

Evaluation of Antioxidant Characterization in Some Microalgae Exposed to Gamma Irradiation

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ABSTRACT

Natural antioxidant substitutes have gained popularity as alternatives to synthetic antioxidants in recent years. Microalgae are considered as an alternative natural source of antioxidants with interesting bioactive compounds. One of the fascinating bioactive features of the microalgae is their antioxidant activity. The purpose of this study was to assess the antioxidant characterization of three microalgae: *Arthrospira platensis*, *Scenedesmus obliquus*, and *Chlorella vulgaris*, after treatments with gamma irradiation at a dose of 200, 300, and 700 Gy, respectively. The phytochemical examination of the three algae revealed that the maximum total antioxidant activity in *S. obliquus* at 300 Gy was 73.8 mg g⁻¹ ascorbic acid, followed by *A. platensis* at 700 Gy by 62.9 mg g⁻¹ ascorbic acid. The minimum activity was noticed for *C. vulgaris* at 200 Gy by 46.7 mg g⁻¹ ascorbic acid as compared to the control. In addition, a significant increase was detected in the activity via γ -irradiation treatment of the three algae as compared to the control. Their levels were 2.26, 2.02, and 1.49 mg g⁻¹ FW in *S. obliquus* (300 Gy), *A. platensis* (700 Gy), and *C. vulgaris* (200 Gy). The antioxidant capacity of DPPH[•] (2,2-diphenyl-1-picrylhydrazyl), ABTS^{•+} [2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity, and ferric-reducing antioxidant power (FRAP) indicated high values in *S. obliquus* at 300 Gy, followed by *A. platensis* at 700 Gy, and *C. vulgaris* at 200 Gy, as compared to the control. Fifteen polyphenol fractions (ten phenolics and five flavonoids) were identified, which increased significantly by gamma irradiation treatment. Therefore, treatments of *A. platensis*, *S. obliquus*, and *C. vulgaris* with the optimum dose of γ -irradiation increased significantly the antioxidant characterization and the content of polyphenol fractions, which directly or indirectly can help maintain the health of living beings as they are natural, safe, cheap, available, and easy to obtain.

INTRODUCTION

Gamma radiation is a naturally occurring electromagnetic radioactive wave with a short wavelength and is the most energetic type of electromagnetic radiation (Moussa, 2001; Khodary & Moussa, 2003; Sreedhar *et al.*, 2013). Due to their widespread availability and potent penetrating capability, gamma rays have been shown to be more affordable and efficient than other ionizing radiations. Gamma irradiation was used for the enhancement of different plant species (Moussa,

