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Influence of γ-Irradiation on Some Phytochemical Constituents of *Scenedesmus obliquus*

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ABSTRACT

This study explores the effects of γ-irradiation on various phytochemical constituents of *Scenedesmus obliquus*, including proanthocyanidins, saponins, β-carotene, proteins, vitamins (B_1, B_2, B_3, B_6, A, and K), amino acids, and fatty acids. Cultures of *S. obliquus* were subjected to a γ-irradiation dose of 300 Gy and were analyzed after 20 days of growth. The results indicated significant changes in the phytochemical profile of the irradiated algae. Proanthocyanidins and saponins levels increased, suggesting an enhanced secondary metabolite production. In addition, β-carotene content increased, indicating an improved antioxidant capacity. Protein levels were significantly higher, reflecting an increased biosynthetic activity. Vitamin analysis showed substantial increases in the contents of B_1, B_2, B_3, B_6, A, and K, highlighting the potential nutritional benefits of γ-irradiated *S. obliquus*. Amino acid profiling revealed higher concentrations of essential amino acids. Fatty acid analysis identified twelve fatty acids, predominantly composed of fatty acid methyl esters, which include both saturated and unsaturated fatty acids with carbon chain lengths ranging from C12 to C24. These findings suggest that γ-irradiation at 300Gy can effectively enhance the phytochemical constituents of *S. obliquus*, potentially improving its applications in nutrition and biotechnology.

INTRODUCTION

Recently, there has been an increasing interest in applying low doses of γ-irradiation, which have potent penetrating capability and are more affordable and efficient than other ionizing radiation, to stimulate biological processes in microalgae and to alter the composition and concentration of bioactive compounds, potentially enhancing their antioxidant properties, which 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)**. γ-irradiation treatment increased the growth, nutritional value, phytochemical composition, and economic value of *S. obliquus* **(Abomohra *et al.*, 2016; Shabana *et al.*, 2017; Al-Habeeb *et al.*, 2024)**.

Experimental evidence shows that ROS contribute to the rise in lipid content in microorganisms **(Tale *et al.*, 2017)**. Organisms under stress have developed various defensive mechanisms to prevent oxidative damage. These include non-enzymatic antioxidants, such as phenols and proline, as well as enzymatic antioxidants like glutathione reductase, superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase **(Zhao & Li, 2014)**. *Scenedesmus* is a genus of green algae belonging to the Chlorophyceae class. They are non-motile and colonial. They are among the most prevalent components of phytoplankton found in freshwater environments **(Al-Habeeb *et al.*, 2024)**. The antioxidant potential of *S. obliquus* is significant due to its high content of carotenoids, chlorophylls, and polyphenols. Free radicals are neutralized by antioxidants, which reduce oxidative stress and may therefore decrease the risk of chronic illnesses **(De Das *et al.*, 2019; Lee *et al.*, 2020)**. Metabolic profiling of bioactive phytochemicals and antioxidants in γ-irradiated *S. obliquus* involves the analysis and characterization of various chemical compounds, and their biological activities. γ-irradiation is often used to enhance the nutritional and therapeutic properties of microalgae like *S. obliquus* **(El-Sheekh & Hamouda, 2014)**. Microalgae are among the most important natural biochemical contents for food, pharmaceuticals, and cosmetics, in addition to being potential sources of vitamins, amino acids, proteins, lipids, and minerals for humans. Most microalgae can also be an untraditional source of these compounds instead of artificial antioxidants **(Shabana *et al.*, 2017; Rani *et al.*, 2021)**. The antioxidants as a natural compound from algae have increased due to their natural, eco-friendly, cost-effective, high efficiency, availability, and easy to obtain, avoiding the use of synthetic compounds that have many toxic adverse effects and very excessive costs **(Lourenço *et al.*, 2019)**.

