BIOSYNTHESIS OF PHENOLIC COMPOUNDS AND WATER SOLUBLE VITAMINS IN CULANTRO (*Eryngium foetidum* L.) PLANTLETS AS AFFECTED BY LOW DOSES OF GAMMA IRRADIATION

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Abstract. Explants obtained from *in-vitro* propagated plantlets of Culantro (*Eryngium foetidum* L.) were exposed to four dose levels of γ-irradiation (0.0, 10.0, 20.0 and 40.0 Gy) to investigate the biosynthesis of phenolic compounds and water soluble vitamins in Culantro fresh plantlets. Among six identified phenolic compounds, the content of *p*-cumaric acid was the highest in the extracts, followed by caffeic acid, coumarin, benzoic acid, salicylic acid and apigenin. Significant increases were observed at dose 40.0 Gy (61.66 mg/g d.w. for flavonoids, 18.02 mg/g d.w, for flavonone and 5.06 mg/g d.w for anthocyanin) compared to control. On the other hand, the flavonols were decreased by increasing the irradiation dose. Vitamin C was increased in irradiated samples and this increase was in correlation with irradiation dose level. Thiamin, riboflavin and nicotinic acid were enhanced by the applied dose level 10 Gy. In addition, folic acid was enhanced by the dose levels 20 and 40 Gy and not detected for the control and 10 Gy treatments. Meanwhile, pyridoxine was decreased by increasing the irradiation dose level. The results obtained suggested that both low doses of γ-irradiation and tissue culture technique could be used to produce plantlets with high amount of phenolic compounds and water soluble vitamins.

Keywords: *Eryngium foetidum* L., gamma irradiation, phenolic compounds, water soluble vitamins

INTRODUCTION

For alternatives production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites [31]. Culantro *(Eryngium foetidum* L.) has been reported as a medicinal plant which is used in traditional medicine for fevers and chills, vomiting and diarrhea [17]. The leaves and roots are boiled and water drunk for pneumonia, flu, diabetes, constipation and malaria fever. Culantro has been acclaimed as health foods due to its high content of calcium, iron, carotene, riboflavin, proteins and vitamins [3]. Gamma rays have played an important role in the producing new mutants with improved properties which can produce higher amounts of commercially important metabolites [32]. Polyphenolic compounds are among the most talked about dietary ingredients nowadays. Polyphenols belong to a class of phytochemicals, occur in nature as mixtures of esters, ethers, or free acids and found in high concentrations in a wide variety of plants. Caffeic, ferulic and *p*-coumaric acids are *trans*-cinnamic acids that occur naturally in their free forms or as a family of mono or diesters with (-) quinic acid, collectively known as chlorogenic acids [27]. Phenolic compounds have reduced risk of cardiovascular disease and cancer prevention [16, 26]. Phenolic compounds are responsible for the brightly colored pigments of many fruits and vegetables. They protect plants from diseases and ultraviolet radiation helping preventing damage to seeds until they germinate [35]. Previous phytochemical investigations on the *Eryngium* genus indicated the presence of flavonoids [15], essential oils [30], coumarins [11] and rosmarinic acid derivatives [23]. Flavonoids are polyphenolic compounds which are categorized into flavonols, flavones, flavanones, isoflavones, and catechins. Variation in flavonoid content at different doses of irradiation treatment [29] may be due to difference in the loss of flavonoids by gamma irradiation and de novo synthesis of flavonoids. However, for other plant metabolites, diverse effects of irradiation on some important ingredients have been reported [20, 21]. In recent years, because of health interest of phenolic compound as antioxidant activities, gamma rays have been used in order to enhance phenolic acids in cloves and nutmeg [21]. Also, γ-irradiation has been successfully applied to induce changes in the phenolic content of almond skin extract [14]. In addition, Aly and Mohamed [1] provided that γ-irradiation doses ranging from 50-150 Gy successfully activate total phenolic content, sinapin, glutathione, ascorbic acid and α-tochopherol content in maize callus tissue. Low doses of irradiation prevented the loss of anthocyanin while, higher doses decreased the content of anthocyanin in grape pomace [2]. Vitamins are essential nutrients found in foods, the requirements are small but they perform specific and vital functions essential for maintaining health. B-complex vitamins and ascorbic acid are water-soluble vitamins that are not stored in the body and must be replaced each day. Vitamin C is one of the most important vitamins for human nutrition that is supplied by fruits and vegetables. Vitamin B6 is an essential metabolite in all organisms. It can act as a coenzyme for numerous metabolic enzymes and has recently been shown to be a potent antioxidant [39]. Riboflavin is a strong oxidant which contains several sites susceptible to attack by reactive species such as hydrated electrons and free radicals [13]. However, riboflavin appears to be very resistant to radiation in grass prawns. Also, riboflavin is quite stable to chemical attack and is reversibly reduced to dihydroriboflavin by reducing agents. Moreover, riboflavin is normally bound to proteins which protect the prosthetic groups from being attacked directly or indirectly by irradiation [40]. There is no information available in the literature on the response of Culantro phenolic acid and water soluble vitamins to low doses of γ-irradiation. This study illustrates the enhancement effect of low doses of γ-irradiation on phenolic compounds and water soluble vitamins.