2006; Vanhoudt *et al.*, 2014) and stimulating the growth of *A. platensis* (Moussa *et al.*, 2015). Low doses of γ -radiation may increase enzymatic activity and most physiological activities, which results in stimulating the rate of cell division (Moussa, 2011; Moussa & Abdul Jaleel, 2011). Recently, there has been a rising interest in the use of relatively low doses of gamma irradiation to activate biological processes in microalgae (Tale *et al.*, 2017; Ermavitalini *et al.*, 2017a, b; Moisesescu *et al.*, 2019; Almarashi *et al.*, 2020). Water makes up 90% of an infant's body and 70% of an adult's; therefore, its chemical change by ionizing radiation is a substantial consideration in relation to the chemical effects of ionizing radiation (Halliwell & Gutteridge, 2004). When water is exposed to ionizing energy, a variety of free radicals are produced, including ionized water molecules ($\text{H}_2\text{O}^{+\bullet}$), H^\bullet and OH^\bullet radicals. Following a series of processes, ionized molecules create secondary reactive oxygen species (ROS) such as H_2O_2 and $\text{O}_2^{\bullet-}$, which ultimately lead to oxidative stress (Lee *et al.*, 2009; Moussa & Mohamed, 2011; Moussa & Amira, 2018; Mohamed *et al.*, 2023). Experimental evidence shows that ROS contribute to the rise in lipid content in microorganisms (Yilancioglu *et al.*, 2014; Tale *et al.*, 2017; Shi *et al.*, 2017). The organism under stress has developed a variety of defensive mechanisms, such as non-enzymatic antioxidants viz. phenols and proline, as well as enzymatic antioxidants including glutathione reductase, ascorbate peroxidase, superoxide dismutase, peroxidase, and catalase to prevent oxidative damage (Zhao & Li, 2014). Microalgae are one of the most valuable sources of natural biochemical contents for food, pharmaceutical, and cosmetics in addition to being potential sources of protein, lipids, vitamins, amino acids, and minerals for humans. Humans can get most of their antioxidants from plants; however, most microalgae can also be an untraditional source of these compounds instead of artificial antioxidants (Rani *et al.*, 2021). Arif *et al.* (2023) suggested that some microalgae are potential sources of carotenoids that have high antioxidant activity and have a potential source of food in the future. The green microalgae *Scenedesmus obliquus* have antimicrobial properties (Danielli *et al.*, 2019). Agam *et al.* (2022) concluded that *S. platensis* is considered a very potential source of antioxidants and phytonutrients, and according to Food and Drug Substances in the USA, *Spirulina* is recorded as a great food supplement due to its enrichment of vitamins, antioxidants, and phytonutrients. *Chlorella vulgaris* is employed in biofuels like biodiesel and bioethanol as well as in antibiotics, food, and pharmaceuticals (Novoveska *et al.*, 2019). Furthermore, *in vitro* tests on *C. vulgaris* reveal anticancer capabilities (Ragaa *et al.*, 2022).

The aim of this manuscript was to assess the antioxidant capacity of DPPH \cdot (2,2-diphenyl-1-picrylhydrazyl), ferric-reducing antioxidant power (FRAP), ABTS \cdot^+ [2,2-azino-bis(3-thylbenzothiazoline-6-sulfonic acid)] radical scavenging activity, total antioxidant capacity, carotenoids, and polyphenol fractions (flavonoid and phenolic) in some microalgae (*A. platensis*, *S. obliquus*, and *C. vulgaris*) in response to γ -irradiation treatment after 20 days of growth.

MATERIALS AND METHODS

Algae cultivation conditions

The algae used in this study (*Arthrospira platensis*, *Scenedesmus obliquus*, and *Chlorella vulgaris*) were obtained from the National Institute of Oceanography and Fisheries, hydrobiology laboratory. The microalgae *S. obliquus* and *C. vulgaris* were cultured in BG-11 media (Muna & Ameel, 2018; Supriya *et al.*, 2023). While, *A. platensis* was cultivated using modified Zarrouk medium (Aboelkheir *et al.*, 2008). The culture medium was autoclaved for 20 minutes at 121°C

before inoculation using an autoclave (STERIFOW-1341), and the required illumination was provided by sunlight. The solution was continually mixed by an aerator at a rate of 0.5L/ min (Heimix S, Heidolph, Germany), the photoperiod was 16/ 8h of day/ night cycle, a temperature of 30 ± 2 °C, and the pH was adjusted at 7.5 for *S. obliquus* and *C. vulgaris*, 8.5 to 9 for *A. platensis*. The harvested biomass was allowed to precipitate before being filtered using 0.45mm pore size Whatman GF/C filter paper to get a concentrated algae paste (Hamid *et al.*, 2016).

Irradiation of *A. platensis*, *S. obliquus*, and *C. vulgaris*

Volumes of 250mL of *A. platensis*, *S. obliquus*, and *C. vulgaris* of four-day-old culture grown were subjected to ten doses of γ -irradiation (0.0, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 Gy). The γ -irradiation is produced using a Co^{60} source at the Egyptian Atomic Energy Authority in Nasr City, Egypt (Moussa *et al.*, 2015; Moussa & Mohamed, 2015; Moussa *et al.*, 2023). The exposure rate was 0.84 Gy min^{-1} . A precise volume of the dark-adapted, irradiated cells was used to inoculate 750mL of modified Zarrouk media into one liter Erlenmeyer flasks with an initial optical density of 680 nm for 20 days.

