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Effect of γ-Irradiation on Amino Acids, Polyphenols, and Phytochemical Constituents of *Arthrospira platensis*

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ABSTRACT

The use of gamma irradiation to promote phytochemical components in various plants, including microalgae, has gained increasing attention in recent years. The objective of this investigation was to evaluate the metabolic profiling of bioactive phytochemical constituents and to assess antioxidant activities in *Arthrospira platensis* after treatment with γ-irradiation at a dose of 700 Gy following 20 days of growth. The data from this study revealed a significant increase in antioxidant capacities, including $DPPH^{\cdot}$, FRAP, $ABTS^{\cdot+}$, total antioxidant capacity, proanthocyanidins, saponins, β-carotene, protein, amino acids, and polyphenol fractions (flavonoids and phenolics) in γ-irradiated *A. platensis* compared to the control samples. Protein constituents typically include 24 standard amino acids, but some amino acids (such as cysteine and lysine) were not detected in *A. platensis*. However, the alga contains eighteen essential amino acids in sufficient concentrations. In this investigation, metabolomic profiling revealed the identification and quantification of fifteen polyphenol fractions (ten phenolics and five flavonoids) in *A. platensis*. This study supports the potential health benefits of incorporating metabolites from γ-irradiated *A. platensis* into functional foods, medicinal foods, and nutritional supplements, offering numerous advantages for the food industry. Therefore, the use of γ-irradiated *A. platensis* metabolites may contribute, directly or indirectly, to promoting health, as these metabolites are natural, safe, affordable, accessible, and easy to obtain.

INTRODUCTION

The increasing global population has prompted researchers to explore new ecological technologies and food sources. Microalgae, as primary producers in the food chain, thrive in various environments, including freshwater and ocean systems **(Udayan *et al.*, 2021)**. Recently, there has been growing interest in using low doses of γ-irradiation, which has strong penetrating capabilities and is more affordable and efficient than other forms of ionizing radiation. This technique has the potential to stimulate biological processes in

microalgae and to alter the composition and concentration of bioactive compounds, potentially enhancing their antioxidant properties. These enhanced properties are crucial for developing applications in nutrition, pharmaceuticals, and other health-related fields **(Tale *et al.*, 2017; Gabr *et al.*, 2019; Almarashi *et al.*, 2020)**.

Microalgae are vital natural biochemical sources for food, pharmaceuticals, and cosmetics, and serve as potential providers of vitamins, amino acids, proteins, lipids, and minerals for humans. In addition, numerous microalgae can serve as an alternative source of these compounds, replacing artificial antioxidants **(Rani *et al.*, 2016; Shabana *et al.*, 2017)**. Recently, there has been an increasing trend among scientists to use natural antioxidants derived from algae due to their safety, low cost, and ease of use. Furthermore, this approach helps avoid industrial products, which often have harmful effects on human health and can cause various diseases **(Lourenço *et al.*, 2019; Abdel-Farid *et al.*, 2020)**.

Arthrospira platensis is a multicellular, filamentous, non-nitrogen-fixing, spiral-shaped, blue-green photosynthetic microalga. It reproduces via binary fission **(Shabana *et al.*, 2017)**. *A. platensis* is a promising microalga, known for its excellent source of proteins, vitamins, and amino acids, which can be used as a feed supplement and pharmaceutical source **(Moussa *et al.*, 2015)**. γ-Irradiation treatment has been shown to enhance the growth, nutritional value, phytochemical composition, and economic value of *A. platensis* **(Abomohra *et al.*, 2016; Shabana *et al.*, 2017; Al-Habeeb *et al.*, 2024)**. Experimental evidence suggests that reactive oxygen species (ROS) contribute to an increase in lipid content in microorganisms **(Tale *et al.*, 2017)**.

Metabolic profiling of bioactive phytochemicals and antioxidants in γ-irradiated *A. platensis* involves the analysis and characterization of various chemical compounds and their biological activities. γ-Irradiation is commonly used to enhance the nutritional and therapeutic properties of microalgae such as *A. platensis* **(Shabana *et al.*, 2017)**.