MATERIALS AND METHODS

Plantlets of Culantro (*Eryngium foetidum* L.) were kindly provided by Professor Mohamed-Yasseen, Genetic Engineering and Biotechnology Research Institute-Minufia University-Egypt.

Culture medium and conditions

Stem-disc (0.5) cm thick section without shoot primordial explants excised from the *in vitro*-raised shoots were cultivated onto MS [28] basal medium without growth regulators supplemented with Sucrose at 25 g l^{-1}, media were gelled with 7.0 g l^{-1} agar. The pH of the medium was adjusted to 5.8 before the addition of agar. All the media were sterilized by autoclaving at a pressure of 1.06 kg cm^2 for 20 min. Culture jars were incubated at 25±2ºC (relative humidity 80%) with 16/8 h photoperiod under white fluorescent tubes (photosynthetic photon flux of 40 µmol m^{-2} s^{-1}).

Irradiation treatments

After 6 weeks from subculture, the plantlets were exposed to γ- rays at dose levels (0.0, 10.0, 20.0 and 40.0 Gy). Irradiation was performed using a Gamma cell 200 apparatus equipped with a ^{6o}Co γ source with average dose rate-of 0.7Gy/min. at National Center for Radiation Research and Technology, Cairo, Egypt. Immediately after irradiation, explants were aseptically transferred into sterile fresh MS medium, placed in a growth chamber at 26^{o}C with 16 / 8 hrs light/dark period. Subculture has been done four times after irradiation. Samples were taken after 6 weeks from the last subculture and used for the following determinations:

Preparation of plant extract

Plant materials were dried at room temperature and ground to a fine powder. Two grams of each treatment were extracted in 20 ml of methanol by agitation at 200 rpm (24 h), then filtred through a Whatman filter paper No. 1. The solvent was removed under the vacuum at temperature below 50^{o}C and the dried extracts were used for analysis of phenolic content, flavonoids, flavonols, flavonones and anthocyanins.

Total phenolic content

The phenolic contents were determined according to the method of Singleton and Rossi [37] using the Folin-Ciocalteu reagent. Aliquots of 500 µl of each methanolic extract were used for measurements. Phenolic contents of the samples were calculated on the basis of the standard curve of pyrogallol. The results were expressed as mg g^{-1} of pyrogallol equivalents of dry weight of the plantles.

Determination of phenolic compounds

One gm of fresh tissues of each sample were homogenized with methanol 40% and stirred on a shaker. The extract was filtered through a whatman filter paper No. 1 and the solvent was evaporated in vacuum. The dried residues containing phenol compounds were dissolved in a solution consists of methanol: water: acetic acid (40: 59.3: 0.7, v: v: v) and stored in vials. The method suggested by Christian [9] was used as follows:

HPLC analysis was used to detect and determine the phenolic compounds from the plant tissues. The extract was passed through micro-filter 0.45µm. The analysis of phenolic compounds was performed on HPLC model (HP1050). HPLC equipped with UV detector. The separation and determination were performed on C18 column (150x 4.6mm). The mobile phase yielded results of methanol: water: acetic acid, (40: 59.3: 0.7, v/v/v). The wave length of UV detector was 254 nm and the total run time for the separation was approximately 25 min at a flow rate of one ml/min. Identification of phenolic compounds was carried out by comparing retention times and spectral data with those of authentic standards (acceptable matches were 90–100%). Quantification was done by an external standard method, in triplicate.

Total flavonoids content

Aluminum chloride colorimetric method was used for flavonoids determination [6]**.** Each plant extracts (0.5 ml) were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min, and then the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing a serial concentration of quercetin solutions. The results were expressed as mg g^{-1} of quercetin equivalents of dry weight of the plantlets.