Biochemical analysis of *A. platensis*, *S. obliquus*, and *C. vulgaris*

Antioxidant capacity of [DPPH \cdot (2,2-diphenyl-1-picrylhydrazyl) (mg VCE/g DW), ferric-reducing antioxidant power (FRAP)] (mM Fe^{2+} equivalent/g DW), and ABTS \cdot^+ [2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity (mg VCE/g DW) in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation after 20 days of growth was determined by the procedure of Sayed *et al.* (2018) and Mohamed *et al.* (2023). Total antioxidant capacity (TAC, mg g^{-1} Ascorbic acid) in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation after 20 days of growth was determined by the phosphomolybdenum method, and the results were calculated from a standard curve with ascorbic acid as a reference (Prieto *et al.*, 1999).

Photosynthetic pigments (carotenoids) were measured (mg g^{-1} FW) in the acetone algal extracts using a Unico 1201 spectrophotometer (Metzner *et al.*, 1965; Lichtenthaler & Buschmann, 2001). Flavonoid and phenolic fractions were determined by high-performance liquid chromatography (Waters, USA) using the technique described in Abdel-Farid *et al.* (2020).

RESULTS AND DISCUSSION

A. platensis, *S. obliquus*, and *C. vulgaris* were treated with different doses of γ -irradiation at 0.0, 100, 200, 300, 40, 500, 600, 700, 800, 900, and 1000 Gy. Considering how these doses affect the growth curve (optical density at 680 nm) and biomass productivity of the three algae after 14 days of growth, the optimum dose for each algae was estimated (data not included in the text). The optimum doses obtained from γ -irradiation treatments were 700, 300, and 200 Gy for *A. platensis*, *S. obliquus*, and *C. vulgaris*, respectively.

Antioxidant capacity of ABTS \cdot^+ [2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity, [DPPH \cdot (2,2-diphenyl-1-picrylhydrazyl), ferric-reducing antioxidant power (FRAP)], total antioxidant capacity (TAC), and carotenoids in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation after 20 days of growth

The data for antioxidant capacity of DPPH \cdot , ABTS \cdot^+ , FRAP, TAC, and carotenoids in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation after 20 days of growth are listed in Table (1) and Fig. (1).

The phytochemical analysis of the three algae treated with γ -irradiation increased the antioxidant characterization significantly ($P < 0.05$), and the maximum total antioxidant activity in *S. obliquus* at 300 Gy was 73.8 mg g⁻¹ ascorbic acid, followed by *A. platensis* at 700 Gy by 62.9 mg g⁻¹ ascorbic acid. The minimum activity was noticed for *C. vulgaris* at 200 Gy by 46.7 mg g⁻¹ ascorbic acid, as compared to the control samples. Additionally, carotenoid contents increased significantly ($P < 0.05$) by gamma irradiation treatment of the three algae as compared to the control. Their levels were 2.26, 2.02, and 1.49 mg g⁻¹FW in *S. obliquus* (300 Gy), *A. platensis* (700 Gy), and *C. vulgaris* (200 Gy), as compared to the control samples. The antioxidant capacity of DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS^{•+} [2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity, and ferric-reducing antioxidant power (FRAP) indicated high values in *S. obliquus* at 300 Gy, followed by *A. platensis* at 700 Gy, and *C. vulgaris* at 200 Gy compared to the control samples.

The results revealed that antioxidant activity increased significantly ($P < 0.05$) compared to the control samples. *S. obliquus* recorded the highest antioxidant screening results (340, 187, 5.2, and 73.8 mg VCE/g DW for ABTS, DPPH, FRAP mM Fe²⁺ equivalent/g DW, and TAC mg g⁻¹ Ascorbic acid, respectively). These values are higher than results recorded for *A. platensis* (245, 141, 3.3, and 62.9) and *C. vulgaris*. On the other hand, the lowest antioxidant results were 221, 123, 2.2, and 46.7, respectively.

