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Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella staiva* seed

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ABSTRACT

In the present study, the radiation processing of *Nigella sativa* seed samples was carried out at dose levels of 2, 4, 8, 10, 12 and 16 kGy. The extraction yield, total phenolic content and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity of both control and irradiated samples extracted in acetone, methanol and water were assessed. The results showed that the extraction yields increased with an increase in radiation dose for all the test solvents. At 16 kGy the increases were 3.7%, 4.2%, 5.6% and 9.0% for hexane, acetone, water and methanol extracts, respectively. The phenolic content in the acetone extract was found to be increased from 3.7 for the control sample to 3.8 mg/g for the 16 kGy radiation-processed sample. No significant change was observed for the phenolic content of the methanolic extract, while the aqueous extract showed a decrease at dose levels of 12 and 16 kGy. In the control samples, the DPPH radical-scavenging activity was 79.4%, 79.1% and 92.0% for water, acetone and methanol extracts, respectively, at 5 mg/ml concentration. Gamma irradiation enhanced the scavenging activity in acetone and methanol extracts by 10.6% and 5.4%, respectively, at 16 kGy. In summary, gamma irradiation increased the extraction yield and total phenolic content, as well as enhancing the free radical-scavenging activity. In addition, the type of solvent used for extraction also affected the impact of irradiation on antioxidant activity and total phenolic content of *N. sativa* seed.

1. Introduction

Nigella sativa is an annual herbaceous plant which belongs to family Ranunculaceae. The plant commonly grows in the Middle East, Eastern Europe and Western and Central Asia. The English name of the plant is black cumin. In Pakistan, it is commonly known as Kalonji. The *N. sativa* seeds are black in colour and taste slightly bitter. They are frequently used as a spice. They are added as a flavouring agent and preservative to bread, pickles and other dishes (Aljabre et al., 2005). The paste of the seeds along with honey is also used in bakery products and cheese. Nutritional investigations on the seeds showed that they are a good source of potassium, phosphorus, sodium, iron, zinc, calcium, magnesium, manganese and copper (Al-Jassir, 1992). The seeds of the plant are extensively used in traditional medicines in Pakistan, India, China, Saudi Arabia, and the countries bordering the Mediterranean Sea, for the treatment of asthma, cough, bronchitis, headache, rheumatism, fever, kidney and liver disorders, influenza, eczema, and as a diuretic, lactagogue, carminative and vermifuge (Randhawa & Al-Ghamdi, 2002). Recent scientific investigations on the black cumin seeds and its oil indicated a number of pharmacological activities for the plant which include anticarcinogenic (Rooney & Ryan, 2005), antiulcer (Kanter, Demir, Karakaya, & Ozbek, 2005), antibacterial (Morsi, 2000), antifungal (Khan, Ashfaq, Zuberi, Mahmoud, & Gilani, 2003), hepatoprotective (Daba & Abdel-Rahman, 1998), anti-inflammatory, antipyretic and analgesic (Al-Ghamdi, 2001). The plant's active principles contain thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellone, nigellimine-*N*-oxide, nigellidine and α-hedrin (El-Tahir & Bakeet, 2006; Randhawa & Al-Ghamdi, 2002).

However, during processing, storage, transportation and marketing, *N. sativa* seeds are susceptible to microbial contamination, which can result in quality deterioration and economic loss. The contaminated materials may also cause spoilage of the pharmaceuticals and food to which they are added. Al-Jassir (1992) reported that the total aerobic bacterial count was 7×10^7 cfu/g and the yeast and mould counts were 4×10^2 cfu/g in *N. sativa*. El-Kady, El-Maraghy, and Eman (1995) found the presence of high levels of mycotoxins in black cumin samples. Our previous studies

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also indicated the microbial contamination level was above the international limits in commercially available seed samples of the plant (Khattak, Ihsanullah, & Ali, 2004; UNIDO, 1984).

Gamma irradiation as a phytosanitary treatment of food and herbal materials is increasingly recognised throughout the world. It improves the hygienic quality of various foods and herbal materials and reduces the losses due to microbial contamination and insect damage (Farkas, 1998; IAEA, 1992). It reduces the reliance on chemical fumigants and preservatives currently used by the food and pharmaceutical industries. The chances of recontamination are also reduced, as it can be done after packaging.

Gamma irradiation has been successfully used for the decontamination of *N. sativa* seeds by Farrag, El-Bazza, El-Fouly, and El-Tablawy (2000), and Zeinab, Hala, Mohie, and Seham (2001). Our study also showed that gamma irradiation is good phytosanitary treatment of *N. sativa* seed and that it does not have any detrimental affect on the antimicrobial activity of the plant (Khattak, Ihsanullah, & Ali, 2004). There is a growing scientific interest in the influence of irradiation processes on antioxidant activity and the compounds responsible for such activity. Several studies on plant materials showed that gamma irradiation does maintain or enhance antioxidant properties (Byun, Son, Yook, Jo, & Kim, 2002; Jo, Son, Lee, & Byun, 2003). However, some studies have shown that gamma irradiation decreased the antioxidant properties (Ahn et al., 2005; Lampart-Szczapa, Korczak, Nogala-Kalucka, & Zawirska-Wojtasiak, 2003) in plant materials.