The goal of this work was to evaluate the changes of proanthocyanidins, saponins, β-carotene, protein, vitamin contents (B_1, B_2, B_3, B_6, A, and K), amino acids, and fatty acids in response to γ-irradiated *S. obliquus* at a dose of 300Gy after 20 days of growth.

MATERIALS AND METHODS

Strains, growth medium, growth conditions, and γ-irradiation of *Scenedesmus obliquus*

The algae used in this study, *S. obliquus*, were obtained from the National Institute of Oceanography and Fisheries hydrobiology laboratory, Qanater branch, Egypt. The *S. obliquus* microalgae were cultured in BG-11 media **(Al-Habeeb *et al.*, 2024)**. The chemical contents of the BG-11 media used are illustrated in Table (1).

Previous studies have shown that the dose of 300Gy is the best in terms of increasing the growth and productivity of the algae as well as increasing the production of many important and effective biological substances for the algae **(Helal *et al.*, 2023; Al-**

Habeeb *et al.*, 2024). In a preliminary experiment, volumes of 500ml of algal batches having the same concentration of cells (50 × 106 cells/ml) for *S. obliquus* culture grown for 4 days were subjected to γ-Irradiation at a dose of 300Gy. The exposure rate was 0.85Gy/ min using Co^{60} as a gamma-ray source at the Atomic Energy Authority, Nasr City, Egypt **(Al-Habeeb *et al.*, 2024)**.

Table 1. Chemical composition of BG-11 media used

Chemical (g/L)	**BG-11 media**
$NaNO_3$	1.5
K_2HPO_4	3.050
$MgSO_4.7H_2O$	7.500
$CaCl_2.2H_2O$	3.600
Citric acid. $1H_2O$	0.600
Ammonium ferric citrate	0.600
EDTA (disodium salt)	0.100
Na_2CO_3	0.020
Trace metal	1 ml
H_3BO_3	2.860
$MnCl_2.4H_2O$	1.810
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.390
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H_2O$	0.049
Distilled water	1.0 L
Ph	7.5±0.2

Following irradiation, we used a specific volume of the overnight dark-adapted *S. obliquus* cells to inoculate 750ml of BG-11 media into 1L Erlenmeyer flasks. Under sterile control conditions, the culture medium was autoclaved at 121°C for 20 minutes before inoculation using an autoclave (STERIF0W-1362), and the required illumination was supplied by a white fluorescent lamp (110µmol photons $m^{-2}s^{-1}$). The solution was continuously mixed using an aerator at a rate of 0.5 L/min (Heidolph MR Hei-Mix S magnetic stirrer, Germany). The photoperiod was set to a 16/8 hours day/night cycle, with a temperature of 30 ± 2 °C and a pH of 8.5. The harvested biomass after 20 days was allowed to precipitate before being filtered using 0.45 mm pore-size Whatman cellulose filter papers to get concentrated algae paste **(Hamid *et al.*, 2016)**. Samples were taken from the flasks for the physiological and biochemical investigations, and these were either immediately used or fixed in liquid nitrogen for further examination.

Biochemical analysis of *S. obliquus*

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)**. Additionally, total protein content was estimated according to the procedures of **Annadotter *et al.* (2015)**. Quantitative estimation of amino acids was done by the procedure of **Abugrara *et al.* (2020)** by using an automated analyzer for amino acids (Dionex ICS-3000). Fatty acid compositions were determined by a standard capillary gas chromatographic method **(Diraman *et al.*, 2009)**. Vitamins (B_1, B_2, B_3, B_6, A, and K) were estimated according the procedure of **Amidzic *et al.* (2005)**.

Statistical analysis

Analytical statistics were estimated with the SPSS version 17 statistical software package (SPSS Incorporated Company, Illinois, USA). Data were described as means ± SD (standard deviation). The significance was determined to be statistically different when $P \leq 0.05$ **(Abdel-Alim *et al.*, 2023a)**.

