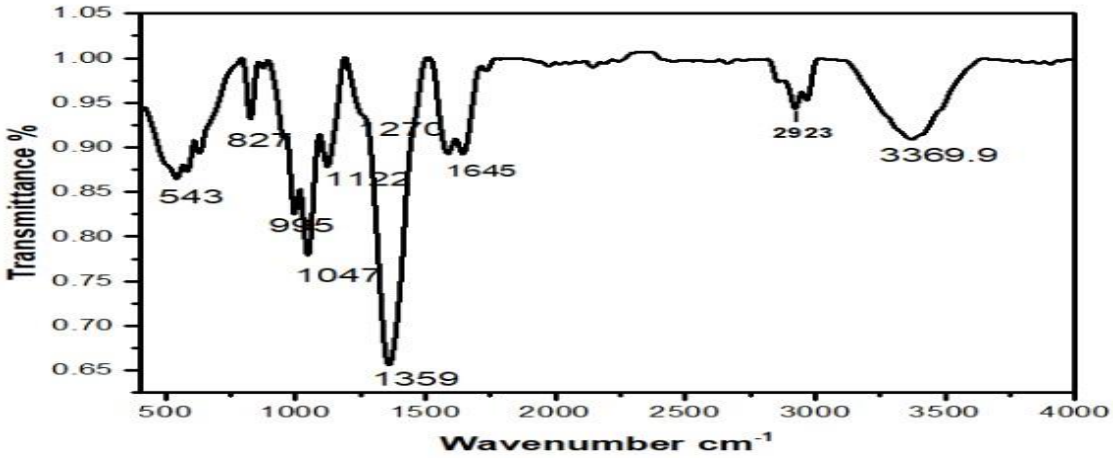

Fig. 3. FTIR spectrum of *C. vulgaris* methanol extract

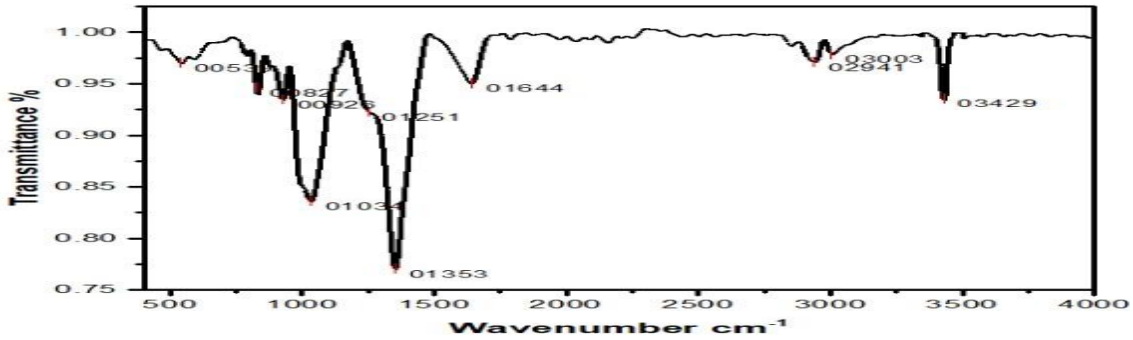

Fig. 4. FTIR spectrum of *A. torulosa* of methanol extract

4. Effect of high light intensity shocks

The impact study of the high light intensity shocks on *Chlorella vulgaris* and *Anabaena torulosa* observed that both microalgal species can tolerate high illumination shocks; 100, 150, 250, and 350µmol photons m^{-2} s^{-1} for 10, 20, and 30min (Tables 4 and 5). As the light intensity shock increases, the growth biomass and the extract mass decrease, while TP and TC increase as well as the antioxidant activity. It was found that,

at conditions of 350µmol photons m^{-2} s^{-1} that last for 20min, TP, TC, and A.O% increases over the control by 20.5, 8.6, and 18.9% for *Chlorella vulgaris* and 20.7, 20.8, and 15.6% for *Anabaena torulosa*, respectively.

Table 4. Effect of high light intensity shocks on some tested biological indicators of *Chlorella vulgaris*