The goal of this study was to assess changes in antioxidant potential, including $ABTS^{\cdot+}$ (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), $DPPH^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, ferric-reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) in γ-irradiated *A. platensis*. Additionally, the study estimated the metabolic profiling of bioactive phytochemicals, including proanthocyanidins, saponins, β-carotene, protein, amino acids, and polyphenol fractions (flavonoid and phenolic) in *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth.

MATERIALS AND METHODS

Growth medium, growth conditions, and γ-irradiation of *A. platensis*

The algae used in this study, *A. platensis*, were obtained from the National Institute of Oceanography and Fisheries Hydrobiology Laboratory, Qanater branch, Egypt. The microalgae *A. platensis* were cultivated using modified Zarrouk medium **(Aboelkheir *et***

***al.*, 2008; Al-Habeeb *et al.*, 2024)**. The chemical composition of used Zarrouk's media is illustrated in Table (1).

Table 1. Chemical composition of Zarrouk's culture media used

Chemical (g/L)	Zarrouk's medium
$NaNO_3$	2.5
K_2HPO_4	0.500
$MgSO_4.7H_2O$	0.200
NaCl	1.000
$CaCl_2.2H_2O$	0.040
$FeSO_4.7H_2O$	0.010
EDTA (disodium salt)	0.080
$NaHCO_3$	16.800
K_2SO_4	1.000
Trace metal	1 ml
H_3BO_3	2.860
$MnCl_2.4H_2O$	1.810
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.017
$CuSO_4.5H_2O$	0.079
Distilled water	1.0 L
pH	9.0±0.2

Previous studies have demonstrated that a γ-irradiation dose of 700 Gy is optimal for enhancing the growth and productivity of *A. platensis*, as well as for increasing the production of many important and effective biological compounds in the algae **(Helal *et al.*, 2023; Al-Habeeb *et al.*, 2024)**. In a preliminary experiment, 500ml batches of *A. platensis* culture, each with a cell concentration of 50 × 10^6 cells/ml, were irradiated with γ-rays at a dose of 700 Gy. The exposure rate was 0.85 Gy/min, using Co-60 as the gamma-ray source at the Egyptian Atomic Energy Authority **(Moussa, 2001; Moussa & Jaleel, 2010; Al-Habeeb *et al.*, 2024)**.

Following irradiation, a specific volume of the overnight dark-adapted *A. platensis* cells was used to inoculate 750ml of Zarrouk medium in a 1-liter Erlenmeyer flask under sterile conditions. Prior to inoculation, the culture medium was autoclaved at 121°C for 20 minutes using an autoclave (STERIF0W-1362). Illumination was provided by a white fluorescent lamp at 110µmol photons $m^{-2}s^{-1}$. The solution was continuously mixed with an aerator at a rate of 0.5L/ min (Heidolph MR Hei-Mix S magnetic stirrer, Germany). The culture was maintained under a 16/8-hour light/dark photoperiod, at a temperature of 30±2°C and pH 8.5.

After 20 days, the biomass was harvested and allowed to precipitate before being filtered through 0.45µm pore-size Whatman cellulose filter papers were used to obtain a

concentrated alga paste **(Hamid *et al.*, 2016)**. Samples were taken from the flasks for physiological and biochemical analysis. These samples were either analyzed immediately or stored in liquid nitrogen for further examination.

Biochemical analysis of *A. platensis*

Antioxidant capacity of DPPH˙, FRAP, and $ABTS^{\cdot+}$ was determined following the procedure of **Sayed *et al.* (2018)** and **Abdel-Alim *et al.* (2023a)**. The TAC was measured using the phosphomolybdenum‖ technique, and the results were calculated using ascorbic acid as a reference on a standard curve **(Prieto *et al.*, 1999)**. Proanthocyanidins (condensed tannin) content was evaluated by the method of **Tyler (1994)**. Saponin content was determined by the reported method of **Obadoni and Ochuka (2001)**. β-carotene was estimated according to the protocol of **Craft and Soares (1992)**. Total protein content was estimated according to the procedures of **Annadotter *et al.* (2015)**. Quantitative estimation of amino acids was done following the method of **Abugrada *et al.* (2020)** by using an automated analyzer for amino acids (Dionex ICS-3000). Flavonoid and phenolic fractions were identified by liquid chromatography with good performance (Waters, USA) using the technique described in **Abdel-Farid *et al.* (2020)**.