RESULTS AND DISCUSSION

Phytochemical constituents (proanthocyanidins, saponins, β-carotene, and protein) in *S. obliquus* treated with and without γ-irradiation (300 Gy) after 20 days of growth

The data demonstrated the presence of various beneficial natural health substances, such as proanthocyanidins (condensed tannins), proteins, β-carotene, and saponins, which increased in *S. obliquus* treated with γ-irradiation (300Gy) compared to the control group after 20 days of growth, as shown in Fig. (1). *S. obliquus*, a species of green microalgae, contains various bioactive compounds such as proanthocyanidins, saponins, β-carotene, and proteins that exhibit a range of anti-inflammatory, antioxidant, antimicrobial, and immune-enhancing properties **(Pizzino *et al.*, 2017)**.

Tannins (proanthocyanidins) exhibit significant and encouraging antioxidant properties, and they may have the potential to protect and preserve biomolecules (DNA, proteins, and lipids) that are subjected to free nitrogen ions, such as peroxynitrite ($ONOO^-$), and free oxygen radicals. Additionally, as a strong and crucial defence against oxidative/nitrifying stress linked to a variety of dangerous and damaging diseases, including cancer, neurodegeneration, and cardiovascular disease **(Abdelkarim *et al.*, 2020)**. Proanthocyanidins can scavenge free radicals and can enhance the body's antioxidant defence mechanism, thereby reducing oxidative stress in organs viz. the liver and kidneys by mitigating lipid peroxidation and preserving antioxidant enzyme activities **(Bagchi *et al.*, 2003)**.

Saponins have been shown to reduce lipid peroxidation, stimulate the body's endogenous antioxidant defenses, reduce oxidative stress by scavenging free radicals, and improve antioxidant enzyme levels **(Mohesien *et al.*, 2023)**. β-carotene supports the maintenance of cellular antioxidant enzymes, thereby stimulating the body's overall antioxidant capacity **(Krinsky & Yeum, 2003)**. β-carotene is a potent antioxidant that reduces oxidative stress by neutralizing free radicals **(Miazek *et al.*, 2022)**.

Proteins have a variety of ways to function as antioxidants, including chelating heavy metal ions, scavenging free radicals, and repairing damaged molecules. Besides, proteins can support the synthesis and activity of endogenous antioxidant enzymes, thereby enhancing the overall antioxidant defense system **(Abdel-Alim *et al.*, 2023b)**. Many antioxidants, including polyphenols, saponins, β-carotene, and vitamin E, have been studied in recent years for their potential or actual benefits against oxidative stress **(Pizzino *et al.*, 2017)**. In medicine, saponins are used as potent antioxidants, antibacterial, hyperglycemia, anticancer, hypercholestrolaemia, antiinflammatory, and reduction in weight **(Bishop *et al.*, 2020)**.

