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Proximate composition, phytochemical profile and free radical scavenging activity of radiation processed *Emblica officinalis*

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Abstract

Medicinal plants and their products are often prone to microbial contamination. Gamma irradiation is a well-recognized phytosanitary treatment for the elimination of bacteria, moulds and insects. The present study was carried out to see the effect of gamma radiation treatment on the proximate nutrients, ascorbic acid, tannins, saponins, flavonoids, phenolics and alkaloidal content, as well as on the DPPH radical scavenging activity of *Emblica officinalis*. The radiation treatment up to the dose level of 12 kGy showed increase in the levels of phenolics and flavonoids. No effect of irradiation was observed on the concentrations of saponins and alkaloids. Tannin content remained unaffected at low doses. Gamma irradiation also enhanced the DPPH scavenging activity and extraction yields of the methanol and aqueous extracts of the samples. The proximate analysis showed no significant effect on the levels of moisture, protein, fiber and carbohydrates. The crude fats increased with the increase in gamma irradiation dose. The data suggest that gamma irradiation up to 12 kGy could safely be used to sanitize the *Emblica officinalis* fruits and it may also be beneficial for enhancing the certain biological activities and phytochemicals.

Introduction

Recent worldwide penchant for the use of natural compounds has extremely augmented the importance of plant materials. They are getting more and more attention than ever as these have now numerous applications in industries like pharmaceutical, nutraceutical, food and cosmetic etc. *Emblica Officinalis* Gaertn is among the widely used medicinal plants throughout the world. It belongs to family Euphorbiaceae. The specie is found in Pakistan, Bangladesh, India, Sri Lanka, China and Malaysia (Bhattacharya *et al*., 1999; Scartezzini and Speroni, 2000; Khan, 2009). In Pakistan it is known as Amla and amlaki. It has small, fleshy and edible fruits. The fruits have astringent, sour and bitter taste. These are extensively used in pickles, chutneys, jams, jellies, vinegar and juices (Parmar and Kaushal, 1982). Traditionally the fruits are used as a tonic, laxative, anodyne, aphrodisiac, diuretic, antipyretic and rejuvenative. These are useful for the treatment of diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, peptic ulcer, erysipelas, leprosy, inflammations, dizziness, anemia, emaciation, hepatopathy, jaundice, strangury, diarrhoea, dysentery, hemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair (Parmar and Kaushal, 1982; Khan, 2009). The flowers of the plants are used as diuretic and for the treatment of typhoid and fever (Malik *et al*., 2010). The whole plant of *Emblica officinalis* is renowned for the treatment of cancer, lowering cholesterol and enhancing memory (Khan, 2009). Scientific studies on the plant showed that it has antioxidant, antipyretic, analgesic, hypolipidaemic, chemoprotective, cytoprotective, antimicrobial, antitussive, immunomodulatory and gastroprotective activities (Saeed and Tariq, 2007; Khan, 2009; Malik *et al.*, 2010; Gavatia *et al.*, 2012). Scientific investigation on the plant showed a wide spectrum of biologically active compounds (Liu *et al*., 2008; Khan, 2009; Arora *et al*., 2012; Gavatia *et al*., 2012).

A recent study conducted by Choudhary (2011) divulged a number of fungal species in *Emblica officinalis* Gaerth samples, of which the *Aspergillus flavus* was the most dominant. The investigation also disclosed the occurrence of aflatoxins in the plant materials. Likewise, Akhund *et al.* (2010) reported a total of 19 genera and 42 fungal species from the fruits of Amla. They described that the most common contaminating fungal species were from the genera

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of *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium* and *Curvularia*. Kumari *et al.* (2009) also affirmed a high level of microbial contamination in a Triphala, a mixture of *Emblica officinalis*, *Terminalia chebula* and *Terminalia bellirica*. The presence of microbial contaminants may affect the efficacy and stability of the active compounds. This may also lead to the spoilage of traditional herbal preparations and pharmaceutical drugs to which they are added. Further, the presence of pathogenic microorganisms in plant materials can precariously affect the human health.