µmol photons m^{-2} s^{-1}	**Time/min.**	**CDW mg/50ml**	**EDW mg/50ml**	**TP mg/1gm**	**TC mg/1gm**	**A.O. %**
100 (Control)	10	65.2±0.5^{Aa}	22.5±0.3^{Aa}	67.0±0.3^{Dc}	80.7±0.3^{Db}	65.7±0.4^{Dc}
	20	64.0±0.5^{Ab}	21.7±0.3^{Ab}	68.3±0.3^{Da}	82.3±0.3^{Da}	68.7±0.4^{Da}
	30	62.7±0.5^{Ac}	19.8±0.3^{Ac}	66.3±0.3^{Db}	82.0±0.3^{Da}	67.7±0.4^{Db}
150	10	62.8±0.5^{Ba}	20.9±0.3^{Ba}	70.0±0.3^{Cc}	82.3±0.3^{Cc}	70.0±0.4^{Cc}
	20	60.7±0.5^{Bb}	19.7±0.3^{Bb}	73.4±0.3^{Ca}	85.3±0.3^{Ca}	73.7±0.4^{Ca}
	30	59.7±0.5^{Bc}	18.3±0.3^{Bc}	72.3±0.3^{Cb}	84.5±0.3^{Cb}	71.7±0.4^{Cb}
250	10	61.8±0.5^{Ca}	19.5±0.3^{Ca}	75.3±0.3^{Bc}	86.7±0.3^{Bc}	74.7±0.4^{Bc}
	20	59.2±0.5^{Cb}	18.3±0.3^{Cb}	78.7±0.3^{Ba}	88.4±0.3^{Ba}	77.3±0.4^{Ba}
	30	59.0±0.5^{Cc}	17.0±0.3^{Cc}	77.7±0.3^{Bb}	86.2±0.3^{Bb}	75.7±0.4^{Bb}
350	10	59.8±0.5^{Da}	17.9±0.3^{Da}	79.3±0.3^{Ac}	87.5±0.3^{Ac}	79.3±0.4^{Ac}
	20	58.2±0.5^{Db}	16.8±0.3^{Db}	82.3±0.3^{Aa}	89.4±0.3^{Aa}	81.7±0.4^{Aa}
	30	57.5±0.5^{Dc}	15.2±0.3^{Dc}	80.3±0.3^{Ab}	88.5±0.3^{Ab}	80.3±0.4^{Ab}
LSD Light		1.07	1.35	1.76	1.76	1.63
LSD Time		1.02	0.56	0.66	0.66	1.14

Values are means ± SE (n=3). Data are analyzed using Multivariate (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage, LSD= least significant difference.

Table 5. Effect of high light intensity shocks on some tested biological indicators of *Anabaena torulosa*

µmol photons m^{-2} s^{-1}	**Time/min.**	**CDW mg/50ml**	**EDW mg/50ml**	**TP mg/1gm**	**TC mg/1gm**	**A.O. %**
100 (Control)	10	75.7±0.4^{Aa}	21.7±0.2^{Aa}	66.2±0.4^{Da}	63.0±0.3^{Dc}	64.8±0.3^{Dc}
	20	78.3±0.4^{Aa}	24.0±0.2^{Aa}	66.0±0.4^{Da}	64.3±0.3^{Da}	66.0±0.3^{Da}
	30	77.0±0.4^{Ab}	23.0±0.2^{Ab}	65.1±0.4^{Db}	62.4±0.3^{Db}	65.1±0.3^{Db}
150	10	76.7±0.4^{Ba}	20.7±0.2^{Ba}	67.5±0.4^{Cc}	64.3±0.3^{Ca}	65.8±0.3^{Cc}
	20	76.0±0.4^{Ba}	19.3±0.2^{Ba}	70.0±0.4^{Ca}	67.7±0.3^{Ca}	68.3±0.3^{Ca}
	30	75.0±0.4^{Bb}	18.3±0.2^{Bb}	71.0±0.4^{Cb}	66.7±0.3^{Cb}	66.7±0.3^{Cb}
250	10	73.0±0.4^{Ca}	17.0±0.2^{Ca}	75.0±0.4^{Bc}	68.3±0.3^{Bc}	69.3±0.3^{Bc}
	20	72.7±0.4^{Ca}	16.0±0.2^{Ca}	77.8±0.4^{Ba}	72.7±0.3^{Ba}	73.3±0.3^{Ba}
	30	71.0±0.4^{Cb}	15.3±0.2^{Cb}	76.4±0.4^{Bb}	71.3±0.3^{Bb}	72.3±0.3^{Bb}
350	10	69.3±0.4^{Da}	15.1±0.2^{Da}	76.5±0.4^{Ac}	73.7±0.3^{Ac}	74.3±0.3^{Ac}
	20	68.0±0.4^{Da}	14.0±0.2^{Da}	79.7±0.4^{Aa}	77.7±0.3^{Aa}	76.3±0.3^{Aa}
	30	66.0±0.4^{Db}	13.0±0.2^{Db}	78.3±0.4^{Ab}	75.3±0.3^{Ab}	75.3±0.3^{Ab}
LSD Light		1.11	2.06	3.33	3.33	3.77
LSD Time		1.41	0.91	1.16	1.16	1.50

Values are means ± SE (n=3). Data are analyzed using Multivariate (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
LSD= least significant difference, CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage.