The overall antioxidant capacities of plant extracts were examined using superoxide radical scavenging tests, DPPH, ABTS, and FRAP (Su et al., 2021). Montone et al. (2018) identified 25 sequenced peptides with angiotensin-converting enzyme inhibitory activities. They added the conceivable antioxidant was found in *Scenedesmus obliquus*. Four of these peptides, in particular, have shown strong DPPH radical scavenging activity. Moreover, proteins from all *Scenedesmus obliquus* showed significant antioxidant activity using the ABTS radical scavenging method (Afify et al., 2018). According to Marecek et al. (2017), the DPPH and ABTS techniques are both effective tools for measuring antioxidant activity. *A. platensis*, *S. obliquus*, and *C. vulgaris* investigated were noticeably able to quench the DPPH and ABTS radicals and serve as powerful reductants. These antioxidant activities are likely related to their phenolic and flavonoid contents, where these potent compounds are electron and/or hydrogen donors, and they therefore can react with the DPPH and ABTS free radicals to convert them into more stable products. In agreement with our results, the recent contributions of Devi et al. (2011) and Ismail et al. (2016) pointed out that both phenolic and flavonoids are common in seaweeds and have a wide niche of free radical scavenging and biological activities. Furthermore, Ismail (2017) concluded that there is a significant positive correlation between the DPPH radical scavenging activity and total phenolics and flavonoids in brown seaweed, *Sargassum linifolium*. Raja et al. (2016) highlighted a significant positive correlation between ABTS radical quenching activities and ferric-reducing antioxidant capacities in the brown macroalga *Eisenia arborea*.

Carotenoids which are red, yellow, or orange pigments that are insoluble in water are found in most photosynthetic organisms. Due to their distinctive qualities, particularly the health advantages and novel methods for manufacturing, microalgal carotenoids are receiving more and more attention from scientists worldwide (Novoveska et al., 2019). Microalgal carotenoids are safe and non-toxic colorants that are frequently employed as nutritional supplements, anticancer agents, and enhancers for antibodies synthesis (Ng et al., 2011).

Table 1. Antioxidant capacity of [DPPH[•](2,2-diphenyl-1-picrylhydrazyl) (mg VCE/g DW), ABTS^{•+}[2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity (mg VCE/g DW), ferric-reducing antioxidant power (FRAP)] (mM Fe²⁺ equivalent/g DW), total antioxidant capacity (TAC, mg g⁻¹ Ascorbic acid) and carotenoids (mg g⁻¹FW), in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation after 20 days of growth

Algal species	Dose (Gy)	ABTS	DPPH	FRAP	TAC	Carotenoids
<i>A. platensis</i>	0.0	201±12.1 ^e	127±6.3 ^d	2.1±0.1 ^c	41.2±3.1 ^e	1.06±0.05 ^f
	700	245± 9.4 ^c	141±8.3 ^c	3.3±0.2 ^b	62.9±2.3 ^b	2.02±0.08 ^b
<i>S. obliquus</i>	0.0	312±18.4 ^b	152±9.1 ^b	3.1±0.2 ^b	51.6±3.1 ^c	1.73±0.10 ^c
	300	340±10.8 ^a	187±12.1 ^a	5.2±0.3 ^a	73.8±5.1 ^a	2.26±0.18 ^a
<i>C. vulgaris</i>	0.0	187±11.2 ^f	99±6.9 ^e	1.0±0.1 ^d	34.2±1.1 ^f	1.23±0.09 ^e
	200	221±14.7 ^d	123±7.4 ^d	2.2±0.1 ^c	46.7±2.1 ^d	1.49±0.11 ^d

Values are represented as mean ± SD of samples in triplicate. Means assigned the same superscript letters in each column are not-significant different ($P > 0.05$), whereas others with different superscript letters are significant different ($P < 0.05$).