Earlier R & D work has shown that the irradiation has a clear edge over other phytosanitary treatments, because of its usefulness in controlling a wide range of microorganisms and insects. The present study was undertaken to determine the effect of different doses of gamma irradiation on the chemical constituents and biological activity of the *Emblica officinalis*.

Materials and Methods

Plant samples

The dried fruits of *Emblica officinalis* Gaertn were purchased from the local market of Peshawar. The fruits were cleaned, cut into small pieces and ground in a grinder (Retch Muhle-Germany) and passed through the sieves of mesh size 30. The powdered samples were packed in clear polyethylene pouches and sealed with electric sealer PFS 300 (Ladder, China).

Irradiation

A Co-60 research gamma radiation source, ISSLEDIOVATE (former USSR), installed at NIFA Peshawar, was used for radiation. The plant samples were irradiated to dose levels of 3, 6, 9 and 12 kGy. The irradiation was carried out at ambient conditions (20-35°C, RH 40-85%). The dose rate was 0.76 kGy per hour, as determined with a Fricke dosimeter. After irradiation, the samples were stored at room temperature.

Preparation of extracts

The irradiated and control samples (50 g each) of *Emblica officinalis* were separately extracted in methanol and water (3 × 150 ml) using a Soxhlet extractor. All the extracts were filtered through Whatman No. 1 filter paper, combined and concentrated to dryness under reduced pressure at 45°C. The dry extracts obtained with each solvent were weighed. Extraction yields for each solvent were calculated by subtracting the dry weight of plant material residue after extraction from the weight of the original plant materials. The extracts were stored at 4°C until further processing. The extracts obtained in methanol were used for the estimation of total phenolics, flavonoids and for the free radical-scavenging assay.

Proximate analysis

The plant samples were analyzed in triplicate for moisture, crude protein, crude fat, fiber and ash on a dry weight basis, according to AOAC (1990). The moisture was determined in a drying oven at 105°C until constant weight. Analysis of crude fat was carried out using petroleum ether (bp. 40–60°C) in a Soxtec system HT (Tecator, Sweden). Determination of crude protein (% N x 6.25) was performed by the micro-Kjeldahl method. Ash contents were estimated by heating the samples at 550°C and crude fiber by digestion with acid and alkali using Fibertec system (Tecator, Sweden). Carbohydrates (%) contents were estimated by using a difference method (Odhav et al., 2007), by subtracting the sum of the percent of moisture, fat, protein and ash from 100. The analysis for ascorbic acid was conducted by titration method using 2,6-dichlorophenol-indophenol.

Estimation of phenolic content

The total phenolic contents of plant samples were estimated by the Folin-Ciocalteau colorimetric method (Khattak *et al*., 2008). In a test tube, 200 µl of the filtered methanolic extract was added to 4 ml of 2% aqueous sodium carbonate solution and mixed thoroughly. Then 200 µl of 50% Folin-Ciocalteau reagent was added to the mixture. The mixture was allowed to stand for 1 hour shaking and absorbance of the green-blue complex was measured at 750 nm in a spectrophotometer against blank. The results were expressed as milligram of gallic acid equivalents per gram (mg/g) of the dry extract.

Determination of alkaloids

The alkaloidal contents were determined using the method of Harborne (1973). Five gram of the powdered sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The solution was covered and incubated at room temperature for 3 hours. Afterward, the solution was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract and the precipitates were collected and washed with dilute ammonium hydroxide and then filtered. The residues were dried and weighed.

Determination of saponins

The method used for saponin estimation was that of Obadoni and Ochuko (2002). The plant sample (8

g) was put into a flask and 100 ml of 20% aqueous ethanol was added. The sample was heated over a water bath at 55°C for 4 hours with incessant stirring. The mixture was filtered and the re-extracted with 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a separating funnel (250 ml). Thirty ml of diethyl ether was added and shaken vigorously. The ether layer was discarded and afterward 60 ml of n-butanol was added to the aqueous layer. The process was repeated. The collective n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight.

Determination of tannins

Tannin contents were determined using the method of Van–Burden and Robinson (1969). Two grams powdered sample was weighed into a 100 ml flask. 100 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. The solution was filtered and 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M ferric chloride in 0.1 N hydrochloric acid and 0.008 M potassium ferrocyanide. The absorbance of the solution was measured at 630 nm within 10 min.