5. Effect of temperature shocks at different durations

Regarding the effect of temperature shocks through the dark period of incubation on *Chlorella vulgaris* and *Anabaena torulosa*. The results in Tables (6, 7) illustrate that high and low temperatures affect positively carotenoid and phenolic production. Both microalgal isolates were able to grow and induce over-production of biologically active metabolites at 17 and 43^{o}C for three, six, and nine hours. The most potent temperature that triggers the highly production of total phenolics and total carotenoids was 43^{o}C for 6h. The values of TP, TC, and A.O% were 24.0%, 13.7%, and 19.0% of the control samples for *C. vulgaris* and 27.3%, 23.2%, and 18.6 % for *A. torulosa*.

Table 6. Effect of temperature shocks on some biological aspects of *Chlorella vulgaris*

Temp/^{o}C	Time/h	CDW mg/50ml	EDW mg/50ml	TP mg/g	TC mg/g	A.O. %
25 (Control)	3	67.3±0.4^{Aa}	22.7±0.4^{Aa}	67.3±0.3^{Cc}	81.4±0.4^{Cc}	66.0±0.4^{Cc}
	6	66.7±0.4^{Ab}	22.3±0.4^{Ab}	68.7±0.3^{Ca}	82.7±0.4^{Ca}	68.7±0.4^{Ca}
	9	65.4±0.4^{Ac}	20.3±0.4^{Ac}	66.3±0.3^{Cb}	82.0±0.4^{Cb}	67.7±0.4^{Cb}
17	3	63.7±0.4^{Ba}	20.8±0.4^{Ba}	76.7±0.3^{Bc}	83.7±0.4^{Bc}	74.3±1.4^{Bc}
	6	62.2±0.4^{Bb}	19.7±0.4^{Bb}	80.7±0.3^{Ba}	86.7±0.4^{Ba}	78.3±0.4^{Ba}
	9	60.0±0.4^{Bc}	18.7±0.4^{Bc}	79.3±0.3^{Bb}	85.7±0.4^{Bb}	76.3±0.4^{Bb}
43	3	62.3±0.4^{Ca}	19.8±0.4^{Ca}	76.7±0.3^{Ac}	89.3±0.4^{Ac}	79.7±0.4^{Ac}
	6	60.8±0.4^{Cb}	18.5±0.4^{Cb}	85.3±0.3^{Aa}	94.0±0.4^{Aa}	81.8±0.4^{Aa}
	9	58.4±0.4^{Cc}	17.0±0.4^{Cc}	83.7±0.3^{Ab}	91.3±0.4^{Ab}	80.3±0.4^{Ab}
LSD Temperature		1.41	1.28	5.08	5.08	5.00
LSD Time		1.22	0.93	1.80	1.80	1.18

Values are means ± SE (n=3). Data are analyzed using Multivariate (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage, LSD= Least significant difference.

Table 7. Effect of temperature shocks on some biological aspects of *Anabaena torulosa*

Temp/^{o}C	Time/h.	CDW mg/50ml	EDW mg/50ml	TP mg/gm	TC mg/gm	A.O. %
25 (Control)	3	78.4±0.3^{Aa}	24.0±0.3^{Aa}	67.3±0.8^{Cc}	63.0±0.4^{Cc}	64.3±0.5^{Cc}
	6	77.2±0.3^{Ab}	23.3±0.3^{Ab}	66.0±0.8^{Ca}	64.7±0.4^{Ca}	66.0±0.5^{Ca}
	9	75.3±0.3^{Ac}	22.0±0.3^{Ac}	65.1±0.8^{Cb}	62.3±0.4^{Cb}	65.1±0.5^{Cb}
17	3	77.3±0.3^{Ba}	21.3±0.3^{Ba}	68.7±0.8^{Bc}	69.3±0.4^{Bc}	69.3±0.5^{Bc}
	6	75.7±0.3^{Bb}	19.3±0.3^{Bb}	74.3±0.8^{Ba}	73.3±0.4^{Ba}	72.0±0.5^{Ba}
	9	74.3±0.3^{Bc}	18.3±0.3^{Bc}	70.7±0.8^{Bb}	71.3±0.4^{Bb}	71.7±0.5^{Bb}
43	3	74.0±0.3^{Ca}	18.0±0.3^{Ca}	75.7±0.8^{Ac}	75.0±0.4^{Ac}	73.7±0.5^{Ac}
	6	72.7±0.3^{Cb}	16.7±0.3^{Cb}	84.0±0.8^{Aa}	79.7±0.4^{Aa}	78.3±0.5^{Aa}
	9	70.3±0.3^{Cc}	15.3±0.3^{Cc}	81.3±0.8^{Ab}	77.0±0.4^{Ab}	76.0±0.5^{Ab}
LSD Light		1.20	3.00	4.88	4.88	4.25
LSD Time		1.42	1.22	1.00	1.00	1.44

Values are means ± SE (n=3). Data are analyzed using Multivariate (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage, LSD= least significant difference.