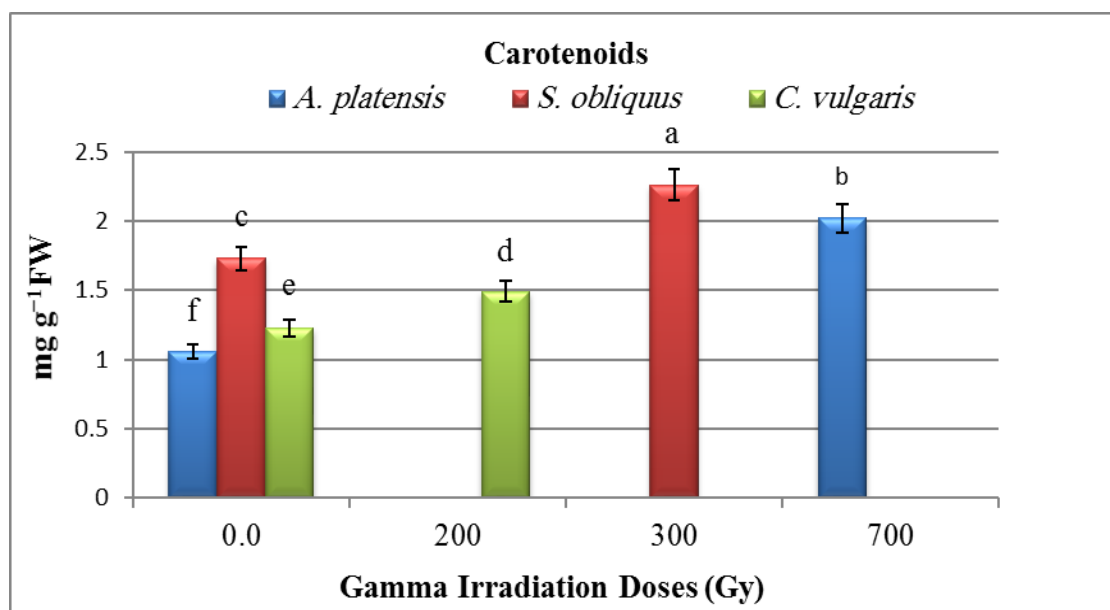


Fig. 1. Antioxidant capacity of carotenoids in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation after 20 days of growth

Microalgae-derived carotenoids are non-toxic colorants that are frequently used as nutritional supplements, anticancer agents, and catalysts for increasing antibody synthesis. Carotenoids are therefore in greater demand and have more applications in numerous industries (Liu *et al.*, 2016) and as a result, more and more studies focus on increasing the production of carotenoids in microalgae (Kuo *et al.*, 2012; Reyes *et al.*, 2014; Liu *et al.*, 2016). Gamma radiation increased the synthesis of carotenoids, which were often increased in stressful situations to protect chlorophyll from photooxidative degradation (Kovács & Keresztes, 2002; Moussa *et al.*, 2015; Effat *et al.*, 2017).

Identification and quantification of polyphenol fractions (flavonoid and phenolic) by HPLC in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation after 20 days of growth

The studied microalgae in this study (*A. platensis*, *S. obliquus*, and *C. vulgaris*) have naturally occurring polyphenol amounts of 10 phenolic and 5 flavonoid fractions, which are both potent effective antioxidants. After the three species of microalgae (*A. platensis*, *S. obliquus*, and *C. vulgaris*) were exposed to gamma irradiation at 700, 300, and 200 Gy, respectively, the phenolic compounds, benzoic acid, had the minimum values at 99, 78, and 92.5 $\mu\text{g/gDW}$, respectively. Salicylic acid (474 $\mu\text{g/gDW}$) had the highest concentration of phenolic compounds in *A. platensis*, whereas gallic acid concentrations in *S. obliquus* and *C. vulgaris* were 597 and 539 $\mu\text{g/gDW}$, respectively.