The content of flavonols

Flavonols were determined by Yermakov *et al.* [42] method. The quercetin calibration curve was prepared by mixing 2ml of various concentrations of ethanolic solutions of quercetin with 2ml (20 mg/ml) aluminium trichloride and 6 ml (50 mg/ml) sodium acetate. The absorbance at 440 nm was read after 2.5 h. The same procedure was used for 2ml of plant extract (10 mg/ml) instead of quercetin solution. All determinations were carried out in triplicates. The content of flavonols was calculated using a standard curve obtained from various concentration of quercetin. The results were expressed as mg g^{-1} of quercetin equivalents of dry weight of the plantles.

Determination of flavonones

One ml methanolic extract of samples were separately mixed with 2 ml of 50.5 mM 2,4-dinitrophenylhydrazine (1.0 mg 2,4-dinitrophenylhydrazine dissolved in 100 ml MeOH with 2 ml H_2SO_4 conc) and 2 ml of methanol. The contents were heated for 50 min at 50°C on a water bath, and then allowed to cool to room temperature. The solution was mixed with 5 ml of 1% potassium hydroxide (W/V) in 70% ethanol (V/V). The absorbance of the filtrate was measured at 495 nm. The values are reported as naringenin equivalents (NE) by the following equation.

[NE]mg/ml = (13:77 x A_{486}-0.7554) x Vml/W. As previously reported by Kosalec *et al*. [22]**.**

Where A was the absorbance, V; was the extract volume and W; was the sample weight

Total anthocyanins contents

Total anthocyanin contents in samples extract were determined by using pH differential method [7]. Absorbance was measured using a Shimadzu Spectrophotometer (Jasco V-530) at 510 nm and 700 nm in buffers at pH 1.0 and 4.5, using A = [(A_{510}-A_{700})

at $pH_{1.0}$ - (A_{510}- A_{700}) at pH 4.5] with a molar extinction coefficient of cyaniding 3-glucoside (29,600). Results were expressed as milligrams of cyaniding 3-glucoside equivalents, in the samples extract, per 100 gram of dry samples.

Total anthocyanin (mg $100g^{-1}$) = ΔA/€L x MW x D x V/G

Where ΔA is absorbance, € the cyaniding3 glucoside molar extinction coefficient (26,900), L the cell path length (1cm), MW the molecular weight of anthocyanin (449.2) ,D a dilution factor, V the final volume (ml) and G the sample weight (g).

Determination of water soluble vitamins content by HPLC

Liquid chromatography was performed with a Shimadzu (Kyoto, Ja-pan) system. Shimadzu software was used to calculate peak areas. The sample (20 µl) was injected into the HPLC with a syringe (Hamilton, Reno, NV, USA). The HPLC column used was a reversed-phase Discovery C18 (150 mm × 4.6 mm, 5 µm) from Supelco (Bellefonte, PA, USA). The column eluate was monitored with a photodiode-array detector at 280 nm. The mobile phase was filtered through a 0.45 µm membrane and degassed by sonication before use. The mobile phase was 0.1 mol^{-1} KH_2PO_4 (pH 7): methanol, 90:10. The flow-rate was 0.7 ml min^{-1}. The column was operated at room temperature (25°C). Chromatographic peak data were integrated up to 39 min. Identification of compounds was achieved by comparing their retention times and UV spectra with those of standards which injected with the samples. Concentrations of the water soluble vitamins (ascorbic acid, thiamin, riboflavin pyridoxine, folic acid and nicotinic acid) were calculated from integrated areas of the sample and the corresponding standards [8].

Statistical analysis

All analysis were performed in triplicate (n=3) and statistical analysis was done using SPSS (version 15) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

RESULTS

We tested the influence of γ-irradiation at dose levels (0.0, 10.0, 20.0 and 40.0 Gy) on biosynthesis of some (phenolic compounds, flavonoids, anthocyanins and water soluble vitamins in Culantro fresh plantlets) as shown in Tables 1-2 and Figs. 1-4.

The enhancement effect of γ-irradiation on phenolic compounds:

Significant P<0.05 increase in total phenolic contents was induced by different doses of γ-irradiation. The maximum increase was observed with a dose level of 40 Gy (18.32±0.11 mg g^{-1} d.w), as shown in (Table I). The methanolic extracts of phenolic compounds from Culantro plantlets were identified by comparison of their retention times and UV spectra with those of known standards and the contents of these phenolic compounds, as shown in (Table I). Among six identified phenolic compounds, *p*-cumaric acid (3-(4-hydroxyphenyl)-2-propenoic acid) verified the superiority as the principal compound in Culantro plantlets. *P*-cumaric acid was increased by increasing the irradiation dose and reached to the maximum increase with dose level of 40 Gy (11.7±0.11 mg g^{-1} f.w) in comparison to unirradiated control (8.8±0.23 mg g^{-1} f.w.). While apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one) was found as a minor component which was detected only with the dose level of 10.0 Gy (3.2±0.15 mg g^{-1} f.w).

