Egypt. J. Chem. **Vol. 65,** No. SI:13B pp. 995 - 999 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effect of gamma radiation on chemical composition, phytochemical constituents and antioxidants of *Portulaca oleracea* seeds

Soad Hasanin[1], A. G. ELshahawy [1], Hamed El-Shora [2], Abu Bakr El-Bediwi [1]

[1]Physics Department, Faculty of Science, Mansoura University, 35516 Mansoura, Egypt

[2]Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt.

Abstract

Extract of *Portulaca oleracea* seeds is considered important for drug development. Gamma radiation is a form of ionizing radiation that affects plant cells at different rates depending on the dosage, radio- sensitivity, types of plants, and physiological condition of plants. This work aimed to study the effect of gamma radiation with different doses (3, 5, 7, 9, and 11 KGy) on phenolic compounds, superoxide dismutase enzyme activity, chemical composition, and molecular structure of *Portulaca oleracea* seeds. The results show that gamma radiation significantly affected phenolic and flavonoid contents which increased at 3, 5 KGy, but decreased at 7, 9, and 11 KGy gradually. Tannin content increased at 3, 5, and 7 KGy but decreased at 7, 9, and 11 KGy. Superoxide dismutase activity increased gradually. The carbohydrates decreased and protein content also varied after exposure to gamma radiation. The radiation affected the arrangement, size, interconnection, and orientation of the seeds molecules

Keywords: *Portulaca oleracea* seeds, Gamma radiation, Phenolic, Superoxide dismutase, Carbohydrates, Protein, FTIR, and SEM.

Introduction

Portulaca oleracea (L) plant belongs to Portulacaceae family. *Portulaca oleracea* has been used as an ingredient in food and herbal medicines owing to the high content of phenolics, flavonoids and organic acids [1]. Gamma radiation is a form of electromagnetic radiation with very shortwave lengths, acting as a source of ionizing energy, where the isotope Cobalt-60 is the most common source for irradiation processes [2]. There is a harmful effect on a biological system caused by gamma radiation, by the direct ionization or indirect action which generates highly reactive oxygen species (ROS) [3]. The effect of gamma radiation on the growth and development of plants varied from stimulatory to inhibitory depending on radiation doses, physiology [4], and the morphology of the plant.

Generally, high doses of gamma radiation can be lethal to plants [4]. The biological effect of gamma radiation is based on the interaction with atoms or molecules in the cell particularly water to produce free radicals [5], which can damage or modify important components of plant cells, where they affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose [5]. These effects also include changes in the plant cell structure and metabolism as dilation of thylakoid membranes, alteration of photosynthesis, modulation of the anti-oxidative system and accumulation of phenolic compounds [6]. The irradiation of seeds with high doses of gamma radiation also disturbs the synthesis of protein, leaf gas exchange, water exchange, hormone balance and enzyme activity [7]. Seeds of Zea mays, *Lathyrus chrysanthus Boiss*, *Moluccella laevis L*, soybean, peanut and sesame were exposed to gamma radiation for varying doses, and their growth, internal structure, antioxidants, and vitamins changed [8–11]. The present research aimed to study the biological effects of gamma radiation on phenolic compounds, flavonoids, tannins, and superoxide dismutase contents of *Portulaca oleracea* seeds.

3. Experimental methods

3.1. Radiation source: The gamma radiation system used in this study is located at the Egyptian Atomic Energy Authority in the unit of gamma irradiation. Seeds of *Portulaca oleracea* were exposed to different doses of gamma radiation (3, 5, 7, 9, and 11 kGy) with a dose rate of 0.863 kGy/h. The seeds were scanned by scanning electron microscope (JEOL JSM-6510LV, Japan) while the molecular structure

*Corresponding author e-mail: soadhasanin@yahoo.com.(Soad Hasanin)

Receive Date: 11 August 2022, Revise Date: 19 August 2022, Accept Date: 31 August 2022

DOI: 10.21608/EJCHEM.2022.155710.6729

was studied by Nicolet™ iS™ 10 FT-IR Spectrometer from the USA.