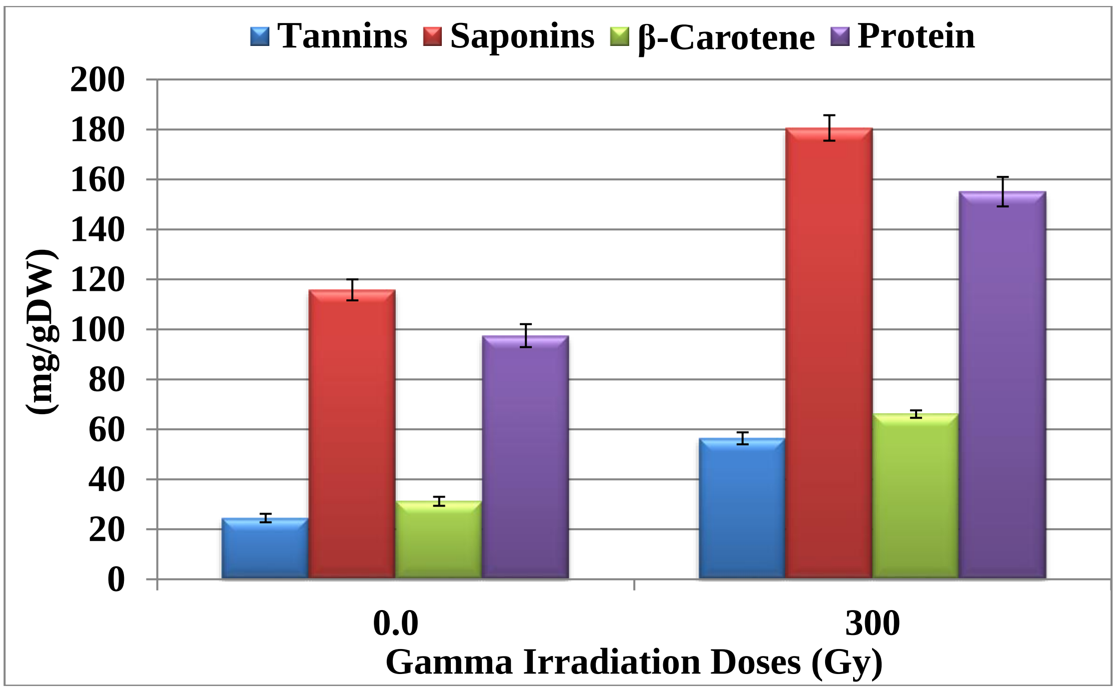

Fig. 1. Quantitative analysis for proanthocyanidins (condensed tannins), saponins, β-carotene, and protein in *S. obliquus* treated with and without γ-irradiation (300 Gy) after 20 days of growth. The values are means of at least three replicates ± standard deviation (SD)

Vitamin contents of B_1, B_2, B_3, B_6, A, and K in *S. obliquus* treated with and without γ-irradiation (300 Gy) after 20 days of growth

The results for vitamin contents of B_1, B_2, B_3, B_6, A, and K in *S. obliquus* treated with and without γ-irradiation after 20 days of growth are listed in Fig. (2). The data demonstrated that the various vitamin concentrations (A, K, and B groups) were significantly increased by the γ-irradiation dose (300 Gy) as compared with the control, which is similar to the results of **Abomohra *et al.* (2016)** and **Shabana *et al.* (2017)**.

Vitamin A has antioxidant properties that help neutralize reactive oxygen species generated. This reduces oxidative stress, provides support for the immune system, and protects cellular components such as DNA, proteins, and lipids from damage **(Unsal *et al.*, 2020)**. B vitamins participate in the detoxification processes by supporting liver function and protecting the nervous system **(Abdelaziz *et al.*, 2013)**. The activation of other precursors or related chemicals may be connected to the increase in vitamins. For instance, **Abomohra *et al.* (2016)** found that increased γ-irradiation up to a dose of 2.0 kGy resulted in an increase in carotenoids contents (as provitamin A), which may be related to the increased vitamin A contents.

Vitamin E, another antioxidant, has been studied for its protective effects, possibly through its capacity to neutralize the free radicals and reduce inflammation **(Sugimoto *et al.*, 2006)**. Moreover, vitamin D is involved in regulating the immune system's **(Bishop *et al.*, 2021)**.