6. Effect of different nitrogen concentration within incubation time

Nitrogen is considered one of the main sources of nutrients in BG-11 medium constituents. BG-11 medium with three concentrations (0.5, 1.5, and 2.5g/ L) of $NaNO_3$ were incubated separately at different durations: 2, 4, and 6 days of incubation. *C. vulgaris* and *A. torulosa* were able to grow and produce phenolic and carotenoids which directly increase the antioxidant capacity either in nitrogen limitation or nitrogen excession. As shown in Tables (8, 9), the ANOVA revealed that, six days of incubation with nitrogen concentration 0.5g/ L was the best-chosen factors that increase TP, TC, and A.O.% over the control ones by 36.0%, 22.5%, and 22.4% for *C. vulgaris* and 38.5%, 38.3%, and 27.5% for *A. torulosa*.

Table 8. Effect of nitrogen concentrations on some biological aspects of *Chlorella vulgaris*

Conc (g/L)	Days	CDW mg/50ml	EDW mg/50ml	TP mg/gm	TC mg/gm	A.O. %
1.5 (Control)	2	60.3±0.4^{Ac}	18.7±0.3^{Ac}	61.3±0.6^{Cc}	77.7±0.6^{Cc}	61.0±0.6^{Cc}
	4	65.0±0.4^{Ab}	21.0±0.3^{Ab}	65.3±0.6^{Cb}	80.3±0.6^{Cb}	64.3±0.6^{Cb}
	6	67.3±0.4^{Aa}	22.7±0.3^{Aa}	67.7±0.6^{Ca}	83.0±0.6^{Ca}	68.3±0.6Ca
0.5	2	57.3±0.4^{Cc}	16.3±0.3^{Cc}	77.3±0.6^{Ac}	89.3±0.6^{Ac}	68.3±0.6^{Ac}
	4	60.3±0.4^{Cb}	18.0±0.3^{Cb}	85.3±0.6^{Ab}	94.0±0.6^{Ab}	78.6±0.6^{Ab}
	6	62.3±0.4^{Ca}	20.3±0.3^{Ca}	92.0±0.6^{Aa}	101.7±0.6^{Aa}	83.6±0.6^{Aa}
2.5	2	59.0±0.4^{Bc}	17.3±0.3^{Bc}	63.0±0.6^{Bc}	83.7±0.6^{Bc}	63.0±0.6^{Bc}
	4	61.7±0.4^{Bb}	21.0±0.3^{Bb}	78.0±0.6^{Bb}	87.7±0.6^{Bb}	71.7±0.6^{Bb}
	6	63.7±0.4^{Ba}	22.7±0.3^{Ba}	82.0±0.6^{Ba}	92.0±0.6^{Ba}	76.3±0.6^{Ba}
LSD Conc.		1.44	1.22	5.44	5.44	6.33
LSD Days		2.11	1.88	3.66	3.66	1.92

Values are means ± SE (n=3). Data are analyzed using Multivariate (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage, LSD= Least significant difference.

Table 9. Effect of nitrogen concentrations on some biological aspects of *Anabaena torulosa*

Conc (g/L)	Days	CDW mg/50ml	EDW mg/50ml	TP mg/gm	TC mg/gm	A.O. %
1.5 (Control)	2	69.7±0.4^{Ac}	17.7±0.3^{Ac}	61.3±0.6^{Cc}	57.7±0.6^{Cc}	60.3±0.5^{Cc}
	4	73.0±0.4^{Ab}	21.7±0.3^{Ab}	65.0±0.6^{Cb}	62.7±0.6^{Cb}	63.0±0.5^{Cb}
	6	78.0±0.4^{Aa}	24.3±0.3^{Aa}	66.7±0.6^{Ca}	65.0±0.6^{Ca}	65.1±0.5^{Ca}
0.5	2	67.7±0.4^{Cc}	15.7±0.3^{Cc}	77.7±0.6^{Ac}	66.7±0.6^{Ac}	74.7±0.5^{Ac}
	4	69.3±0.4^{Cb}	19.3±0.3^{Cb}	85.0±0.6^{Ab}	79.7±0.6^{Ab}	77.3±0.5^{Ab}
	6	75.7±0.4^{Ca}	21.7±0.3^{Ca}	90.0±0.6^{Aa}	86.7±0.6^{Aa}	80.3±0.5^{Aa}
2.5	2	69.3±0.4^{Bc}	16.7±0.3^{Bc}	67.0±0.6^{Bc}	62.0±0.6^{Bc}	69.3±0.5^{Bc}
	4	70.7±0.4^{Bb}	19.3±0.3^{Bb}	69.0±0.6^{Bb}	75.3±0.6^{Bb}	71.7±0.5^{Bb}
	6	77.7±0.4^{Ba}	22.7±0.3^{Ba}	73.3±0.6^{Ba}	78.7±0.6^{Ba}	72.3±0.5^{Ba}
LSD Conc.		1.00	0.66	9.55	9.55	5.77
LSD Days		2.11	2.77	4.33	4.33	4.55

Values are means ± SE (n=3). Data are analyzed using Multivariate (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage, LSD= Least significant difference.