3.2. Extraction method: The seeds of *Portulaca oleracea* were purchased from the Egyptian Ministry of Agriculture. The seeds were dried at 45 °C in the oven for 1 day for complete dryness. A sample (5 gm) of the dried seeds of the plant was settled in a conical flask (250 mL), then 50 mL of methanol was added. The conical flask was retained in a horizontal water bath with shaking at 200 rpm and 30 °C for four hours. The prepared extracts were filtered using Whatman filter paper No. 1 (Whatman Int. Ltd., Kent, UK) and a Buchner funnel and stored at 5 °C for further analysis.

3.3. Phenolic content: To measure the phenolic content in *Portulaca oleracea* seed extract, the Folin-Ciocalteu (F-C) assay was used following the procedure reported by Wolfe *et al* and Issa *et al*. [12,13], in which the standard curve of gallic acid was used.

3.4. Flavonoid content: The flavonoid content in *Portulaca oleracea* seeds was determined by aluminum chloride spectrophotometrically following the standard curve of catechin [14].

3.5. Tannin Content: The tannin content in *Portulaca oleracea* seeds was carried out following the procedure of vanillin-hydrochloride assay [15].

3.6. Superoxide dismutase: Superoxide dismutase (SOD) was assayed by a photochemical method as described by Giannopolitis and Ries [16].

3.7. Chemical composition Determination

The chemical composition including moisture, ash, fiber, and protein was determined according to the method described by AOAC (2019)

3.7.1. **Determination of moisture content**

Moisture content was determined in the following manner. A sample (2g) of the plant was dried at 105 °C in the oven to a constant weight. Then the moisture content percentage was calculated [17].

3.7.2. **Determination of ash content**

Ash content was determined by placing 2g of dried seeds in a silica melting- pot and turn on at 600 ºC in a muffle oven to a fixed weight. Then the percentage of ash content was calculated [17].

3.7.3. **Determination of fiber content**

Fiber content was estimated by mixing 2g of dried seeds with 0.5g asbestos, then 200 ml of sulphuric acid (1.25% v/v) was added. The mixture was boiled for 30 minutes, then filtered through a melting pot. The precipitates were boiled again with aqueous sodium hydroxide solution (200 ml, 1.25% w/v) for 30 minutes, and the filtration was repeated in the same manner. The remains were washed with hot water followed by diethyl ether and dried at 110 ºC to fixed weight. Fiber content percentage was calculated by subtraction of ash content from the weight of the digested sample [18].

3.7.4. **Determination of protein content**

Protein content was determined by digesting 0.5g of dried seeds with 8 ml of concentrated sulfuric acid in a beaker in the presence of 2.14 g of the digestion mixture [1 kg of potassium sulfate and 60 g of mercuric oxide (red)]. Then the solution was treated with 10 mL (40%) NaOH solution. The liberated NH_3 was received in 10 ml. of 1% boric acid in the presence of 2 drops of Tachero indicator (1.25 g methyl red + 0.32 g methylene blue in 1 liter of 90% ethanol). Then ammonia was titrated with 0.01 N sulfuric acid. The total nitrogen was estimated and the protein content was calculated using 6.25 as a protein factor [19].

3.7.5. **Determination of carbohydrate content**

Carbohydrates content was calculated by difference from the following equation: Carbohydrates content % = 100 - [% protein + % ash + % lipids + % fiber].

3.8. Statistical analysis

Data were analyzed by SPSS software using analysis of variance (ANOVA) and differences among means were determined for significance at $P < 0.05$ using Tukey's test.