Statistical analysis

Statistical analysis was estimated with the SPSS version 17 statistical software package (SPSS Incorporated Company, Illinois, USA). Data were described as means ± SD (standard deviation). *Post- hoc* tests were performed after finding a significant difference in an ANOVA test. The significance was determined to be statistically different when $P \leq 0.05$ **(Moussa & Galad, 2015; Abdel-Alim *et al.*, 2023)**.

RESULTS AND DISCUSSION

Antioxidant capacity of DPPH˙, $ABTS^{\cdot+}$, FRAP, and TAC in *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth

The data for antioxidant capacity of DPPH˙, $ABTS^{\cdot+}$, FRAP, and TAC in *A. platensis* treated with and without γ-irradiation at a dose of 700 Gy after 20 days of growth are listed in Table (2). The antioxidant capacity of $ABTS^{\cdot+}$, DPPH˙, FRAB, and TAC significantly increased in *A. platensis* treated with γ-irradiation (700 Gy) compared to non-irradiated samples (control). These results are concomitant with the results of **Abomohra *et al.* (2016)** and **Shabana *et al.* (2017)**.

Even though oxidant molecules have a physiological function, oxidative stress (OS) may be the cause of a number of disorders in humans. OS arises when antioxidant levels are either excessively high or too low, causing damage to molecules, tissues, and cellular levels. Antioxidant substances may therefore offer a means of controlling OS and/or preserving an appropriate redox balance **(Silvestrini *et al.*, 2023)**.

The TAC assay evaluates the cumulative antioxidant potential of all antioxidants present in a sample, providing a comprehensive measure of its potential to counteract oxidative stress. It is believed that antioxidants offer a number of beneficial health effects including the prevention of many diseases **(Abdel-Karim *et al.*, 2020)**. Antioxidants, as measured by $DPPH^{\cdot}$, $ABTS^{\cdot+}$, FRAP, and TAC assays in *A. platensis*, play a vital role in countering oxidative stress and inflammation by mitigating the damaging effects of ROS **(Abdel-Karim *et al.*, 2020; Silvestrini *et al.*, 2023)**.

Significantly, *A. platensis* has demonstrated the ability to act as a potent reductant, effectively quenching DPPH and ABTS radicals **(Marecek *et al.*, 2017)**. The high levels of phenolic and flavonoid compounds in *A. platensis* are likely responsible for its antioxidant properties. These molecules are strong electron or hydrogen donors, capable of reacting with free radicals like DPPH and ABTS to form more stable compounds **(Aslanbay *et al.*, 2024)**. *A. platensis* has shown strong antioxidant activity, with inhibition rates of 89% for ABTS and 85% for DPPH **(Shalaby & Shanab, 2013)**. Additionally, the radical scavenging activity of DPPH and ABTS helps inhibit apoptotic cell death **(Chu *et al.*, 2010)**. The FRAP value of *A. platensis* exceeds that of other algae **(Safari *et al.*, 2020)**. The total antioxidant capacity (TAC) assay measures the cumulative antioxidant effect of all compounds in a sample, providing an overall indication of its ability to neutralize reactive oxygen species (ROS). *A. platensis* has high TAC levels, further supporting its strong antioxidant potential **(El-Chaghaby *et al.*, 2019)**.

Table 2. Antioxidant potential of $DPPH^{\cdot}$(2,2-diphenyl-1-picrylhydrazyl) (mg VCE g^{-1}DW), $ABTS^{\cdot+}$(2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (mg VCE g^{-1}DW), ferric-reducing antioxidant power (FRAP) (mM Fe^{2+} equivalent/g DW), total antioxidant capacity (TAC, mg g^{-1} ascorbic acid) in *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth

Algae	Dose (Gy)	ABTS	DPPH	FRAP	TAC
A. platensis	**0.0**	198±12.1	130±6.6	14.2±0.9	62±5.6
	700	251±14.4	179±14.4	32.7±2.4	98±6.9

VCE: mg vitamin C equivalent. ABTS: ABTS radical scavenging capacity; DPPH: DPPH radical scavenging capacity; and FRAP: ferric-reducing antioxidant power. Values are represented as the mean ± SD of samples in triplicate.