Table 1. Effect of γ-irradiation on total phenolic and phenolic acids of Culantro plantlets.

Phenolic compound	Irradiation dose (Gy)				
	Control	10.0	20.0	40.0	L.S.D 5%
Total phenolic mg g^{-1} d.w	8.91±0.01^{a}	11.25±0.06^{b}	14.61±0.17^{c}	18.32±0.11^{d}	0.13
Coumarin mg g^{-1} f.w	5.61±0.11^{a}	6.33±0.11^{b}	9.13±0.14^{c}	9.33±0.14^{d}	0.07
Caffeic acid mg g^{-1} f.w	8.62±0.06^{c}	5.40±0.14^{a}	7.77±0.44^{b}	7.46±0.09^{b}	0.05
***P*-cumaric** mg g^{-1} f.w	8.86±0.23^{a}	9.36±0.14^{ab}	9.92±0.17^{b}	11.70±0.11^{c}	0.61
Salicylic acid mg g^{-1} f.w	3.53±0.17^{a}	6.21±0.21^{c}	5.50±0.11^{b}	5.10±0.11^{b}	0.59
Benzoic acid mg g^{-1} f.w	6.60±0.11^{b}	3.21±0.11^{a}	3.13±0.14^{a}	ND	0.22
Apigenin mg g^{-1} f.w	ND	3.22±0.15	ND	ND	0.09

$^{a,b,c,\ldots}$Means within same column followed by different letters are significantly different at P<0.05. Values are means of three replicates (±SE); ND - not detected in the samples; L.S.D. - least significant difference.

Effect of γ-irradiation on the flavonoieds, flavonolas, Flavonone and anthocyanin

Significant increases in flavonoieds, flavonone and anthocyanin P<0.05 were observed by applied doses of γ-irradiation (Figs. 1-4) and this increase reached to the maximum increase with the dose of 40 Gy (61.66±2.9 , 18.2±1.80 and 5.6±0.39 mg g^{-1} d.w.) for flavonoids, flavonone and anthocyanin, respectively. On the other hand, the increase of γ-irradiation reduced the content of flavonols and it was 11.86±1.1 mg g^{-1} d.w. for dose of 40 Gy compared to control 18.09±1.78 mg g^{-1} d.w.

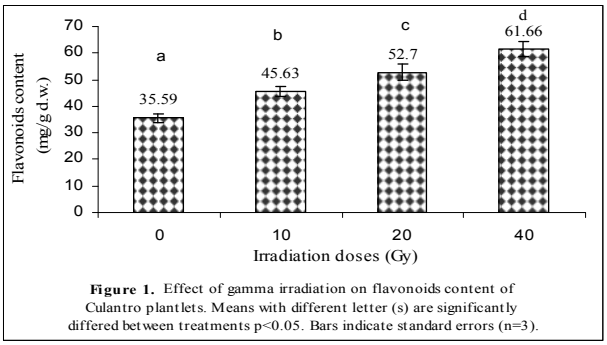

Figure 1. Effect of gamma irradiation on flavonoids content of Culantro plantlets. Means with different letter (s) are significantly differed between treatments p<0.05. Bars indicate standard errors (n=3).

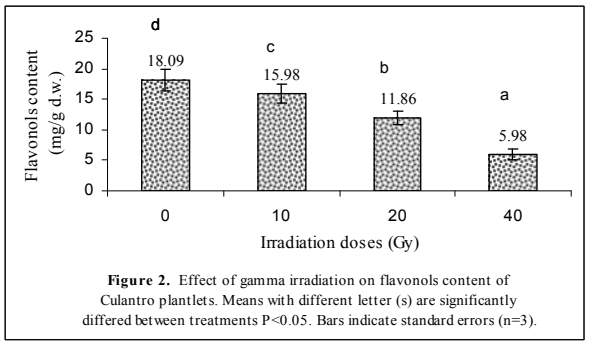

Figure 2. Effect of gamma irradiation on flavonols content of Culantro plantlets. Means with different letter (s) are significantly differed between treatments P<0.05. Bars indicate standard errors (n=3).