Estimation of total flavonoids

Flavonoids contents were determined by following the method of Aiyegroro and Okoh (2010). One ml of the methanolic extract was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6ml of distilled water and kept at room temperature for 30 minutes. The absorbance of the mixture was measured at 420 nm with UV visible spectrophotometer. Quercetin was used as standard (0 – 1 mg/ml). Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound).

Determination of DPPH radical scavenging activity

The antioxidant activity of the samples was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Khattak *et al*., 2008). Eighty µl of the sample at various concentrations was added to 2 ml of DPPH in methanol solution (60 µM) in a test tube and shaken vigorously. After incubation at 37 °C for 40 minutes in the dark, the absorbance of each solution was estimated at 517 nm. The corresponding blank (control) reading was also taken. The results obtained from the radical scavenging experiments were expressed as EC_{50} values. EC_{50} value is the extract concentration at which DPPH radicals were reduced by 50% and calculated from the linear regression analysis.

Statistical analysis

All determinations were obtained from triplicate measurements and results were expressed as means ± standard deviations. The data were analyzed using one-way ANOVA and least significant difference tests for the mean differences between controls and irradiated (3–12 kGy) samples for all the parameters. The Statistical Package for Social Sciences (SPSS) for Windows version (14.0) was used to analyze the data (SPSS Inc., Chicago, IL). Statistical significance was declared at $p < 0.05$.

Results and Discussion

The effect of gamma irradiation at various dose levels on the proximate composition is given in Table 1. The control samples of the plant showed moisture (10.8%), ash (10.0%), crude protein (9.1%), fat (3.8%), fiber (11.8%) and carbohydrate (54.5%). An earlier study (Krishnaveni and Mirunalini, 2011) revealed that the fresh fruit of the plant had 81.2% moisture, 0.5% protein, 0.1% fat, 0.7% ash, 3.4% fiber and 14.1% carbohydrate. Gavatia et al. (2012) reported 6.4% ash in the fruit of the plant. In general, no substantial changes in the proximate constituents amongst the samples were observed. The moisture, protein, fiber and carbohydrate contents of Emblica officinalis remained unchanged ($p > 0.05$) following gamma irradiation up to the dose level of 12 kGy. However, irradiated samples showed significantly higher fat contents ($p < 0.05$)., and the increase was found to be largely dose dependent The complexes fat contents seem to be converted into smaller fragments after gamma irradiation. Ash contents were found same for control and irradiated samples except for the sample irradiated at the dose of 12 kGy. There is no information available in the literature on the effect of ionizing radiation on the proximate nutrients of the plant. However, our previous study (Khattak *et al*., 2009) on the rhizomes of Nelumbo nucifera showed nearly similar results. No considerable changes were noted for the proximate constituents of the plant samples up to the dose levels of 6 kGy.

The ascorbic acid contents of the fruits of Emblica officinalis were determined on dry weight basis and results are presented in Fig. 1. The vitamin C content of control samples was 2035.7 ± 92.7 mg/100 g. Earlier, Khan (2009) reported 600 mg/100 g vitamin C in the fresh pulp of the fruit. After gamma radiation processing, the vitamin C contents were found to

Table 1. Effect of different doses of gamma irradiation on the proximate composition of *Emblica officinalis*

Nutrients	Radiation doses (kGy)				
	Control	3	6	9	12
Moisture (%)	10.8 ± 1.4 a	10.1 ± 0.7 a	10.8 ± 1.4 a	11.1 ± 1.6 a	10.3 ± 1.4a
Protein (%)	9.1 ± 0.5a	9.3 ± 0.2a	9.5 ± 0.2a	8.8 ± 0.7a	8.9 ± 0.9a
Fat (%)	3.8 ± 0.1a	4.1 ± 0.1b	4.2 ± 0.0b	4.4 ± 0.0c	4.7 ± 0.1d
Ash (%)	10.0 ± 0.4a	10.2 ± 0.3a	10.3 ± 0.4a	10.5 ± 0.4a	11.3 ± 0.1b
Fibre (%)	11.8 ± 0.2a	11.5 ± 0.2a	11.6 ± 0.1a	11.3 ± 0.3a	11.3 ± 0.3a
Carbohydrate (%)	54.5 ± 1.1a	54.9 ± 1.3a	53.6 ± 1.8a	53.9 ± 0.4a	53.4 ± 1.0a