Phytochemical constituents (proanthocyanidins, saponins, β-carotene, and protein) in *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth

The data demonstrated the presence of several beneficial natural health substances, including proanthocyanidins (condensed tannins), proteins, β-carotene, and saponins, which were found to increase in *A. platensis* treated with γ-irradiation at a dose of 700 Gy compared to the control group after 20 days of growth, as shown in Fig. (1).

A. platensis, a species of green microalgae, contains various bioactive compounds such as proanthocyanidins, saponins, β-carotene, polyphenols, and proteins. These compounds exhibit a range of anti-inflammatory, antioxidant, antimicrobial, and immune-enhancing properties, which offer potential or actual benefits against oxidative stress **(Pizzino *et al.*, 2017; Elazab *et al.*, 2024)**.

Tannins (proanthocyanidins) are particularly notable for their significant antioxidant properties. They have the potential to protect biomolecules (such as DNA, proteins, and lipids) from oxidative damage caused by free nitrogen ions (e.g., peroxynitrite) and free oxygen radicals. Additionally, proanthocyanidins act as a crucial defense against oxidative and nitrifying stress, which is associated with a variety of diseases, including cancer, neurodegeneration, and cardiovascular diseases **(Abdel-Karim *et al*., 2020)**.

Proanthocyanidins can scavenge free radicals, thereby improving the body's defense system. This helps reduce oxidative stress in organs viz. the liver and kidneys via mitigating lipid peroxidation and preserving antioxidant enzyme activities **(Bagchi *et al.*, 2003; Elazab *et al.*, 2024)**.

Saponins, another important bioactive compound, have been shown to reduce lipid peroxidation, stimulate endogenous antioxidant defenses, and scavenge free radicals, thus reducing oxidative stress. Additionally, they improve antioxidant enzyme levels **(Mohesien *et al.*, 2023)**. β-carotene also supports cellular antioxidant enzymes, stimulating the body's overall antioxidant capacity. It is a potent antioxidant that reduces oxidative stress by neutralizing free radicals **(Krinsky & Yeum, 2003; Miazek *et al.*, 2022)**.

Proteins have various antioxidant functions, including chelating heavy metal ions, scavenging free radicals, and repairing damaged molecules. Additionally, proteins support the synthesis and activity of endogenous antioxidant enzymes, enhancing the overall antioxidant defense system **(Abde-Alim *et al.*, 2023b)**. Many antioxidants, such as polyphenols, saponins, β-carotene, and vitamin E, have been extensively studied for their potential benefits against oxidative stress **(Pizzino *et al*., 2017)**. In medicine, saponins are used as potent antioxidants and for various other health benefits, including antibacterial, anticancer, anti-inflammatory, and weight reduction properties **(Abdel-Karim *et al.*, 2020)**.

Spirulina, which contains saponins, is known for its health-promoting properties **(Habib *et al.*, 2008)**. In addition to its antioxidant effects, saponins in *A. platensis* contribute to its antibacterial, anticancer, and anti-inflammatory properties **(Abdel-Karim *et al.*, 2020)**.

β-carotene, a potent antioxidant and precursor of vitamin A, is abundant in *A. platensis*, making it an important nutrient for combating oxidative stress and supporting eye health **(Wu *et al.*, 2016)**. *A. platensis* contains β-carotene at levels thirty times greater than carrots **(Soni *et al*., 2017)**.

A. platensis is also known for its high protein content, which can comprise 60-70% of its dry weight. The protein in *Spirulina* is complete, containing all essential amino acids (EAAs), making it an excellent dietary supplement, especially for vegetarians and vegans **(El-Chaghaby *et al.*, 2019)**. Proteins act as antioxidants by scavenging free radicals and repairing damaged molecules. Additionally, they support the synthesis and activity of endogenous antioxidant enzymes, further enhancing the body's antioxidant defense system **(Asagba *et al.*, 2007)**.