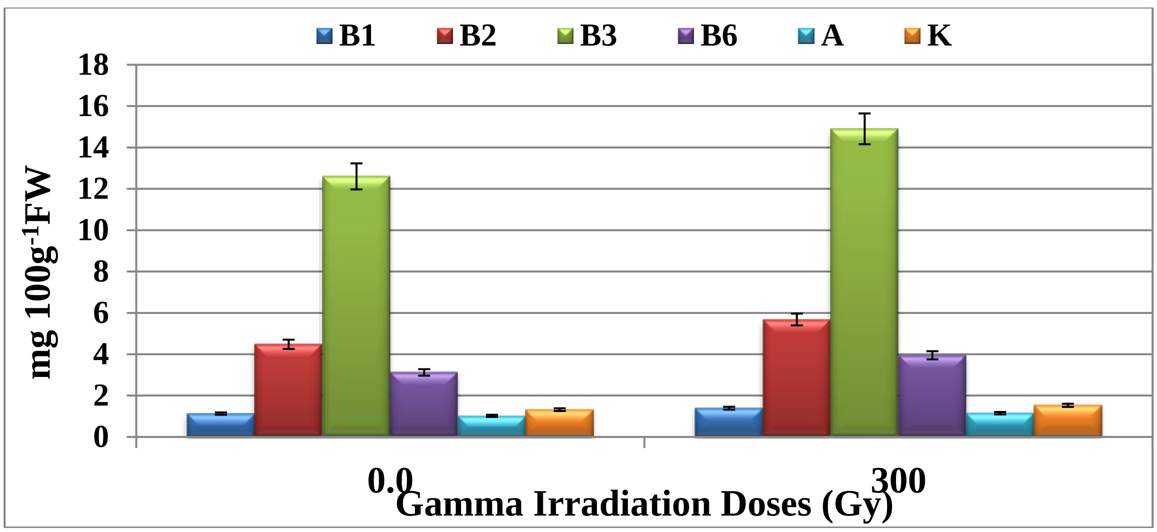

Fig. 2. Vitamin contents of B_1, B_2, B_3, B_6, A, and K in *S. obliquus* treated with and without γ-irradiation (300 Gy) after 20 days of growth. The values are means of at least three replicates ± standard deviation (SD)

Amino acid contents in *S. obliquus* treated with and without γ-irradiation (300 Gy) after 20 days of growth

Findings regarding the amino acid composition of *S. obliquus* treated with and without **γ**-irradiation after 20 days of growth are listed in Fig. (3). The amino acid composition of γ-irradiated *S. obliquus* increased significantly compared to the control samples. There are typically 24 standard amino acids found as constituents of proteins. *S. obliquus* had twenty amino acids. 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 essential amino acids (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 essential amino acid (EAA) metabolism is protein synthesis. Leucine, phenylalanine, methionine, histidine, tryptophan, threonine, valine, lysine, and isoleucine are the nine essential amino acids (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 since 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 reactive oxygen species-induced oxidative damage **(Newsholme *et al.*, 2012)**.

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)**.

Fatty acids composition in *S. obliquus* treated with and without γ-irradiation after 20 days of growth

Information about the fatty acid composition of *S. obliquus* is given in Fig. (4). The data revealed the presence of twelve identified fatty acids. Moreover, fatty acid methyl esters mostly include saturated and unsaturated fatty acids with carbon chain lengths from C12 to C24 in *S. obliquus,* in agreement with **El-Sheekh and Fathy (2009)**.

Exposure of microalgae to γ-irradiation increases the levels of saturated and monounsaturated fatty acids **(Tale *et al.*, 2018; Abo-State *et al.*, 2019; Oliver *et al.*, 2020)**.

It is further hypothesized that γ-irradiation-induced ROS accumulation may upregulate the lipid biosynthetic pathway, which could be a possible mechanism by which γ-irradiation may cause lipid accumulation **(Tale *et al.*, 2018)**. γ-irradiation increases the lipid accumulation of *S. obliquus* **(Abo-State *et al.*, 2019)**. Fatty acids play a significant role in antioxidant defense, anti-inflammatory pathways, membrane integrity, and modulation of cellular signaling. Adequate intake of these fatty acids improves overall health outcomes **(Das, 2006; Moussa *et al.*, 2022)**.