The physiological redox equilibrium depends on the consumption of antioxidants in the diet (Wang *et al.*, 2007). Antioxidant polyphenols investigated in this study can be used as natural supplements in the food industry instead of synthetic antioxidants responsible for many intensively hazardous effects on human health (Carocho *et al.*, 2014). Algae can produce a wide range of primary and secondary metabolites with potent antioxidant properties, such as phenolic compounds, vitamins, and carotenoids (Munir *et al.*, 2016; Mona *et al.*, 2016). The results revealed that microalgae are considered as a good source of strong polyphenol antioxidants (phenolic and flavonoid fractions), which agrees with the findings of Abdel-Daim *et al.* (2018) and Rani *et al.* (2021), who could identify and make use of natural antioxidant items that are pharmaceutically effective and have minimal or no adverse effects while dealing with various diseases. As dietary polyphenols function as antioxidants, they reduce oxidative stress and neutralize ROS. Consequently, the apigenin, kaempferol, caffeic acid, and quercetin found in *A. platensis*, *S. obliquus*, and *C. vulgaris* reduce the oxidative damage brought on by gamma irradiation treatment (Mohamed *et al.*, 2019; Abdel-Farid *et al.*, 2020; Abdel-Hamid *et al.*, 2021). γ -irradiation treatments increased the phenolic content of *Mucuna pruriens* (Bhat *et al.*, 2007). The strong antioxidant properties of phenolic compounds, which function as scavengers of ROS created under the stress of gamma irradiation, are one theory put in to explain this increase (Effat *et al.*, 2017). Numerous investigations on some plant species and the snow alga *Chlamydomonas nivalis* have supported strong potent antioxidant activities (Devi *et al.*, 2011). According to Wright *et al.* (2001), there are two potential ways in which phenolic chemicals function as antioxidants. The first mechanism included contributing electrons to a free radical atom so that it could form a radical cation, while the second involved transferring a hydrogen atom to a free radical. The antioxidant properties and antiinflammatory activity of kaempferol have been reported (Karthivashan *et al.*, 2013). The biological effects of chlorogenic acid include anticancer, hypolipidemic, antibacterial, antioxidant, and hypoglycemic properties (Sotillo & Hadley, 2002; Santos *et al.*, 2006; Bassoli *et al.*, 2008). Due to the presence of β -carotene, α -tocopherol, and phenolic acids, it has been observed that *Spirulina* sp. offers some antioxidant protection both *in vitro* and *in vivo* (Banskota, 2019). These findings are in agreement with the results of Ali and Doumandji (2017), who stated that although the microalgae are more rudimentary, they are still capable of creating polyphenols that are relatively complex (Klejdus *et al.*, 2010; Li *et al.*, 2011).

Table 2. Estimation of polyphenol (flavonoid and phenolic) fractions ($\mu\text{g/gDW}$) in *A. platensis*, *S. obliquus*, and *C. vulgaris* treated with and without gamma irradiation after 20 days of growth