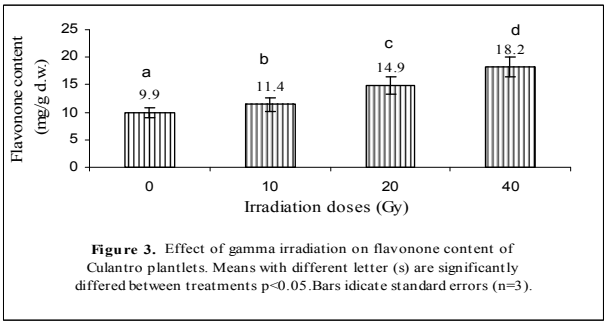

Figure 3. Effect of gamma irradiation on flavonone content of Culantro plantlets. Means with different letter (s) are significantly differed between treatments p<0.05.Bars idicate standard errors (n=3).

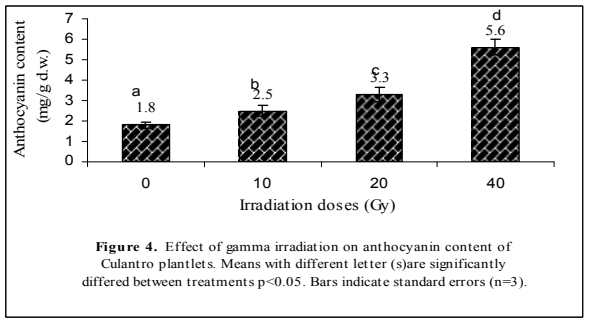

Figure 4. Effect of gamma irradiation on anthocyanin content of Culantro plantlets. Means with different letter (s)are significantly differed between treatments p<0.05. Bars indicate standard errors (n=3).

Low doses of gamma irradiation affects amount of water-soluble vitamins:

The effect of γ-irradiation on ascorbic acid, thiamin, riboflavin pyridoxine, folic acid and nicotinic acid of Culantro plantlets is shown in (Table 2). Vitamin C value in fresh Culantro plantlets was increased in irradiated samples and this increase was in correlation with irradiation dose level and reached to the maximum increase with the dose level of 40.0 Gy (61.48±0.59 mg $100g^{-1}$ f.w.) compared to control (2.24±0.03 mg $100g^{-1}$ f.w.). Thiamin, riboflavin and nicotinic acid of fresh Culantro plantlets were enhanced by the applied dose level of 10 Gy and the values were (2.05±0.05, 3.70±0.11 and 3.05±0.04 mg $100g^{-1}$ f.w.), respectively. In addition, folic acid was enhanced by the dose levels of 20 and 40 Gy (8.76±0.05 and 12.1±0.11 mg $100g^{-1}$ f.w.), respectively and not detected for the control and 10 Gy treatments. On the other hand, pyridoxine was decreased by increasing the irradiation dose level.

Table 2. Effect of γ-irradiation on ascorbic acid, thiamin, riboflavin pyridoxine, folic acid and nicotinic acid of Culantro plantlets.

Vitamins content mg/100g f.w.	Irradiation dose (Gy)				
	Control	10.0	20.0	40.0	L.S.D 5%
Ascorbic acid	2.24±0.03^{a}	5.62±0.11^{a}	10.81±0.20^{b}	61.48±0.59^{c}	4.48
Thiamin	0.34±0.09^{b}	2.05±0.05^{c}	0.27±0.01^{b}	0.08±0.01^{a}	0.11
Riboflavin	0.04±0.01^{a}	3.70±0.11^{d}	0.79±0.01^{c}	0.30±0.01^{b}	0.19
Pyridoxine	11.79±0.28^{d}	5.47±0.19^{c}	2.78±0.02^{b}	1.86±0.09^{a}	0.17
Folic acid	N.D	N.D	8.76±0.05^{a}	12.1±0.11^{b}	0.19
Nicotinic acid	1.43±0.02^{b}	3.05±0.04^{d}	1.55±0.02^{c}	0.52±0.01^{a}	0.08

a,b,c,.....Means within same column followed by different letters are significantly different at P<0.05; Values are means of three replicates (±SE); ND - not detected in the samples; L.S.D. - least significant difference.