4. Results and discussion

4.1. Secondary metabolites

4.1.1. Phenolic content

Table 1 showed that the phenolic content in *Portulaca oleracea* seeds was increased at 3 and 5 KGy by 7.03% and 12.35%, respectively but decreased at 7, 9 and 11 KGy by 25.61%, 44.73%, and 53.55%, respectively after exposure to different gamma radiation doses. The results show a significant change of $P < 0.001$. Phenolic was increased due to free radical production by gamma radiation, therefor these compounds increased to scavenge the free radicals which destroy the cells [20], the decrease in phenolics content after exposure to high doses may be due to inhibition of their biosynthetic enzymes particularly phenylalanine ammonia - lyase or may be due to breaking down the flavonoid molecules [21].

Table 1: Phenolic content in *Portulaca oleracea* seeds before and after exposure to gamma radiation.

Radiation dose (KGy)	Phenolic content (mgg^{-1} gallic acid)
Normal	321.24
3	343.83
5	360.91
7	238.98
9	177.56
11	149.21

4.1.2. Flavonoid content

Flavonoid content in *Portulaca oleracea* seeds increased at 3 and 5 KGy, by 14.9% and 24.47%, but decreased at 7, 9 and 11 kGy by 8.39%, 42.46% and

59.79% as presented in Table 2. The results show a significant change of $P < 0.001$. Flavonoid content was increased due to free radical production by gamma radiation, therefor these compounds increased to scavenge the free radical which destroys the cells. The reduction in flavonoid content after treatment with high doses may be due to inhibition of their biosynthetic enzymes [22]. A great change in the O-H band caused a change in flavonoids. Gamma radiation owns enough energy to broking the chemical bonds that cause photochemical reactions, which leads to a variation in flavonoid content for seeds. The effect of radiation on the structure of phenol compounds leads to a change in flavonoid content.

Table 2: Flavonoid content in *Portulaca oleracea* seeds before and after exposure to gamma radiation.

Radiation dose (KGy)	Flavonoid content (mgg^{-1} catechine)
Normal	189.26
3	217.45
5	235.58
7	173.39
9	108.90
11	76.07

4.1.3. Tannin content

The tannin content in *Portulaca oleracea* seeds increased at 3, 5 and 7 KGy by 4.86%, 5.1% and 1.54% after exposure but decreased at 9 and 11KGy by 33.77% and 52.51%, respectively, as shown in Table 3. The results show a significant change of $P < 0.001$. A significant change in tannins is due to the reactivity of tannins with protein and metal ions, in addition, tannins protect the cells against oxidative stress resulting from gamma radiation [23]. The effect of radiation on the structure of phenol compounds leads to a change in tannins' content.

Table 3: Tannin content in *Portulaca oleracea* seeds after exposure to gamma radiation.

Radiation dose (KGy)	Tannin content (mgg^{-1} tannic acid)
Normal	37.037
3	38.84
5	38.92
7	37.61
9	24.53
11	17.59

4.2. Enzymatic antioxidants

4.2.1. Superoxide dismutase (SOD)

SOD activity in the extract of *Portulaca oleracea* seeds was greatly affected by exposure to gamma radiation. Table 4 shows that it increased after exposure to different doses from 234.4 Umg^{-1} to 864.7 Umg^{-1} at 0 to 11 kGy, respectively. The results show a significant change of $P < 0.001$. The increase may be due to the production of reactive oxygen species (ROS), particularly superoxide radicals. This enzyme scavenges the superoxide radical as a sort of defense system for the cell. The enzyme acts as a good therapeutic agent against reactive oxygen species-mediated diseases [24]. The superoxide dismutase which scavenges ROS and protects lipids, proteins and nucleic acids is activated when plants respond to oxidative injury [25].

Table 4: Superoxide dismutase activity of *Portulaca oleracea* seeds before and after exposure to gamma radiation.

Radiation dose (KGy)	Superoxide dismutase (Umg^{-1})
Normal	243.39
3	435.74
5	538.82
7	650.06
9	722.38
11	864.26

4.3. Chemical composition

Table 5 shows, the chemical composition of *Portulaca oleracea* seeds after exposure to 3 and 11 kGy gamma radiation doses, where carbohydrates decreased but protein varied results, which protein increased at 3 KGy and decreased at 11KGy. The reduction of carbohydrates may be due to the inhibitory radiation effect on the photosynthetic enzyme and consequently on the photosynthetic enzyme system. The increase of protein content after treatment with 3 KGy may be due to induction of amino acid incorporation to protein or induction of enzymes involved in protein synthesis. However, the reduction of protein content after treatment with 11 KGy may be due to protein proteolysis or inhibition of enzymes of protein synthesis [26].