The values presented in this table are on dry weight basis. Values are means ± standard deviations of three determinations. Means with different letters within the same row are significantly different ($p < 0.05$).

be decreased considerably ($p < 0.05$). The contents were 2020.0, 1901.7, 1863.7 and 1672.7 mg/100 g for samples treated with 3, 6, 9 and 12 kGy radiation doses, respectively. Our earlier study (Khattak *et al*., 2009) on Nelumbo nucifera displayed a significant decrease in the vitamin C content of irradiated samples. Similarly, a study conducted by Wen *et al.* (2006) showed decreases in the vitamin C content following radiation for lycium fruit. They found no vitamin C after applying 8 and 14 kGy radiation doses. In contrast, Hajrae *et al*. (2007) reported that vitamin C content remains unchanged after radiation at dose levels of 1 and 2 kGy in minimally processed green gram and garden pea sprouts.

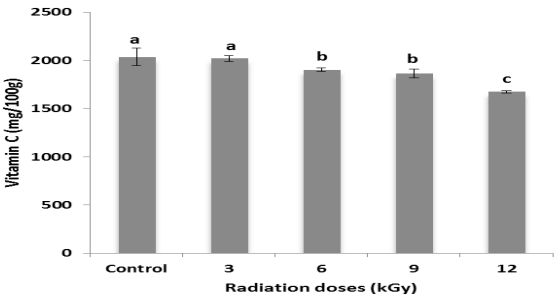

Figure 1. Effect of different doses of gamma radiation on the vitamin C content of *Emblica Officinalis* fruit. Values are means of triplicate determinations (n = 3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

The dry weight yields of both methanolic and aqueous extracts of Emblica officinalis were determined and given in Fig 2. The non-irradiated methanol extract of the plant showed 26.7% dry weight yield. In contrast to our results, Gavatia *et al.* (2012) reported comparatively low extraction yields of the plant in alcohol (15.56%). In general, radiation treatment resulted in significant increases in the extraction yields and the increase in dry weight yields was found to be dose dependent ($p < 0.05$). The samples treated with gamma rays at doses of 3, 6, 9 and 12 kGy showed 29.0, 30.7, 33.7 and 34% extraction yields, respectively. The increase in extraction yields could be attributed to easy release of soluble ingredients from degraded complex forms after radiation processing. The water extract of control sample showed the lowest extraction yield (30.7%). Previously, Gavatia *et al*. (2012) reported 24.35% yield for water extract of the plant. The data showed that the dry weight yields of the aqueous extracts of irradiated samples were significantly high ($p < 0.05$) as compared to that of the control sample. Highest extraction yield was recorded for sample treated with 12 kGy radiation dose (42.3%). The results of the present study came in consistence with our other previous investigation (Khattak *et al*., 2009), that showed significant increases in the extraction yields of methanolic and acetone extracts of lotus rhizome following gamma-radiation treatment even at comparatively low doses (1, 2, 4 and 6 kGy). Comparable results demonstrating upsurge in the extraction yields have been also reported by Kim *et al*. (2000). They observed that the extraction yield in Korean medicinal herbs, using various solvents, increased by 5–30% with a 10 kGy of gamma radiation dose.