DISCUSSION

Screening of Methanolic extracts of irradiated Culantro fresh plantlets at dose levels of (0.0, 10.0, 20.0 and 40.0 Gy) showed the presence of six identified phenolic compounds, coumarin (2H-chromen-2-one , 1-benzopyran-2-one), caffeic acid (3,4-Dihydroxy-cinnamic acid), *p*-cumaric acid (3-(4-hydroxyphenyl)-2-propenoic acid), salicylic acid (2-Hydroxybenzoic acid), benzoic acid (Benzenecarbo-xylic acid) and apigenin (5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one as indicated in (Table 1). The accumulation of phenolic compounds in cells was demonstrated and explained by the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by γ-irradiation [38, 41]. The ability of gamma irradiation to increase polyphenolic acids in plant metabolites has also been observed in soybeans samples treated with γ-irradiation at levels ranging from 50 to 150 Gy increased free polyphenolic acids [41]. Meanwhile, Siddhuraju *et al.* [36] attributed such increase in polyphenolic acids to higher extractability by depolymerization and dissolution of cell wall polysaccharides due to gamma irradiation. Moreover, Oufedjikh *et al.* [29] indicated that γ-irradiation was known to stimulate the activity of phenylalanine ammonia lyase, which is responsible for the synthesis of polyphenolic acids. Fan *et al.* [12] reported that the free radicals generated in plants during irradiation may act as stress signals and may trigger stress responses in plants, resulting to increased polyphenolic acid synthesis which had notable antioxidative properties. Irradiation exerts its effects as direct and indirect mechanisms, in case of indirect mechanism, radiolysis of water results in the production of free radicals such as hydroxyl radicals, hydroperoxide radicals and hydrated electrons. These radicals may break the

glycosideic bonds of procyanidin trimer, tetramer and hexamer that are present in fruits, leading to the formation of procyanidin monomers, which increase the total phenolic content in irradiated fruits [18, 19].

Flavonoids as one of the most diverse and widespread group of natural compounds, are likely to be the most important natural phenolics. Therefore, the content of flavonoids and some their derivative (flavonols, flavonones and anthocyanins) are also determined in the methanolic extracts of irradiated Culantro fresh plantlets at dose levels of 0.0, 10.0, 20.0 and 40.0 Gy (Figs. 1-4). The increase in anthocyanin may be due to the effect of γ-irradiation which enhances the activities of phenylalanine ammonia-lyase (PAL) and flavonoid glucosyltransferase (GT), the two key enzymes involved in the anthocyanin biosynthesis from phenylalanine, so anthocyanin and flavonoids compounds enhanced under γ-irradiation stress [19]. Low doses of irradiation prevented the loss of anthocyanin, while higher doses decreased the content of anthocyanin in grape pomace [2]. Moreover Beaulieu *et al.* [4] reported that the activity of catechol oxidase, (an enzyme related to the biosynthesis of anthocyanin monomers), was significantly higher in irradiated mushrooms both at low and high doses than in controls. The anthocyanin content of the peaches subjected to irradiation treatments was significantly higher $P<0.05$ than control unirradiated peaches throughout the entire storage period under both the storage conditions. Thus, the irradiation effects on anthocyanin accumulation and enhanced peach fruit colour development as observed in these studies are ethylene mediated via enhanced PAL activity [19]. In addition**,** Variyar *et al.* [41] also suggested a radiation-induced breakdown of glycosides resulting in the release of free isoflavones. Isoflavones are phenolic compounds, and they were increased in their concentration at a low dose of γ-irradiation supported by the corresponding increase in the total phenolic content. In general, polyphenols are thought to deliver health benefits by several mechanisms, including direct free radical quenching, protection and regeneration of other dietary antioxidants, and chelation of metal ions [5, 10].

Vitamins can be accelerated at low doses of γ-irradiation. There has been an interest in the ascorbate ability as an antioxidant to prevent or at least minimize the formation of carcinogenic substances from dietary material. Biosynthesis of ascorbic acid and riboflavin in irradiated corn, chickpea and soybean was found to be more than un-irradiated controls during germination [33, 34]. Ascorbic acid content of microtubers in Shepody cultivar have been significantly increased under γ-radiation dose levels (2, 4, 6 and 8 Gy) in comparison to control [25].

In conclusion, this is a study focused on the enhancement and quantifcation of *Eryngium foetidum* L. phenolic compounds and water soluble vitamins using low doses of γ-irradiation and tissue culture techniques. Low doses of γ-irradiation increased phenolic compounds, flavonoids, anthocyanins, as well as water soluble vitamins biosynthesis were also enhanced. Further studies are needed to investigate the possibility of using low doses of γ-irradiation for potential research and development value in the field of pharmaceutical compounds from different medicinal plants.