Table 5: Chemical composition of *Portulaca oleracea* seeds before and after exposure to gamma radiation.

% Composition	Normal	3 KGy	11 KGy
% Protein	9.8	10.5	5.15
% Carbohydrates	31.79	26.32	25.16
% Fiber	44.59	49.2	58.42
% Fat	6.14	6.63	4.29
% Ash	7.68	7.35	6.98
% N	1.57	1.68	0.82
% Moisture	8.91	8.79	6.35

4.4. Internal structure

4.4.1. FTIR analysis

The IR spectrum, of *Portulaca oleracea* seeds after exposure to gamma radiation, is a graph plot of infrared light % transmittance on the Y-axis against

wavelength on the X-axis shown in (Figure 1). From IR analysis in Table 6, there is a change occurred in position and intensity for the hydroxyl group after exposure to gamma radiation, where the untreated sample was at 3420 (80%), then after treated by 3, 7 and 11 kGy, changed to 3417 (70.6%) 3421 (65.4%) and 3420 (62.6%). This means that the molecular bonds in the *Portulaca oleracea* seeds changed after gamma radiation because each interatomic bond vibrated with several different motions and individual bonds were absorbed in more than one IR frequency. A remarkable change in O-H group specifications means that it is broken or modified. This is because the gamma radiations have enough energy to destroy or modify the bounds of molecules which causes a change in enzymatic and non-enzymatic antioxidants [19].

Table 6: IR analysis of *Portulaca oleracea* seeds before and after exposure to gamma radiation.

Radiation dose (KGy)	Characteristics of O-H Bond	
	Bond position	Bond Int. %
Normal (0)	3420	80%
3	3417	76.6%
7	3421	65.4%
11	3420	62.6%

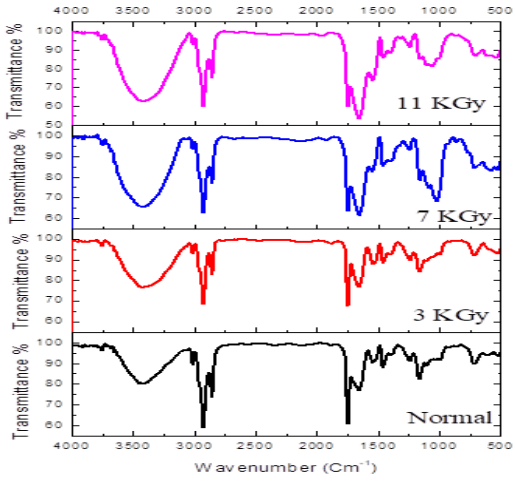

Figure 1: IR spectrum of *Portulaca oleracea* seeds before and after exposure to gamma radiation.

4.4.2. SEM analysis

Scanning electron micrographs (SEM) of *Portulaca oleracea* seeds show a marked effect on the shape, size, interconnection and orientation of molecules after exposure to 3, 7, and 11 kGy (Figure 2). This is because gamma radiation collided with cell-matrix or reacted rapidly with almost all structural and functional organic molecules, resulting in breaking or destroying, or modifying bonds of molecules, which consequently change the internal structure. These structural changes and cell disruptions were due to the oxidative stress caused by the gamma radiation and the production of free radicals interacting with the molecules [27].