Scientific investigations on *N. sativa* showed that it has strong antioxidant activity (Burits & Bucar, 2000; Machmudah, Shiramizu, Goto, Sasaki, & Hirose, 2005; Ramadan, Kroh, & Morsel, 2003; Thippeswamy & Naidu, 2005; Yu, Zhou, & Parry, 2005). However, to the best of our knowledge, the effect of gamma irradiation on the free radical-scavenging activity and phenolic content of *N. sativa* seed has not been investigated. The present study was undertaken to investigate the effect of gamma irradiation at various dose levels on the free radical-scavenging activity, total phenolic content and extraction yield of *N. sativa* seed in acetone, water and methanol extracts.

2. Materials and methods

2.1. Materials

The seed samples of *N. sativa* were purchased from the local market in Peshawar, Pakistan. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), gallic acid and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co., (St. Louis, MO). All the solvents and other chemicals used were of analytical grade from Sigma and Merck (Darmstadt, Germany).

2.2. Sample preparation

The seeds were cleaned and ground in a grinder (Retch, Haan, Germany). The ground material was passed through a 30-mesh sieve and aerobically packed (100 g each) in low density polyethylene pouches (thickness, 0.1 mm).

2.3. Gamma irradiation

The polyethylene packed samples of *N. sativa* were irradiated using gamma rays from a cobalt-60 radiation source (Issledovatel, Russia) at NIFA Peshawar in January 2007. The dose rate was 0.96 kGy per hour, as determined with a Fricke dosimeter. The dose levels applied were 2, 4, 6, 8, 10, 12 and 16 kGy. At each dose level, three pouches containing 100 g of seeds were irradiated. The irradiations were carried out at room temperature.

2.4. Preparation of plant extracts

The irradiated and control samples (100 g each) of *N. sativa* were separately extracted in hexane, acetone, methanol and water using a Soxhlet extractor. All the extracts were filtered through Whatman No. 1 filter paper and concentrated under vacuum at 45 °C. The dry extract obtained with each solvent was weighed. Extraction yields for each solvent were calculated by subtracting the dried weight of plant material residues after extraction from the weight of the original plant material. The extracts thus obtained in acetone, methanol and water were used for the estimation of total phenolic content and for the free radical-scavenging assay. The extracts were stored at 4 °C until further processing.

2.5. Determination of total phenolic content

The total phenolic content of the acetone, methanol and water extracts of irradiated and control samples of the plant were determined using the Folin–Ciocalteu reagent. In a test tube, 100 μl of the extract (5–0.1 mg/ml) was added to 2 ml of 2% aqueous sodium carbonate solution and mixed well. Then 100 μl of 50% Folin–Ciocalteu reagent was added to the mixture. After shaking, it was kept for 1 h and the absorbance of the green-blue complex formed was measured at 750 nm against a blank control. All spectrophotometric work was performed using an Ultraspec 3000 UV/visible spectrophotometer (Pharmacia, Uppsala, Sweden). The total phenolic contents were calculated on the basis of a calibration curve of gallic acid. The results were expressed as gallic acid equivalents (mg) per gram of dry weight of the extracts.

2.6. Free radical-scavenging assay with DPPH

The antioxidant activity of the irradiated and non-irradiated control samples of the methanolic, aqueous and acetone extracts were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. DPPH is a stable free radical with a purple colour and has a maximum absorption at 517 nm. The free radial-scavenging assay is based on the decolouration of the compound when reduced by a free radical scavenger. The assay was carried out according to the method described by Rai, Wahile, Mukherjee, Saha, and Mukherjee (2006), with slight modifications. About 100 μl of each extract (from 100 to 2 mg/ml) was added to 1.9 ml of DPPH in methanol solution (150 μM) in a test tube and shaken vigorously. After incubation at 37 °C for 35 min in the dark, the absorbance of each solution was determined at 517 nm. The corresponding blank (control) reading was also recorded. The free radical-scavenging activity was expressed as percentage scavenging of the DPPH by the plant extracts and calculated as %DPPH radical-scavenging activity = (absorbance of control − absorbance of sample) × 100/absorbance of sample.

For better comparison of the DPPH-scavenging activity of the *N. sativa* seed extracts, with respect to radiation doses, the results obtained from the DPPH radical experiments were also expressed as EC_{50}. The EC_{50} value is the extraction concentration at which 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals are reduced by 50%. A low EC_{50} value is indicative of strong DPPH-scavenging activity. This is calculated from linear regression analysis.