REFRENCES

[1] Aly, A.A., Mohamed, A.A., (2005): Natural antioxidants in maize callus tissue as enhanced by gamma irradiation. Egyptian Journal of Radiation Science and applications, 18: 221-236.

[2] Ayed, N., Yu, H.L., Lacroix, M., (1999): Improvement of anthocyanin yield and shelf-life extension of grape pomace by gamma irradiation. Food Reasearch International, 32: 539-543.

[3] Bautista, O.K., Kosiyachinda, S., Rahman, A.S.A., Soenoeadji, I., (1988): Traditional vegetables of Asia. Asian Food Journal, 4: 47–58.

[4] Beaulieu, M., Béliveau, G., D'Aprano, G., Lacroix, M., (1999): Dose rate effect of gamma irradiation on phenolic compounds, poly phenol oxidase (PPO) and browning of mushrooms Agaricus bis porus. Journal of Agricultural and Food Chemistry, 47: 2537-2543.

[5] Bravo, L., (1998): Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews, 56: 317-333.

[6] Chang, C.C., Yang, M.H., Wen, H.M, Chern, J.C., (2002): Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10: 178-182.

[7] Cheng, G.W., Breen, P.J., (1991): Activity of phenylalanine ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. Journal of the American Society for Horticultural Science, 116: 865-869.

[8] Cho, C.M., Ko, J.H., Cheong, W.J., (2000): Simultaneous determination of water-soluble vitamins excreted in human urine after eating an overdose of vitamin pills by a HPLC method coupled with a solid phase extraction. Talanta, 51: 799-806.

[9] Christian, G., (1990): HPLC Tips and Tricks. Great Britain at the Iden Press, Oxford, 608 p.

[10] Cieslik, E., Greda, A., Adamus, W., (2006): Content of polyphenols in fruit and vegetables. Food Chemistry, 94: 135-142.

[11] Erdelmerier, C.A., Sticher, O., (1985): Coumarin derivatives from *Eryngium campestre* L. Planta Medica, 51: 407–409.

[12] Fan, X., Toivonen, P.M.A., Rajkowski, K.T., Sokorai, K.J.B., (2003): Warm water treatment in combination with modified atmosphere packaging reduces undesirable effects of irradiation on the quality of fresh-cut iceberg lettuce. Journal of Agricultural and Food Chemistry, 51: 1231-1236.

[13] Fujimaki, M., Morita, M., (1968): Radiation chemistry of foods. I. Reaction rate constants of some foods constituents with hydrated electrons and hydroxyl radicals. Agriculture Biological Chemistry, 32: 574-579.

[14] Harrison, K., Were, LM., (2007): Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of almond skin extracts. Food Chemistry, 102: 932–937.

[15] Hohmann, J., Pall, Zs., Gunther, G., Mathe, I., (1997): Flavonolacyl glycosides of the aerial parts of *Eryngium campestre*. Planta Medica, 63: 96.

[16] Hollman, P.C.H., (2001): Evidence for health benefits of plant phenols: local or systemic effects? Journal of the Science of Food and Agriculture, 81: 842–852.