7. Effect of sodium chloride (NaCl) concentrations

An ongoing study examined the impact of four NaCl concentrations—50mM, 100mM, 150mM, and 200mM—on algal growth. As shown in Tables (10 and 11), the biomass of *Chlorella vulgaris* and *Anabaena torulosa* decreased progressively with increasing salinity. Biomass values were 61.7±0.7, 61.7±0.7, 60.7±0.7, 59.7±0.7, and 57.7±0.3mg for *C. vulgaris*, and 76.3±0.9, 75.7±0.3, 74.2±0.2, and 72.3±0.3mg for *A. torulosa*, compared to the untreated controls, which were 63.3±0.7mg and 78.0±0.6mg, respectively.

Notably, there was a significant increase in total protein (TP), total carbohydrate (TC), and antioxidant activity (A.O.%) relative to the control. For *C. vulgaris*, increases were observed at 150mM NaCl: 61.4% (TP), 31.0% (TC), and 23.4% (A.O.%). For *A. torulosa*, the highest increases were seen at 100 mM NaCl: 50.0% (TP), 47.2% (TC), and 20.1% (A.O.%).

Table 10. Effect of salinity on some biological aspects of *Chlorella vulgaris*

Conc (mM)	CDW mg/50ml	EDW mg/50ml	TP mg/gm	TC mg/gm	A.O. %
0 (Control)	63.3 ±0.7^{a}	21.7±0.3^{a}	67.7 ±0.3^{e}	83.3 ±0.9^{e}	68.3±0.3^{e}
50	61.7±0.7^{ab}	20.2±0.4^{b}	92.0 ±0.0^{d}	101.0±1.0^{d}	79.3±0.3^{d}
100	60.7 ±0.7^{b}	18.2±0.4^{c}	100.3 ±0.3^{c}	104.0±0.6^{c}	81.3±0.3^{c}
150	59.7 ±0.7^{b}	16.3±0.3^{d}	109.3 ±0.3^{a}	109.0±0.6^{a}	84.3±0.6^{a}
200	57.7 ±0.3^{c}	15.2±0.4^{d}	107.3 ±0.3^{b}	106.3±0.3^{b}	82.7±0.3^{b}
LSD	2.00	1.50	2.00	2.33	1.33

Values are means ± SE (n=3). Data are analyzed using one-way analysis of variance (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage, LSD= Least significant difference.

Table 11. Effect of salinity on some biological aspects of *Anabaena torulosa*

Conc (mM)	CDW mg/50ml	EDW mg/50ml	TP mg/gm	TC mg/gm	A.O. %
0 (Control)	78.0 ±0.6^{a}	23.0 ±0.6^{a}	66.3 ±0.3^{e}	65.0±0.0^{e}	67.7±0.3^{e}
50	76.3±0.9^{b}	19.3 ±0.3^{b}	82.7 ±0.7^{d}	86.3±0.3^{d}	74.7 ±0.3^{d}
100	75.7±0.3^{bc}	17.3±0.9^{bc}	99.3 ±0.3^{a}	95.7±0.3^{a}	81.3 ±0.3^{a}
150	74.2 ±0.2^{c}	16.0±1.0^{cd}	96.3 ±0.3^{b}	92.7±0.9^{b}	80.0 ±0.6^{b}
200	72.3 ±0.3^{d}	14.7±0.7^{d}	94.3 ±0.3^{c}	89.0±0.3^{c}	77.7 ±0.3^{c}
LSD	1.66	2.66	2.00	2.66	1.33

Values are means ± SE (n=3). Data are analyzed using one-way analysis of variance (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage, LSD= Least significant difference.

8. Effect of different doses of gamma radiation

After different gamma radiation doses exposure, though the ability of *Chlorella vulgaris* and *Anabeana torulosa* to grow, lower growth biomass, and lower extract mass were spotted contrast to the non-irradiated microalgal isolates (Tables 12, 13). A converse relation was revealed between radiation doses and biomass as well as methanol extracts. On the other hand, by rising gamma radiation doses, the mass of total phenolics as well as carotenoids increase. The highest recorded percentages of TP, TC, and A.O.% over the control were 105.1%, 71.3%, and 40.1% at 1.5 KGy for *C. vulgaris* and 104.3%, 104.1%, and 39.0% at 1 KGy for *A. torulosa*, respectively.