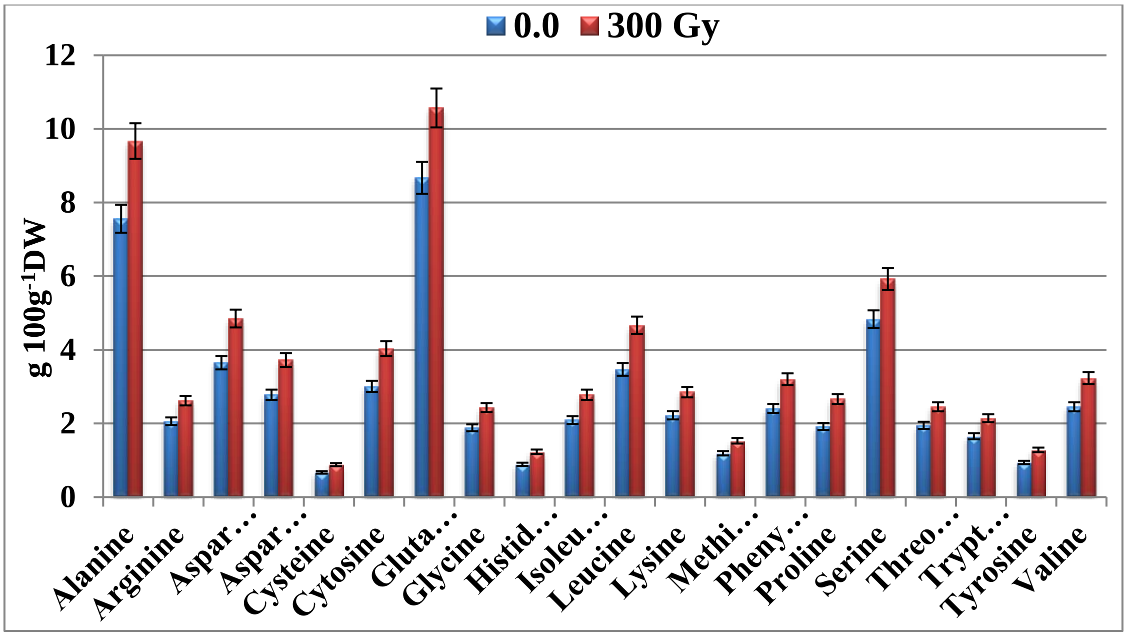

Fig. 3. Amino acid contents of *S. obliquus* treated with and without γ-irradiation (300 Gy) after 20 days of growth. The values are means of at least three replicates ±standard deviation (SD)

It has been demonstrated that linoleic acid possesses anti-inflammatory and antioxidant properties. It has the ability to modify immunological responses and reduce oxidative stress **(Alam *et al.*, 2021)**. Fatty acids enhance the antioxidant defense system. They increase the activity of antioxidant enzymes such as CAT, SOD, and GPx, which help neutralize reactive oxygen species **(Das, 2006)**.

Dietary intake of specific fatty acids could potentially act with high effects against oxidative stress **(Su *et al.*, 2018)**. Algae are essential to the earth's primary productivity, as they produce a range of valuable nutrients in addition to oxygen. Polyunsaturated fatty acids are one type of nutrition that accumulates in *S. obliquus* and can eventually be ingested by humans and other animals via the food chain **(Chen *et al.*, 2023)**. Thus, *S. obliquus* demonstrated considerable potential in pharmaceutical and nutraceutical applications because of their biopigment, protein, appropriate high productivity of lipid, long-chain polyunsaturated fatty acids, antioxidant activity, and α-linolenic acid **(Khodadadianzaghmari *et al.*, 2024)**.