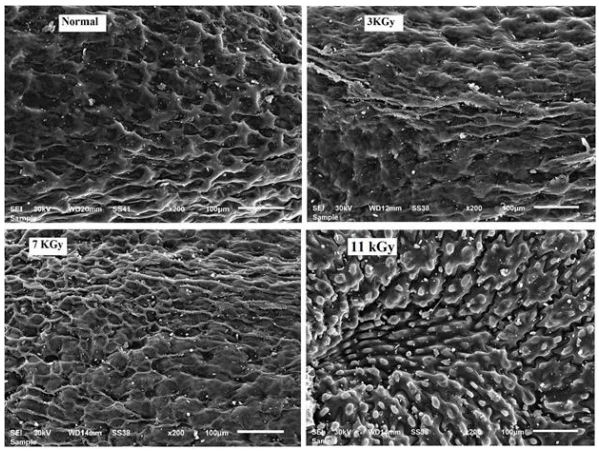

Figure 2: SEM of *Portulaca oleracea* seeds before and after exposure to gamma radiation.

Conclusion

The high gamma radiation doses have a remarkable effect on orientation, size, arrangement or interconnection due to the higher penetrating power of gamma radiation into seed cells. These changes in the chemical composition include carbohydrates, protein, fat and fiber. Also, the change included phenolic compounds, flavonoids, tannins and superoxide dismutase in *Portulaca oleracea* seeds. Thus, gamma radiation could be applied at lower doses for increasing the antioxidant compounds and consequently increasing the antioxidant activity.

References

[1] Zhou, Y. X., Xin, H. L., Rahman, K., Wang, S. J., Peng, C., & Zhang, H. (2015). *Portulaca oleracea* L.: a review of phytochemistry and pharmacological effects. *Biomed Research International*, *2015*.

[2] Chmielewski, A. G. (2019). Radiation crosslinking for the cable, rubber and healthcare products industry. In: *Radiation Effects in Polymeric Materials* (pp. 369-391). Springer, Cham.

[3] Gudkov, S. V., Grinberg, M. A., Sukhov, V., & Vodeneev, V. (2019). Effect of ionizing radiation on physiological and molecular processes in plants. *Journal of Environmental Radioactivity*, *202*, 8-24.

[4] Yadav, V. (2016). Effect of gamma radiation on various growth parameters and biomass of canscora decurrens dalz. *International Journal of Herbal Medicine*, *4(5)*, 109-115.

[5] Kebeish, R., Deef, H. E., & El-Bialy, N. (2015). Effect of gamma radiation on growth, oxidative stress, antioxidant system, and alliin producing

gene transcripts in *Allium sativum*. *International Journal of Research Studies in Biosciences*, 3(3), 161-174.

[6] Hamideldin, N., & Eliwa, N. E. (2015). Gamma radiation and sodium azide influence on physiological aspects of maize under drought condition. *Basic Rese. J. Agric. Scie. Review*, *4(1)*, 5-13. [7] Hanafiah, D. S. (2021). Germination and seedling growth of Kipas Putih soybean (*Glycine max* [L.] Merril) in various dosage of gamma rays irradiation. In IOP conference series: *Earth and Environmental Science (Vol. 637, No. 1, p. 012052)*. IOP Publishing.

[8] Marcu, D., Damian, G., Cosma, C., & Cristea, V. (2013). Gamma radiation effects on seed germination, growth and pigment content and ESR study of induced free radicals in maize (*Zea mays*). *Journal of Biological Physics*, *39*(4), 625-634.

[9] Beyaz, R., Kahramanogullari, C. T., Yildiz, C., Darcin, E. S., & Yildiz, M. (2016). The effect of gamma radiation on seed germination and seedling growth of *Lathyrus chrysanthus* Boiss under in vitro conditions. *Journal of Environmental Radioactivity*, *162*, 129-133.

[10] Minisi, F. A., El-mahrouk, M. E., Rida, M. E. F., & Nasr, M. N. (2013). Effects of gamma radiation on germination, growth characteristics, and morphological variations of *Moluccella laevis* L. *Am.-Eurasian J. Agric. Environ. Sci*, *13*, 696-704.

[11] Afify, A. E. M. M., Rashed, M. M., Mahmoud, E. A., & EL-Beltagi, H. S. (2011). Effect of gamma radiation on protein profile, protein fraction, and solubility's of three oil seeds: soybean, peanut, and sesame. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *39*(2), 90-98.