Table 12. Effect of gamma radiation on some biological aspects of *Chlorella vulgaris*

Dose (KGy)	CDW mg/50ml	EDW mg/50ml	TP mg/1gm	TC mg/1gm	A.O. %
0 (Control)	64.3±0.3^{a}	21.2±0.2^{a}	66.3±0.3^{e}	82.7±0.3^{e}	68.3±0.3^{d}
0.5	56.3±0.3^{b}	16.0±0.6^{b}	126.0±0.0^{d}	132.3±0.3^{d}	93.1±0.1^{c}
1.0	55.3±0.3^{b}	15.3±0.3^{bc}	132.0±0.6^{b}	137.3±0.3^{b}	94.3±0.3^{b}
1.5	54.0±0.0^{c}	14.5±0.3^{cd}	136.0±0.6^{a}	141.7±0.9^{a}	95.7±0.3^{a}
2.0	53.0±0.6^{c}	13.3±0.3^{d}	129.0±0.6^{c}	135.0±0.0^{c}	93.9±0.5^{c}
LSD	1.33	1.50	3.00	2.33	1.20

Values are means ± SE (n=3). Data are analyzed using one-way analysis of variance (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage, LSD= Least significant difference.

Table 13. Effect of gamma radiation on some biological aspects of *Anabaena torulosa*

Dose (KGy)	CDW mg/50ml	EDW mg/50ml	TP mg/1gm	TC mg/1gm	A.O. %
0 (Control)	77.0±0.6^{a}	22.0±0.6^{a}	67.7±0.3^{e}	66.0±0.6^{e}	68.0±0.0^{d}
0.5	67.3±0.3^{b}	15.0±0.0^{b}	122.7±0.3^{d}	119.7±0.3^{d}	90.4±0.3^{c}
1.0	66.0±0.0^{c}	14.3±0.3^{b}	138.3±0.9^{a}	134.7±0.9^{a}	94.3±0.3^{a}
1.5	65.0±0.0^{c}	13.0±0.0^{c}	130.7±1.2^{b}	129.0±0.6^{b}	92.7±0.3^{b}
2.0	63.7±0.3^{d}	12.6±0.3^{c}	126.0±0.6^{c}	124.3±0.7^{c}	91.0±0.0^{c}
LSD	1.33	1.33	3.33	4.66	1.66

Values are means ± SE (n=3). Data are analyzed using one-way analysis of variance (ANOVA) followed by a, b, c, d Duncan's multiple range test (DMRT).
CDW= cells dry Weight, EDW= extract dry weight, TP = Total phenolic, TC = Total carotenoids, A.O% = Antioxidant percentage, LSD= Least significant difference.

DISCUSSION

Green algae are advantageous to higher plants for the production of bioactive substances as they grow faster, utilize small amounts of water, have high biomass productivity, and fix high volumes of carbon dioxide **(Wu *et al.*, 2023)**.

The extraction yield of carotenoids and phenolics for the green microalga *Chlorella vulgaris* and the cyanobacteria *Anabaena torulosa* is robustly dependent on the extracts

prepared and the polarity of used solvents **(Jerez-Martel *et al.*, 2017)**. **Molnár *et al.* (2013)** stated that methanol solvent is the best for carotenoids extraction from *Chlorella vulgaris*, resulting in high amount of carotenoids when compared to all other solvent extracts and according to **Bhuvana *et al.*, (2019)**, the towering phenolic content was notified in *Chlorella minutissima* methanolic extracts was reported as well as *Anabeana* sp. This finding is also similar to the finding of **Goiris *et al.* (2012)** postulating that the samples with a comparatively high phenolic content (>3 mg G.A.E. g^{-1} biomass) were extracted with a polar solvent.

Koh *et al.* (2019) reported that, in addition to chlorophyll a, other types of chlorophyll—such as b, c, and d—are found in microalgae, while chlorophylls f and d are present in certain cyanobacteria. Chlorophyll *a* is considered the primary pigment in algal cells, while other chlorophylls serve as accessory pigments. Phycobilins contribute to additional light absorption in the 490–650nm range, according to **Stadnichuk *et al.* (2015)**. The phycobilins found in cyanobacteria differ from those in Rhodophyta and are named accordingly: R-phycoerythrin, R-phycocyanin, C-phycoerythrin, and C-phycocyanin **(Greenwold *et al.*, 2019)**.

Another important group of accessory pigments is the carotenoids, which absorb light in the 400–500nm range **(Ladygin, 2014)**. Carotenoids are widespread in nature, with over 1,100 types identified to date, as noted by **Wan *et al.* (2021).** Based on the presence of oxygen, carotenoids are classified into xanthophylls—oxygen-containing pigments—and carotenes, which lack oxygen. The most common microalgal carotenoids include β-carotene and the xanthophylls zeaxanthin, lutein, and violaxanthin **(Novoveská *et al.*, 2019)**. Synthesis and structure of microalgae carotenoids can be affected by the seasonal change of the the aquatic environment such as salinity, water temperature, nutrients availability, and light.