2.7. Statistical analysis

All determinations were obtained from triplicate measurements and results were expressed as mean ± standard deviation. The data were analysed using one-way ANOVA and least significant difference tests for the mean differences between controls and irradiated (2–16 kGy) samples for all the parameters. The

Statistical Package for Social Sciences (SPSS) for Windows version (14.0) was used to analyse the data (SPSS Inc., Chicago, IL). Statistical significance was declared at $p < 0.05$, or mentioned otherwise.

3. Results and discussion

The seed samples of *N. sativa* were exposed to gamma radiation at dose levels of 2, 4, 8, 10, 12 and 16 kGy. The effect of irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of the seed samples of *N. sativa* were studied for all irradiated and control (non-irradiated) samples.

3.1. Extraction yields

The extraction yields of *N. sativa* seed in hexane, acetone, methanol and water were determined and are shown in Table 1. Solvent extraction significantly affected the dry weight yield in control samples of the plant. Hexane extracts showed the highest extraction yield (31.9%). While, non-irradiated control samples extracted with acetone, methanol and water gave extract yields of 24.6%, 28.7% and 25.4%, respectively. Radiation treatment resulted in significant increases in the extraction yields for all the test solvents ($p < 0.05$). The hexane extract showed a linear increase in the dry weight with increase of the gamma radiation dose (Table 1). Similarly, the extraction yield in acetone extract was increased from 24.6% (control) to 28.8% for the 16 kGy radiation-processed sample ($p < 0.05$). The dry weight yield in the water extract for samples irradiated at 2–4 kGy, were found to be the same as that of the control ($p > 0.05$), while it increased to 31.0% at higher doses (16 kGy). The methanol extracts also showed increased yields after exposure to radiation. The increase in the dry weights of extracts following irradiation might be due to degradation of some high molecular weight components, and changing these components from non-soluble to soluble ones in the test solvents. The measured effect of irradiation was stronger in the methanolic extraction, which is able to extract both polar and semipolar compounds. Overall, the results show that gamma irradiation up to 16 kGy is an effective method for enhancing extract yields in *N. sativa*.

The increase in extraction yields with radiation treatment has also been reported by Huang and Mau (2007). Similarly, Kim, Yook, and Byun (2000) found an increase in the extraction yields after treating medicinal herbs with gamma irradiation. They observed that the total extraction yield in Korean medicinal herbs using various solvents, increased by 5–30% with a 10 kGy dose of gamma irradiation. Our study showed that at 16 kGy, increases in extraction yield were 3.7%, 4.2%, 9.0% and 5.6% for hexane, acetone, methanol and water, respectively (Table 1). The difference in increase in extraction yields, as compared to that reported in the literature, may be due to different chemical composition of the plants.

Table 1
Effect of gamma irradiation on the extraction yield of *Nigella sativa* seed

Radiation dose (kGy)	Extraction yield (%, w/w)			
	Hexane	Acetone	Methanol	Water
0	31.9 ± 0.8^{a}	24.6 ± 1.1^{a}	28.7 ± 0.4^{a}	25.4 ± 0.4^{a}
2	32.0 ± 0.4^{a}	25.7 ± 1.1^{a}	29.0 ± 0.2^{a}	25.2 ± 0.5^{a}
4	33.4 ± 0.5^{ab}	26.0 ± 0.8^{a}	29.8 ± 0.6^{ab}	25.2 ± 0.3^{a}
8	33.7 ± 0.8^{b}	26.5 ± 0.6^{ab}	31.1 ± 1.0^{b}	27.0 ± 0.7^{ab}
10	35.0 ± 0.6^{b}	26.5 ± 0.6^{ab}	33.0 ± 0.4^{c}	27.9 ± 0.8^{b}
12	35.5 ± 0.7^{b}	28.4 ± 0.9^{c}	37.5 ± 0.8^{d}	29.0 ± 1.0^{b}
16	35.5 ± 0.6^{b}	28.8 ± 0.4^{c}	37.7 ± 0.5^{d}	31.0 ± 0.4^{c}

Each value is expressed as mean ± standard deviation($n = 3$). Means with different superscript letters within the same column are significantly different ($p < 0.05$).

3.2. Total phenolic content

Phenolic compounds are hydroxylated derivatives of benzoic and cinnamic acids and contribute to overall antioxidant activities in the plants. The total phenolic content of irradiated and non-irradiated samples of *N. sativa* in different solvents was determined using the Folin–Ciocalteu's phenol reagent. The results are expressed as mg equivalents of gallic acid/g dry weight of extract and given in Fig. 1. Solvent extraction significantly affected the concentration of total phenols in control and irradiated *N. sativa* seed. In both cases, the concentration decreased in the order: methanol > acetone > water. The phenolic contents of the control (non-irradiated) samples were found to be 4.1, 3.7 and 3.6 mg/g for methanol, acetone and water extracts, respectively. It can be inferred from these results that methanol is the most efficient solvent for extracting phenolic compounds from control *N. sativa* seed.