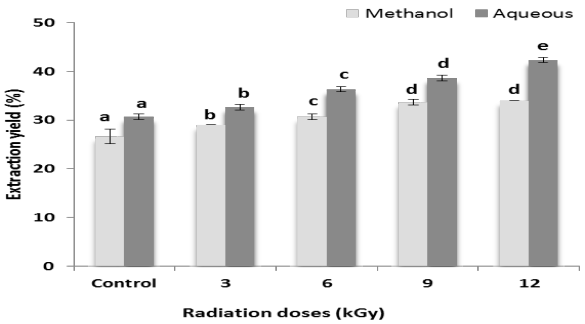

Figure 2. Effect of different doses of gamma radiation on the extraction yields of *Emblica Officinalis* fruit. Values are means of triplicate determinations (n = 3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

Phytochemical analysis of the fruits of *Emblica officinalis* showed the presence of tannins, saponins, flavonoids, alkaloids and phenols (Table 2). The levels of tannin, saponin and alkaloidal contents of control

Table 2. Effect of different doses of gamma irradiation on the phytochemicals of *Emblica officinalis*

Phytochemicals	Radiation doses (kGy)				
	Control	3	6	9	12
Phenolic	193.7 ± 7.0a	215.7 ± 5.7b	213.7 ± 1.5b	228.3 ± 3.1c	236.0 ± 0.0d
Tannins	199.0 ± 6.6a	203.0 ± 4.0a	207.0 ± 7.2a	207.3 ± 3.2a	221.7 ± 2.1b
Saponin	0.4 ± 0.0a	0.4 ± 0.1a	0.4 ± 0.0a	0.5 ± 0.0a	0.5 ± 0.0a
Flavonoid	30.0 ± 2.6a	33.2 ± 1.5a	38.6 ± 0.6b	43.1 ± 1.5c	47.8 ± 1.2d
Alkaloids	3.5 ± 0.2a	3.6 ± 0.3a	3.8 ± 0.1a	3.7 ± 0.1a	3.8 ± 0.2a

The values are means ± standard deviations of three determinations. Means with different letters within the same row are significantly different ($p < 0.05$).

samples were 199.0, 0.4 and 3.5 mg/g, respectively. The concentrations of these phytochemicals remained unaffected ($p > 0.05$) up to the gamma radiation dose of 12 kGy. No information is available on the effect of gamma irradiation on the tannin, saponin and alkaloidal contents of the plant. Though, our recent study (Khattak, 2012a) on seven indigenous medicinal plants clearly exhibited the increase in the alkaloidal contents after radiation processing for *Eruca sativa*, *Tagetes patula*, *Ocimum sanctum*, *Zizyphus nummularia* and *Morus nigra*. In case of saponins, all plants except *Carum copticum* showed increase in the contents after radiation treatment. The tannin content of radiated samples of *Zizyphus nummularia*, *Tagetes patula*, *Morus nigra*, *Bryophyllum pinnatum* remained unaffected up to the dose level of 12 kGy, while these substances increased in irradiated samples of *Ocimum sanctum*, *Eruca sativa* and *Carum copticum*. Difference in the effects of gamma irradiation on the phytochemicals may be due to differences in structure of the compounds and types of the plants. The phenolic contents of the control samples were 193.7 mg/g. In the past, Charoenteeraboon *et al.* (2010) reported 34.2 g/100 g polyphenolic contents in the water extract of the fruit of *Emblica officinalis*. All the irradiated samples showed significantly higher phenolic contents ($p < 0.05$). The concentrations of phenolics were 215.7, 213.7, 228.3 and 236.0 mg/g for 3, 6, 9 and 12 kGy treated samples, respectively. The increase in phenolic contents could be attributed to easy release of active ingredients from their radiation degraded complex forms. Earlier, Kumari *et al*. (2009) reported increase in the phenolic contents (2.16 to 2.87 times) of irradiated *Triphala samples* (a mixture of *Emblica officinalis*, *Terminalia chebula* and *Terminalia bellirica*). Our earlier study (Khattak *et al*., 2008) on Nigella sativa seed showed that the phenolic content in radiation processed samples increased with the increases in radiation doses (2–16 kGy) for acetone extract. However in case of water extracts, phenolic content showed a slight decrease at 16 kGy treated sample. In contrast to this, Koseki *et al*. (2002) reported a significant decrease in the amount of total phenolic compounds in dehydrated rosemary after irradiation at doses between 10 and 30 kGy, with respect to controls. The difference in the effect of radiation on total phenolic content may be due to plant type, geographical locations, environmental conditions, state of the sample (solid or dry), phenolic content opus, extraction solvent, extraction techniques, temperature, dose of gamma irradiation, etc. No significant differences ($p > 0.05$) in the flavonoid contents were observed for control (30.0 mg/g) and 3 kGy irradiated sample (33.3 mg/g). Earlier, Gavatia *et al*. (2012) reported 24.2 mg/g of flavonoids in the fruit of the plant. Samples treated with higher doses showed gradual increase in the flavonoid content. The levels of flavonoids were 33.2, 38.6, 43.1 and 47.8 mg/g in 3, 6, 9 and 12 kGy irradiated samples. Observed increase in phenolic and flavonoid contents may be beneficial for antioxidant properties of the fruit of *Emblica officinalis*.