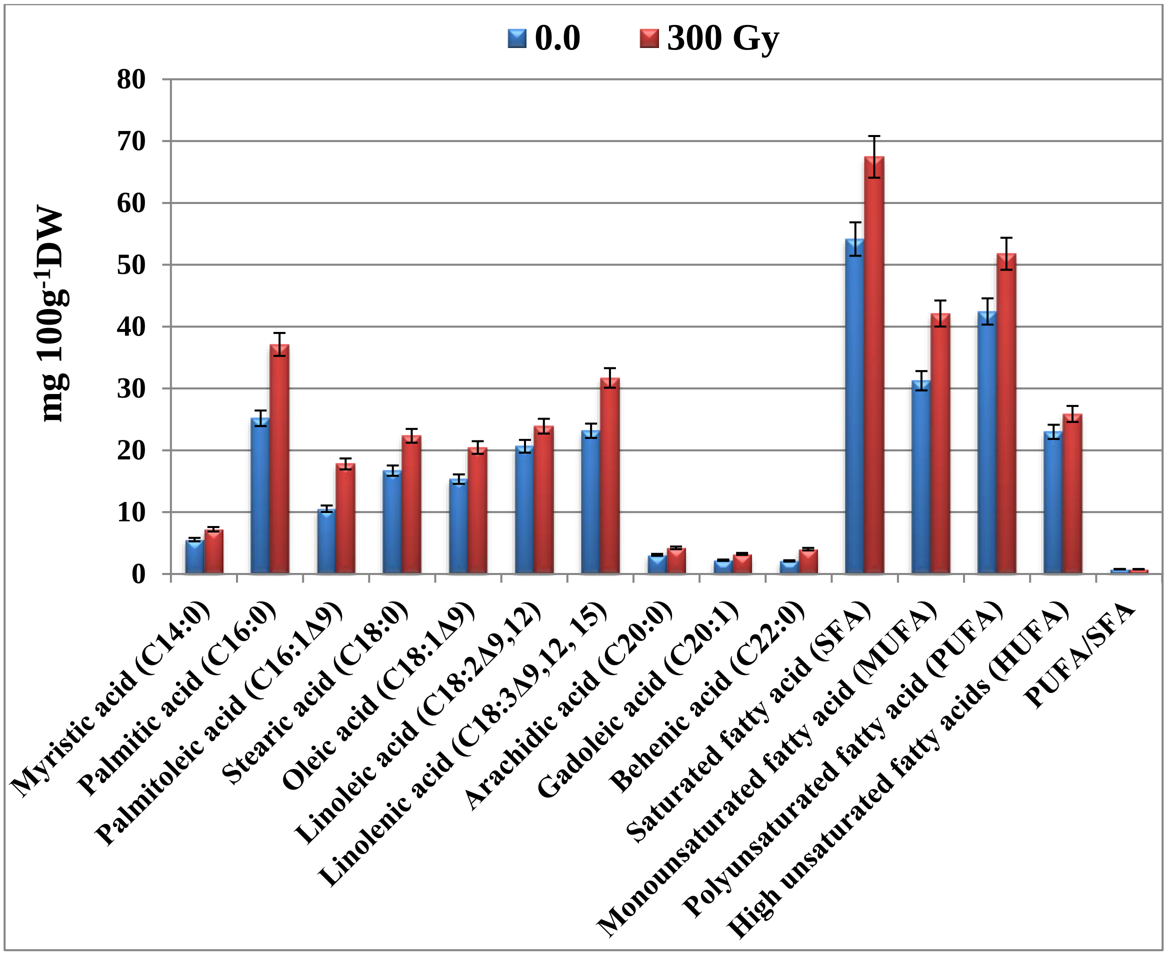

Fig. 4. Fatty acid compositions in *S. obliquus* treated with and without gamma irradiation (300 Gy) after 20 days of growth. The values are means of at least three replicates ± standard deviation (SD)

CONCLUSION

According to the findings of this investigation, γ-irradiation (300Gy) has significantly increased the contents of proanthocyanidins, saponins, β-carotene, protein, vitamin contents (B_1, B_2, B_3, B_6, A, and K), amino acids, and fatty acids in *S. obliquus* compared to the control samples. Since *S. obliquus* has many benefits for the food business, this article advocates using it to improve health perspectives in the development of several therapeutic foods, functional foods, and nutritional supplements. The metabolites of γ-irradiated *S. obliquus* are inexpensive, readily available, safe, and natural; thus, their use may contribute directly or indirectly to the maintenance of health.

Conflicts of interest

We have no conflicts of interest to disclose.

ABBREVIATION

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

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