[12] Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, *51*(3), 609-614.

[13] Dhabian, S. Z., & Jasim, R. S. (2021). Anticancer and antioxidant activity of the greenly synthesized zinc nanoparticles composites using aqueous extract of *Withania somnifera* plant. *Egyptian Journal of Chemistry*, *64*(10), 3-4.

[14] Yayinie, M., Atlabachew, M., Tesfaye, A., Hilluf, W., Reta, C., & Alemneh, T. (2022). Polyphenols, flavonoids, and antioxidant content of honey coupled with chemometric method: geographical origin classification from Amhara region, Ethiopia. *International Journal of Food Properties*, *25*(1), 76-92.

[15] Poudel, M., & Rajbhandari, M. (2020). Phytochemical analysis of *Ampelopteris prolifera* (Retzius) Copeland. *Nepal Journal of Science and Technology*, *19*(1), 78-88.

[16] Hu, W. H., Song, X. S., Shi, K., Xia, X. J., Zhou, Y. H., & Yu, J. Q. (2008). Changes in electron transport, superoxide dismutase, and ascorbate peroxidase isoenzymes in chloroplasts and mitochondria of cucumber leaves as influenced by chilling. *Photosynthetica*, *46*(4), 581-588.

[17] Park, Y. W., & Bell, L. N. (2004). Determination of moisture and ash contents of foods. *Food Science and Technology-New York-Marcel Dekker-*, *138*(1), 55.

[18] Rahman, M. H. (2020). Production of functional textile filaments from chemically modified cellulose fibers (Doctoral dissertation).

[19] Ebrahim, R., Abdelrazek, A., El-Shora, H., & El-Bediwi, A. B. (2022). Effect of ultraviolet radiation on molecular structure and photochemical compounds of *Salvia hispanica* medical seeds. *AIMS Biophysics*, *9*(2), 172-181.

[20] Pradhan, B., Baral, S., Patra, S., Behera, C., Nayak, R., MubarakAli, D., & Jena, M. (2020). Delineation of gamma irradiation (^{60}Co) induced oxidative stress by decrypting antioxidants and biochemical responses of microalga, chlorella sp. *Biocatalysis and Agricultural Biotechnology*, 25, 101595.

[21] Gheysarbigi, S., Mirdehghan, S. H., Ghasemnezhad, M., & Nazoori, F. (2020). The inhibitory effect of nitric oxide on enzymatic browning reactions of in-package fresh pistachios (*Pistacia vera* L.). *Postharvest Biology and Technology*, 159, 110998.

[22] Ma, H., Xu, X., Wang, S., Wang, J., & Wang, S. (2022). Effects of microwave irradiation of *Fagopyrum tataricum* seeds on the physicochemical and functional attributes of sprouts. *LWT*, *165*, 113738.

[23] Choi, J., & Kim, W. K. (2020). Dietary application of tannins as a potential mitigation strategy for current challenges in poultry production: A review. *Animals*, *10(12)*, 2389.

[24] Bratovcic, A. (2020). Antioxidant enzymes and their role in preventing cell damage. *Acta Scientific Nutritional Health*, *4(3)*, 01-07.

[25] Qamer, Z., Chaudhary, M. T., DU, X., Hinze, L., & Azhar, M. T. (2021). Review of oxidative stress and antioxidative defense mechanisms in Gossypium hirsutum L. in response to extreme abiotic conditions. *Journal of Cotton Research*, *4(1)*, 1-9.

[26] Hellwig, M. (2020). Analysis of protein oxidation in food and feed products. *Journal of Agricultural and Food Chemistry*, *68(46)*, 12870-12885.

[27] Abramov, A. Y., Potapova, E. V., Dremin, V. V., & Dunaev, A. V. (2020). Interaction of oxidative stress and misfolded proteins in the mechanism of neurodegeneration. *Life*, *10(7)*, 101.