Polyphenols fractions	RT (min.)	<i>A. platensis</i>		<i>S. obliquus</i>		<i>C. vulgaris</i>	
		Dose (Gy)		Dose (Gy)		Dose (Gy)	
		0.0	700	0.0	300	0.0	200
Phenolics							
Benzoic acid	1.1	81 \pm 2.6	99 \pm 1.9	65 \pm 4.1	78 \pm 3.9	82 \pm 4.8	92.5 \pm 8.4
Gallic acid	1.9	393 \pm 19.6	451 \pm 23.3	474 \pm 42.7	597 \pm 33.2	438 \pm 25.7	539 \pm 45.2
Resorcinol	2.1	84 \pm 5.1	119 \pm 5.9	113 \pm 4.6	133 \pm 6.5	100 \pm 6.7	123 \pm 8.6
Chlorogenic acid	3.0	190 \pm 5.9	233 \pm 9.3	247 \pm 18.6	323 \pm 17.5	266 \pm 7.9	335 \pm 30.2
Caffeic acid	3.6	350 \pm 13.4	442 \pm 23.1	74 \pm 3.8	105 \pm 4.2	102 \pm 5.4	129 \pm 5.3
<i>P</i> -Coumaric acid	4.5	198 \pm 11.8	267 \pm 16.2	234 \pm 14.7	280 \pm 16.1	217 \pm 15.4	259 \pm 33.5
Salicylic acid	4.8	346 \pm 19.3	474 \pm 33.2	410 \pm 36.9	488 \pm 41.7	346 \pm 20.9	415 \pm 34.2
Ferulic acid	5.5	291 \pm 18.7	395 \pm 15.3	345 \pm 40.2	456 \pm 29.8	340 \pm 34.4	396 \pm 34.2
Cinnamic acid	6.1	126 \pm 7.5	173 \pm 6.8	150 \pm 16.2	211 \pm 8.7	161 \pm 14.7	188 \pm 12.4
Syringic acid	8.2	132 \pm 5.5	157 \pm 6.6	158 \pm 14.3	189 \pm 13.2	154 \pm 16.3	203 \pm 23.1
Flavonoids							
Catechin	1.1	135 \pm 8.1	166 \pm 9.9	97 \pm 5.9	123 \pm 6.6	101 \pm 10.2	124 \pm 6.3
Kaempferol	2.3	110 \pm 8.8	134 \pm 6.7	131 \pm 7.7	178 \pm 12.7	136 \pm 12.3	182 \pm 5.2
Rutin	3.2	105 \pm 6.4	127 \pm 10.3	122 \pm 6.1	149 \pm 10.3	112 \pm 7.8	154 \pm 9.5
Hesperidin	4.9	149 \pm 8.8	176 \pm 14.2	176 \pm 10.2	241 \pm 9.8	190 \pm 13.3	239 \pm 18.4
Quercetin	6.9	88 \pm 4.4	119 \pm 7.6	65 \pm 3.3	92 \pm 5.6	94 \pm 8.7	124 \pm 8.8

RT (retention time). Values are mean \pm SD of samples in triplicate.

Polyphenols exhibit a wide range of biological effects as a consequence of their antioxidant properties. Due to their antioxidant capabilities, polyphenols in marine algae species display a wide variety of biological effects that can be useful in lowering oxidative processes harmful to health (Abdel-Daim *et al.*, 2018; Rani *et al.*, 2021).

The human cell experiences oxidative stress during disease conditions or when it is not receiving the best nutrition. It is possible for the ROS to start lipid peroxidation and harm other biomolecules in these conditions. Thus, it is suggested that phenolic compounds play a role in the prevention of diabetes mellitus, cancer, and neurological illnesses in addition to the protection against cancer and cardiovascular disease (Urquiaga & Leighton, 2000).

CONCLUSION

This study concluded that gamma irradiation has the ability to increase the antioxidant activity of DPPH, ABST, FRAP, and TAC in microalgae such as *Scenedesmus obliquus*, *Spirulina platensis*, and *Chlorella vulgaris*, with the highest activity in *S. obliquus*, followed by *S. platensis*, and *C. vulgaris*. In addition, carotenoid content and polyphenol (flavonoid and phenolic) fractions increased by gamma irradiation treatment in the three algae as compared to the control samples. Antioxidant polyphenol fractions (flavonoid and phenolic) investigated in this study are highly recommended to be used as valuable and natural supplements in the food industry instead of synthetic antioxidants responsible for many intensively hazardous effects on human health. Furthermore, the findings highlighted the possibility of using these microalgae as antioxidants in the future in the pharmaceutical, nutritional supplement, food, and cosmetic industries.

ABBREVIATION

TAC: Total antioxidant capacity

ROS: Reactive oxygen species (ROS)

FRAP: Ferric-reducing antioxidant power

ABTS^{•+}: 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

DPPH[•]: 2, 2-diphenyl-1-picrylhydrazyl

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