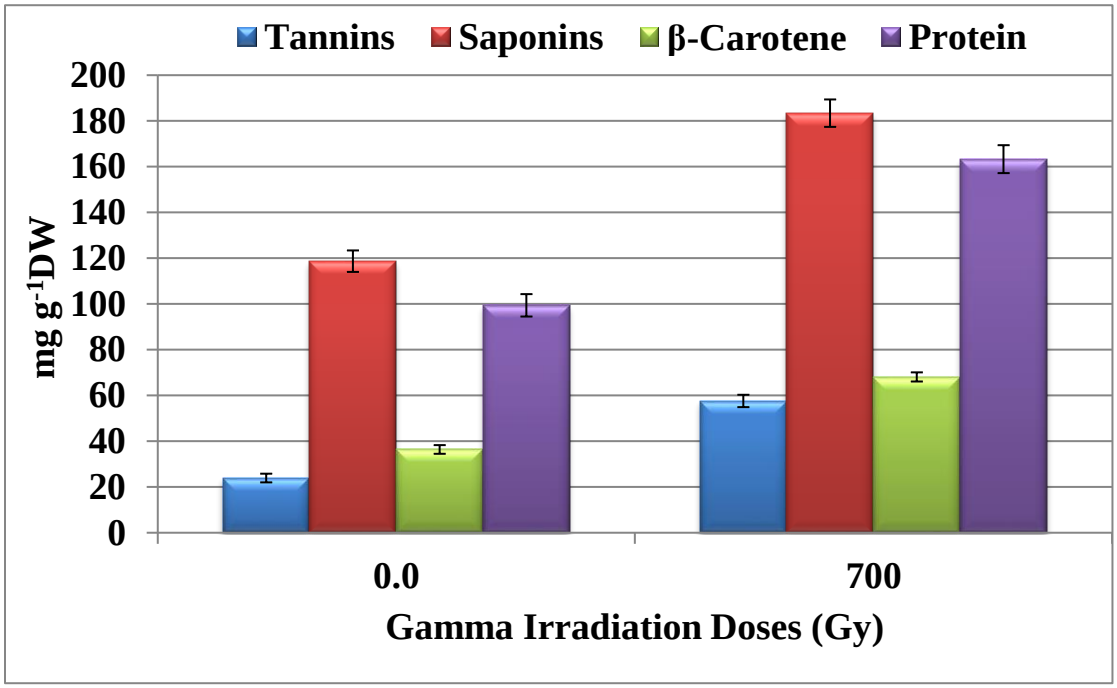

Fig. 1. Quantitative analysis for proanthocyanidins (condensed tannins), saponins, β-carotene, and protein in *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth

Amino acid contents in *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth.

Findings regarding the amino acid composition of *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth are listed in Fig. (2).

The amino acid composition of γ-irradiated *A. platensis* significantly increased as compared to the control samples **(Moussa *et al.*, 2015; Shabana *et al.*, 2017)**. There are typically 24 standard amino acids found as constituents of proteins. However, some of the amino acids are not detected in *A. platensis*, which has eighteen amino acids **(Moussa *et al.*, 2015)**.

Amino acids play significant roles in mitigating oxidative stress through various mechanisms, including detoxification, anti-inflammatory, antioxidant properties, metal chelation, and maintenance of protein synthesis and cellular functions **(Egbujor *et al.*,**

2024). The metabolism of nutrients within cells, especially EAAs, is vital for cellular functions, including the production of energy and maintenance of redox equilibrium in cells. Oxidative stress and cellular damage can result from an EAA deficit **(Li *et al.*, 2023)**.

The main metabolic route for EAAs' metabolism is protein synthesis. Phenylalanine, tryptophan, threonine, leucine, lysine, methionine, valine, isoleucine, and histidine are the nine EAAs that cells use to synthesize proteins necessary for cellular structure, function, and control of cells **(Wu, 2009)**. Ribosomes are cellular organelles that are involved in protein synthesis. According to the genetic code conveyed by the messenger RNA, amino acids are joined by peptide bonds during protein synthesis in a particular order **(Li *et al.*, 2023)**. The decrease in amino acids can damage cellular function and general health, as their availability in the cell is essential for appropriate protein synthesis **(Lopez & Mohiuddin, 2020)**.