[17] Honeychurch, P.N., (1980): Caribbean Wild Plants and Their Uses. Letchworth Press, Barbados, W.I., pp. 70
[18] Hussain, P.R., Meena, R.S., Dar, M.A., Wani, A.M., (2008): Studies on enhancing the keeping quality of peach (*Prunus persica Bausch*) Cv. Elberta by gamma-irradiation. Physics and Chemistry, 77: 473-481.
[19] Hussain, P.R., Wani, A.M., Meena, R.S., Dar, M.A., (2010): Gamma irradiation induced enhancement of phenylalanine ammonia-lyase (PAL) and antioxidant activity in peach (*Prunuspersica Bausch*, Cv. Elberta). Radiation Physics and Chemistry, 79: 982-989.
[20] Kamat, J.P., Venkatachalam, S.R., (2004): *Asparagus Racemosus* and Radioprotection. pp. 78–84. In Ramawat, K.G. (ed.): Biotechnology of medicinal plants vitalizer and therapeutic. Oxford - New Delhi.
[21] Khattak, K.F., Simpson, T.J., Ihasnullah, I., (2008): Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella sativa* seed. Food Chemistry, 110: 967–972.
[22] Kosalec, I., Bakmaz, M., Pepeljnjak, S., Vladimir-Kneževiæ, S. (2004): Quantitative analysis of the flavonoids in raw propolis from northern Croatia. Acat pharma, 54: 65-72.
[23] Le Claire, E., Schwaiger, S., Banaigs, B., Stuppner, H., Gafner, F., (2005): Distribution of a new rosmarinic acid derivative in *Eryngium alpinum* L. and other Apiaceae. Journal of Agricultural and Food Chemistry, 53: 4367-4372.
[24] Lee, J.W., Kim, J.K., Srinivasan, P., Choi, J., Kim, J.H., Han, S.B., Kim, D., Byun, M.W., (2009): Effect of gamma irradiation on microbial analysis, antioxidant activity, sugar content and color of ready-to-use tamarind juice during storage. LWT-Food Science and Technology, 42: 101-105.
[25] Li, H.Z, Zhou, W.J., Zhang, Z., Gu, H.H, Takeuchi, Y., Yoneyama, K., (2005): Effect of γ-radiation on development, yield and quality of microtubers *in vitro* in *Solanum tuberosum* L. Brief Communications, 49: 625-628.
[26] Manach, C., Scalbert, A., Morand, C., Remesy, C., Jimenez, L. (2004): Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition, 79: 727–747.
[27] Marques, V., Farah, A., (2009): Chlorogenic acids and related compounds in medicinal plants and infusions. Food Chemistry, 113: 1370–1376.
[28] Murashige, T., Skoog, F., (1962): A revised medium for rapid growth and bioassays for tobacco tissue cultures. Physiology Plantarum, 15: 473-497.
[29] Oufedjikh, H., Mahrouz, M., Amiot, M.J., Lacroix, M., (2000): Effect of gamma-irradiation on phenolic compounds and phenylalanine ammonia-lyase activity during storage in relation to peel injury from peel of *Citrus clementina* hort. Ex. tanaka. Journal of Agricultural and Food Chemistry, 48: 559-565.
[30] Pala-Paul, J., Copeland, L.M., Brophy, J.J., Goldsack, R.J. (2006): Essential oil composition of *Eryngium rosulatum* P.W. Michael ined.: a new undescribed species from eastern Australia. Biochemical Systematics and Ecology, 34: 796–801.
[31] Rao, R., Ravishankar, G.A., (2002): Plant cell cultures: Chemical factories of secondary metabolites. Biotechnolgy Advances, 20: 101–153.
[32] Sanada, T., (1986): Induced mutation breeding in fruit trees resistant mutant to black spot disease of Japanese pear. Symposia, 25: 87-108.
[33] Sattar, A., Atta, S., Akhtar, M.A., Wahid, M., Ahmad, B., (1991): Biosynthesis of ascorbic acid and riboflavin in radiated germinating chickpea. International Journal for Vitamin and Nutrition Research, 61: 149-154.
[34] Sattar, A., Neelofar, X., Akhtar, M.A., (1992): Radiation effect on ascorbic acid and riboflavin biosynthesis in germinating soybean. Plant Foods for Human Nutrition, 42: 305-312.
[35] Scalbert, A., Williamson, G., (2000): Dietary intake and bioavailability of polyphenols. Journal of Nutrition, 130: 2073S-2085S.
[36] Siddhuraju, P., Makkar, H.P.S., Becker, K., (2002): The effect of ionizing radiation on antinutritional factors and the nutritional value of plant materials with reference to human and animal food. Food Chemistry, 78: 187–205.
[37] Singleton, V.L., Rossi, J.A., (1965): Colorimetry of total phenolics with phosphormolybdic- phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16: 144-158.
[38] Štajner, D., Milošević, M., Popović, B., (2007): Irradiation effects on phenolic content, lipid and protein oxidation and scavenger ability of soybean seeds. International Journal of Molecular Sciences, 8: 618-627.
[39] Tambasco-Studart, M., Titiz, O., Raschle, T., Forster, G., Amrhein, N., Fitzpatrick, T.B., (2005): Vitamin B6 biosynthesis in higher plants. Proeedings of the National Academy of Sciences, 102: 13687–13692
[40] Tobback, P.P., (1977): Radiation chemistry of vitamins. pp. 187-220. In Elias, P.S., Cohen, A.J., (eds.): Radiation Chemistry of Major Food Components, Elsevier Science, New York.
[41] Variyar, P.S., Limaye, A., Sharma, A., (2004): Radiation-induced enhancement of antioxidant contents of soybean (*Glycine max* Merrill). Journal of Agricultural and Food Chemistry, 52: 3385-3388.
[42] Yermakov, A.I., Arasimov, V.V., Yarosh, N.P., (1987): Methods of biochemical analysis of plants. Leningrad: Agropromizdat, pp. 122-142 (in Russian).

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