The free radical-scavenging activity of the methanolic extracts of the plants was analyzed by using DPPH radical. The results obtained from these experiments are expressed as EC_{50} values (µg/ml) and presented in Fig. 3. Low EC_{50} is sign of high DPPH scavenging activity. The methanolic extract of the control sample exhibited the lowest scavenging activity with the highest EC_{50} value of 74.3 µg/ml. Charoenteeraboon *et al.* (2010) studied the DPPH scavenging activity of the water extract of the fruit of *Emblica officinalis* and reported the EC_{50} value of 51.3±16.5 µg/ml. The EC_{50} values were found decreased for radiation treated samples, as compared to that of the control samples, indicating enhancement in free radical activity after radiation treatment. The values were 68.4, 59.7, 53.7 and 48.0 µg/ml for samples irradiated at 3, 6, 9 and 12 kGy doses, respectively. It was clear from the data that radiation treatment has significantly increased ($p < 0.05$) the scavenging activity. Eearlier research studies showed different results for the effect of gamma irradiation on the antioxidant properties of other plants. Kumari *et al.* (2009) reported that the water extracts of irradiated samples of Triphala, a mixture of *Emblica officinalis*, *Terminalia chebula*, and *Terminalia bellirica* showed linear increase in

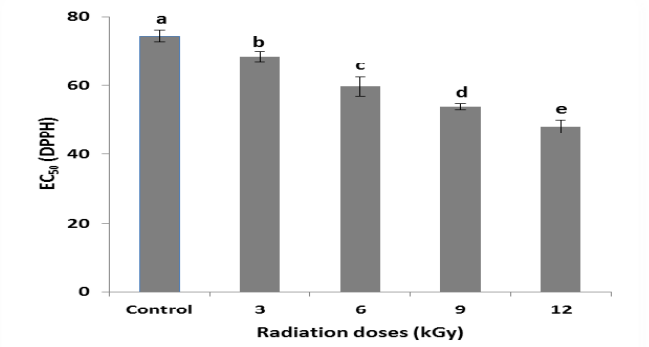

Figure 3. Effect of different doses of gamma radiation on the EC50 value (µg/ml) of the DPPH scavenging activity of *Emblica Officinalis* fruit. Values are means of triplicate determinations (n = 3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

antioxidant properties with the increase in radiation doses up to 25 kGy. Our previous studies (Khattak *et al.*, 2008; Khattak *et al*., 2009; Khattak and Simpson, 2010; Khattak, 2012b) also showed increase in DPPH scavenging activity of *Nelumbo nucifera*, *Nigella sativa*, *Glycyrrhiza glabra* and *Fagonia arabica* after radiation processing. Conversely, Lampart-Szczapa *et al.* (2003) explored that increased doses of radiation decreased the antioxidant effects of lupin seed extracts. Byun *et al*. (2002) detected no significant changes in the scavenging abilities of control as well as 5, 10 and 20 kGy irradiated soybean samples.

Conclusion

There is a growing scientific interest in the influence of irradiation processing on the chemical constituents, biological activities and physical characteristics of the treated plant materials. The present study showed that gamma irradiation of *Emblica officinalis* ensued increase in the levels of certain phytochemicals, which may be beneficial. In addition, the free radical scavenging activity was enhanced in all the radiation treated samples up to the dose levels of 12 kGy. In short, the study suggests that gamma irradiation treatment is effective for quality improvement of the plant materials.

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