Additionally, intracellular amino acid metabolism is essential for preserving the redox equilibrium of cells and shielding them from ROS-induced oxidative damage **(Newsholme *et al.*, 2012; Elgendy *et al.*, 2024)**. Tryptophan, methionine, histidine, lysine, cysteine, arginine, and tyrosine were the seven amino acids with the highest total antioxidative capacity **(Xu *et al.*, 2017)**. Cysteine is a precursor in the synthesis of glutathione, thereby enhancing the body's antioxidant capacity **(Kranich *et al.*, 1998)**. Glutamine supports cellular energy metabolism and enhances the synthesis of glutathione **(Cruzat *et al.*, 2018)**. **Farhi *et al.* (2008)** observed that at low doses of γ-irradiation, the pool of free amino acids increased in Chlorophyceae green microalga. An important function that protein content played in the DNA repair pathway was linked to the increase in amino acid concentration **(Yu *et al.*, 2016)**. In both the animal and human bodies, amino acids are essential for cellular assembly and metabolism to produce proteins, which are then utilized to build various body tissues **(Debnath *et al.*, 2019)**. Except for cysteine and lysine, all of the necessary EAAs are found in Arthrospira in sufficient amounts according to FAO **(Paoletti *et al.*, 1980)**. Glutamic acid, leucine, aspartic acid, arginine, and alanine were the main five primary amino acids found in *A. platensis* **(Uslu *et al.*, 2009; Bashir *et al.*, 2016)**.

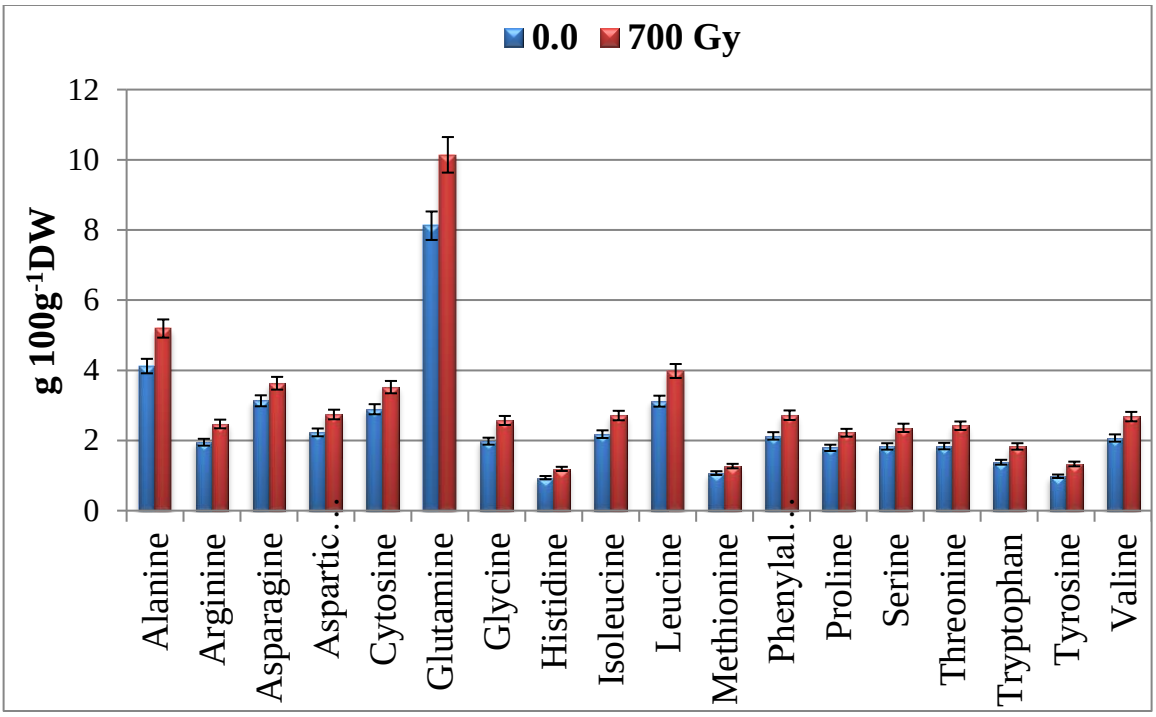

Fig. 2. Amino acid contents of *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth

The values are means of at least three replicates ± standard deviation (SD).

Identification and quantification of polyphenol (flavonoid and phenolic) fractions by HPLC in *A. platensis*, treated with and without γ-irradiation (700 Gy) after 20 days of growth

In this investigation, metabolomics profiling reported that fifteen polyphenol fractions (ten phenolics and five flavonoids) were identified and quantified in *A. platensis* by comparing the HPLC chromatograms of those samples to the standard compounds according to retention time, as listed in Figs. (3, 4).