Klejdus *et al.* (2009) notified that more abundant compound in microalgae compared to cyanobacteria is phenolics. In contrast. **Shalaby (2011)** explained that a large diversity of secondary bioactive compounds as phenolic compounds are produced by cyanobacteria. Phenols are a substantial set of natural products with antioxidant and another biological actions (generated as secondary metabolites by microalgae). However, these compounds are not participating directly in primary processes of algae such as cell division, reproduction, and photosynthesis. These compounds are virtual in algal cell defense versus biotic and abiotic stress **(Mobin *et al.*, 2019)**.

In vitro methods, DPPH scavenging method out of all is the most simple, easy, and rationally costly way for studying the antioxidants activity against the free radical-scavenging **(Shaheen *et al.*, 2023)**. **Jerez-Martel *et al.* (2017)** illustrated that *Leptolyngbya protospira*, *Nostoc* sp., and *phormidiochelate* sp. (cyanobacteria strains) and *Ankistrodesmus* sp., *Caespitella pascheri*, *Spirogyra* sp., and *Euglena cantabrica* (microalgae) displayed DPPH scavenging capacity at a higher concentration of samples. Microalgal biomass is a prosperous naturalistic source of antioxidants, with potential

applications in aquatic feed, human food, medicine, and cosmetics **(de Morais *et al.*, 2015)**. Applications of antioxidant on health have been spured by surveillance that oxidation and free radicals are implicated in many physiological missions causing morbidal conditions, an example of powerful antioxidant is Astaxanthin.

In the present study, at normal growth conditions, the lack of phenolic compounds abundance was noticeable and this may be due to its nature as a secondary metabolite which is usually produced at the late growth phase and sometimes at harsh conditions. This is parallel with **Sadek *et al.* (2017)**, who denoted that there are 13 major components in the chemical composition of methanol extracts of *Spirulina platensis* such as octadecenoic acid methyl ester, 9-octadecanoic acid, and heptadecane at normal growth conditions.

FTIR transmittance spectrum of *C. vulgaris* and *A.torulosa*, by showing characteristic peaks at 1047.66cm^{-1} and 1033.87cm^{-1} marks the existence of carbohydrate C-O-C of polysaccharides or C-O stretch of cyclic ethers or C-F stretch of fluoro-compouds. The peak at 1124.92cm^{-1} ascribe C-O stretching to secondary or tertiary alcohol or PO_3 stretch to phosphate ion. Peaks at 1270.86cm^{-1} and 1266.94cm^{-1} exhibit C-O C-N stretching of aromatic amines or stretching of aromatic ester **(Duygu *et al.*, 2012)**. Peaks at 1359.62cm^{-1} and 1352.75cm^{-1} also reveal O-H bending of phenol or C-O of COO^- of carboxylates or protein CH_2 and CH_3 bending of methyl or lipids ($N(CH_3)_3$ bending of methyl or S-O stretching of sulfate and sulfonic acid. The two bands at 1655.01cm^{-1} and 1652.16cm^{-1} display C-H bending of aromatic compounds or C=O stretching vibration of ketone group and protein amide. Whilst the two peaks at 2923.19cm^{-1}and 2940.36cm^{-1} indicate the existence of CH_2 stretching of lipid and carbohydrate or C-H stretching of alkanes. The peak at 3005.64cm^{-1} also indicates O-H stretch and C=O stretch of carboxylic or C-H stretching of aromatic compounds or alkenes **(Prabakaran *et al.*, 2019)**. Ultimately, the two peaks at 3350.94cm^{-1} and 3330.85cm^{-1} indicate O-H stretching hydroxy group of phenolic or O-H stretch of alcohols or N-H stretching of protein amide A **(Sahayaraj *et al.*, 2015)**. The wide and narrow peaks at 3350.94cm^{-1} and 3330.85cm^{-1} points to the quantity of hydroxyl group (carboxylic acid) either for phenolic or alcohol. The FTIR spectra of the methanolic crude extract showed similar fingerprints of ß-carotene and phycocyanin of *C. vulgaris* and *A. torulosa*, respectively, as studied by **Yusuf *et al.* (2016)** and **Al-Malki (2020)**. The spectral analysis of FT-IR is a tremendous technique to scout about the functional group of the natural metabolite compounds **(Moovendhan *et al.*, 2015)**.

Under stress conditions, excessive obsterics of highly reactive oxygen species (ROS) and hydroxyl radical (OH) produces oxidative damage out of the reaction of biomolecules including DNA with these species **(Rudrapal *et al.*, 2022)**. Pharmacological research studies have manifested that large amounts of free radicals and oxidative stress enhance the over-production of bioactive metabolites to overcome the

harsh conditions. *Chlorella vulgaris* and *Anabaena torulosa* can tolerate high illumination shocks.