The γ-irradiation treatments increased the polyphenol contents compared to the control samples. **Devi *et al.* (2011)** suggested that the increase could be explained by the potent antioxidant properties of phenolic compounds, which function as scavengers of ROS generated under γ-irradiation stress. A. platensis has been shown to have a higher phenolic content following γ-irradiation **(Shabana *et al.*, 2017)**. Polyphenol compounds are responsible for possible antioxidant activities, antimicrobial, anti-inflammatory, anti-viral, anticancer actions and have an important role in human health through their contents of phenols, flavonoids, or carotenoids **(El-Tablawy *et al.*, 2020; Abdel-Karim *et al.*, 2020)**.

There are two potential ways through which phenolic chemicals function as antioxidants **(Wright *et al.*, 2001)**. The first method involves giving a free radical atom an electron to turn it into a radical cation; the second involves transferring a hydrogen atom

to a free radical. According to reports, kaempferol has anti-inflammatory and antioxidant properties **(Karthivashan *et al.*, 2013)**. Numerous biological properties of chlorogenic acid include antibacterial, antioxidant, anticarcinogenic, hypoglycemic, and hypolipidemic effects **(Bassoli *et al.*, 2008)**. The findings of **Ali and Doumandji (2017)**, who observed that microalgae are more primitive and capable of producing relatively complex polyphenols, align with our results. Available data indicate that phenolic compounds offer significant protective effects against cancer, cardiovascular diseases, neurological disorders, and diabetes mellitus **(Urquiaga & Leighton, 2000)**.

Along with flavonoids, phenolic compounds contribute significantly to the antioxidant capacity of algae **(El-Tablawy *et al.*, 2020)** and are associated with a variety of biological activities. This explains their potential for commercial applications in pharmaceutical, medical, and nutritional fields **(Galasso *et al.*, 2019)**. In fact, marine algae are rich in a wide range of structurally diverse and physiologically active metabolite compounds, such as lectins, sulfated polysaccharides, carotenoids, alkaloids, phycobilins, sterols, tocopherols, terpenes, and polyphenolic compounds **(Ghallab *et al.*, 2022b)**. Consistent with our findings, recent studies by **Devi *et al.* (2011)** and **Ismail *et al.* (2016)** have demonstrated that flavonoids and phenolic acids are abundant in seaweeds, exhibiting broad biological activities and powerful free radical scavenging capabilities.

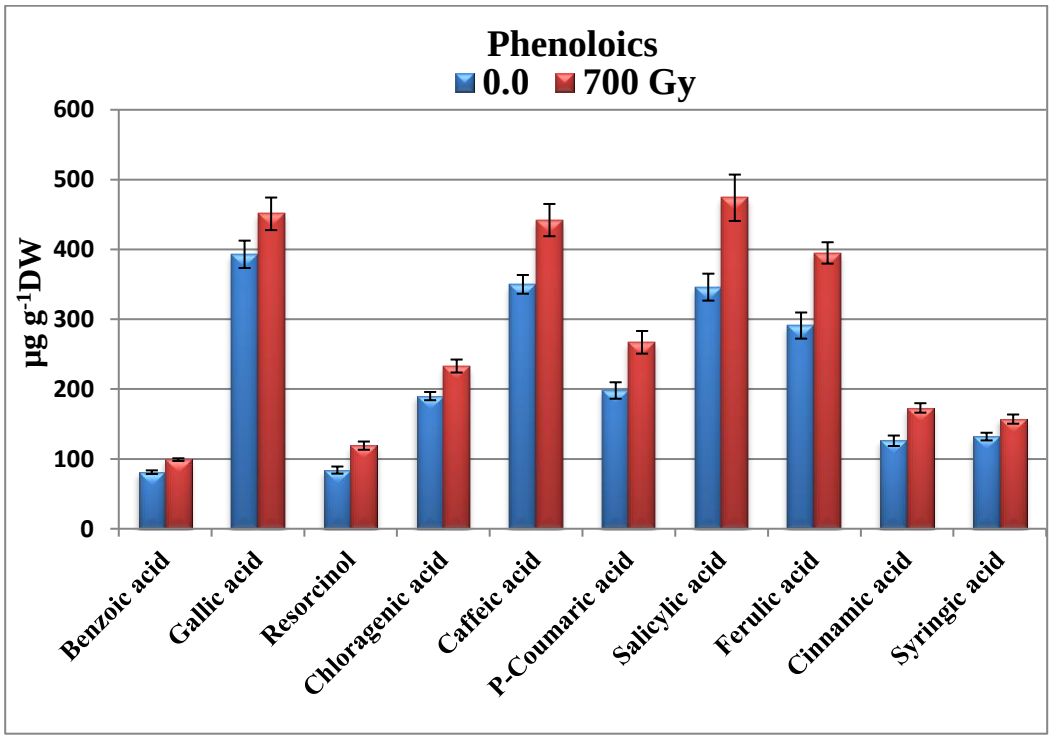

Fig. 3. Estimation of polyphenol fractions (phenolics) in *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth
The values are means of at least three replicates ± standard deviation (SD).

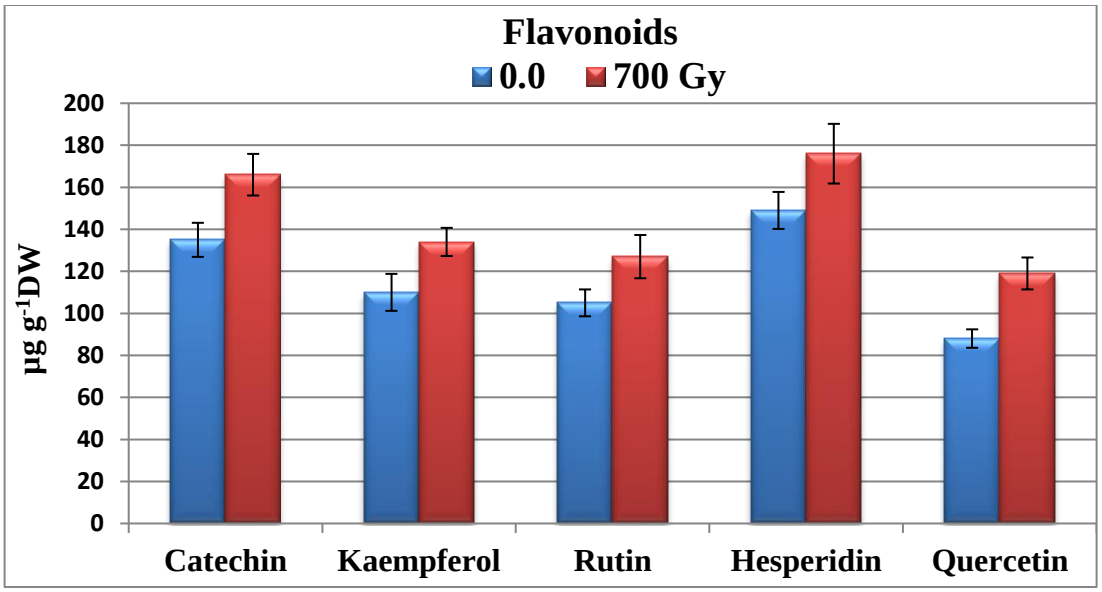

Fig. 4. Estimation of polyphenol fractions (flavonoids) in *A. platensis* treated with and without γ-irradiation (700 Gy) after 20 days of growth
The values are means of at least three replicates ± standard deviation (SD).

CONCLUSION

The findings of this investigation demonstrate that γ-irradiation (700 Gy) treatment significantly enhances the antioxidant potential of DPPH˙, FRAP, ABTS˙⁺, and TAC, as well as the contents of proanthocyanidins, saponins, β-carotene, protein, amino acids, and polyphenols (flavonoids and phenolics) in *A. platensis* compared to the control samples. Given the numerous benefits that *A. platensis* offers for the food industry, this study supports its use in the development of therapeutic foods, functional foods, and nutritional supplements. Since the metabolites of γ-irradiated *A. platensis* are cost-effective, readily available, safe, and natural, their incorporation may contribute both directly and indirectly to the maintenance of health.

Conflicts of interest

We have no conflicts of interest to disclose.

ABBREVIATION

FRAP: Ferric-reducing antioxidant power
ROS: Reactive oxygen species
DPPH˙: 2, 2-diphenyl-1-picrylhydrazyl
TAC: Total antioxidant capacity
ABTS˙⁺: 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

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