Microalgal carotenoids are associated with photosynthesis, where they contribute to light energy absorption and are referred to as primary carotenoids, such as β-carotene. These primary carotenoids are less diverse than secondary carotenoids. Secondary carotenoids are predominantly produced by cells in response to specific environmental conditions, such as prolonged light exposure and high light intensity **(Maltsev *et al.*, 2021)**. For instance, *Dunaliella salina* can accumulate β-carotene up to 14% of its dry weight when subjected to nutrient stress, high light, and high salinity **(Moulton *et al.*, 1987)**. Similarly, *Haematococcus pluvialis* can accumulate large amounts of carotenoids—particularly astaxanthin—reaching up to 203% of its dry weight under stress conditions **(Rao *et al.*, 2007)**.

Faraloni and Torzillo (2013) stated that excess light energy absorbed by cells triggers a defense strategy involving the synthesis of photoprotective carotenoids such as lutein and xanthophyll cycle pigments (e.g., zeaxanthin). Astaxanthin and canthaxanthin are final products of secondary carotenoid biosynthesis in certain microalgae. **López *et al.* (2015)** found that cyanobacteria and microalgae have evolved antioxidant phenolic compounds as part of their stress adaptation mechanisms, particularly under high light intensities. **Barten *et al.* (2021)** reported that in photobioreactors, high light intensities can raise the temperature to as much as 50°C, negatively impacting biomass productivity. Therefore, careful regulation of light energy is essential.

The optimal growth temperature for mesophilic microalgae typically ranges between 20 and 25°C. Deviations from this range, either by increasing or decreasing temperature, are considered stress factors that can enhance the production of carotenoids and phenolic compounds **(Minhas *et al.*, 2016)**. **Napaumpaiporn and Sirikhachornkit (2016)** reported that a temperature shift from 25°C to 37°C led to increased carotenoid accumulation, emphasizing the importance of temperature control to maintain biomass productivity and avoid stress-induced losses.

Kilic *et al.* (2018) observed that extracts from *Dunaliella* sp. grown in media with 0.5g/ L nitrogen exhibited the highest antioxidant activity. Nitrogen depletion and excess induced metabolic shifts, resulting in increased production of phenolic and carotenoid compounds, but also significantly reduced growth rates, consistent with findings by **Campenni *et al.* (2013)**.

Salinity stress also plays a critical role in microalgal metabolism. **Salama *et al.* (2013)** reported that salinity can inhibit photosynthesis, thereby reducing net biomass production. However, *Dunaliella* sp. has been shown to tolerate high salinity levels and still accumulate substantial biomass **(Azachi *et al.*, 2003)**. In the current study, increasing salinity led to a reduction in extract weight, despite a corresponding increase in carotenoid and phenolic content. This aligns with **Minhas *et al.* (2016)**, who described salinity as a challenging stressor that stimulates carotenoid and phenolic synthesis due to

dynamic osmoregulation involving continuous glycerol production. **Salama *et al.* (2013)** further noted that in *Scenedesmus* sp., NaCl stress enhanced the production of lipids and bioactive compounds, including total phenolics and carotenoids.

Radiation is another stress factor influencing microalgal metabolism. **Abomohra *et al.* (2016)** found that exposure to 2 kGy significantly reduced biomass and active substances in *Arthrospira platensis* by 21 and 10%, respectively, compared to the control. However, microalgae can tolerate various radiation levels due to the radioprotective properties of carotenoids and phenolic compounds **(Oh *et al.*, 2016)**. **Pradhan *et al.* (2020)** supported this by demonstrating that *Chlorella vulgaris* exposed to low doses of gamma radiation exhibited increased radioresistance through enhanced production of carotenoids, proteins, and antioxidant compounds.

CONCLUSION

In conclusion, stress-induced shocks represent a promising technique for enhancing the production of bioactive compounds, particularly carotenoids and phenolic compounds. Additionally, the carotenoids and phenolics produced by *Chlorella vulgaris* and *Anabaena torulosa* demonstrated significant protective properties under stress conditions, functioning as potent antioxidants and radioprotective agents.

Author contributions

All authors contributed to the study conception and design. Habiba Nagah Sadek, Hassan Mohamed Gebreel, Hesham Mohamed Abd El Fatah, Loutfy Ali Moussa and Abeer Eman Zakaria performed material preparation, data collection, and analysis. Habiba Nagah Sadek and Abeer Emam Zakaria wrote the first draft of the manuscript. The final drafting, writing, review, and editing of the article in addition to revising it critically for important intellectual content were performed by Habiba Nagah Sadek, Abeer Emam Zakaria, and Hesham Mohamed Abd El Fatah. Finally, all authors read and approved the manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval

Not applicable. This study did not involve any human or animal subjects, therefore no ethical approval is required.

Competing interests

The authors declare no competing interests.

Informed consent

All authors agreed to publication.

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