For radiation-processed samples, the data (Fig. 1) showed significant ($p < 0.01$) increases in the total phenolic contents of irradiated acetone extracts, as compared to that of the control, increasing to 3.8 mg/g for a gamma radiation dose of 16 kGy. It seems that 2–16 kGy of irradiation might induce some chemical reactions in components of *N. sativa* seed, which possibly degrade or decompose large molecules into small phenolic molecules, which are readily soluble in acetone and may also be beneficial for the antioxidant properties of the plant seeds. The differences in total phenolic content of the control and radiation-processed seed samples were statistically insignificant for the methanolic extract. No significant changes in phenolic contents were observed in the water extract following 2, 4, 6, 8 and 10 kGy gamma irradiation. However, the water extract showed a decrease in the phenolic contents at 12 (3.5 mg/g) and 16 kGy (3.5 mg/g) radiation doses ($p < 0.05$).

There is no information available in the literature on the effect of ionising radiation on the phenolic content of *N. sativa* seed. However, for other plant materials, diverse effects of radiation on the phenolic content have been reported. Variyar, Bandyopadhyay, and Thomas (1998) found increased amounts of phenolic acids in irradiated cloves and nutmeg. Harrison and Were (2007) also reported increases in total phenolic content of gamma-irradiated

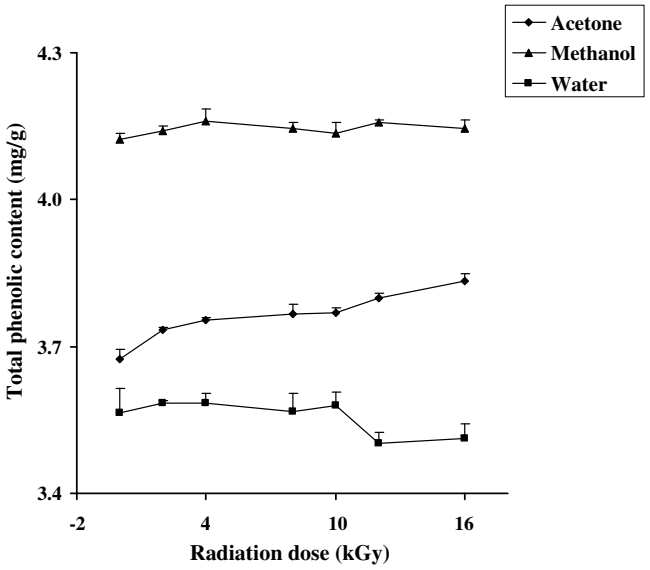

Fig. 1. Effect of gamma irradiation on the total phenolic content (mg/g of dry weight of extract) of *Nigella sativa* seed in acetone, methanol and water extracts. Each value is expressed as mean ± standard deviation ($n = 3$). The vertical bars represent the standard deviation for each data point.

almond skin extract, as compared to the control samples. Similarly, Huang and Mau (2006) reported a higher content of tocopherols in irradiated than in non-irradiated lyophilised mushrooms. These increases in phenolic contents were associated with the degradation of tannins (Variyar, Bandyopadhyay, & Thomas, 1998) and changes in the conformation of the molecules (Topuz & Ozdemir, 2004), as a result of the irradiation treatment. In contrast, Koseki et al. (2002) reported a decrease in the amount of total phenolic compounds in dehydrated rosemary after irradiation doses of 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 and environmental conditions, state of the sample (solid or dry), phenolic content composition, extraction solvent, extraction procedures, temperature, dose of gamma irradiation, etc.

3.3. Free radical-scavenging activity

Free radicals are extremely reactive species and are known to damage proteins, cause breakdown of DNA strands, initiate the peroxidation of various compounds and thus lead to many health problems and degenerative diseases, such as cancer, inflammation, atherosclerosis and accelerated ageing. Plants are well recognised for their potential antioxidants constituents, which include flavonoids, tannins and lignin etc. These compounds therefore play a significant role in health promotion.

The radical-scavenging activity of the irradiated and control seed samples were analysed in acetone, methanol and water extracts, using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The reduction in the DPPH concentration is a measure of scavenging activity. For all the control samples, the scavenging activity was found to increase ($p < 0.05$) with concentration between 0.1 and 5 mg/ml (Figs. 2–4). A comparison of the three solvents showed differences in scavenging activity at all concentrations, in the order: methanol > water > acetone. At 5 mg/ml concentration, the DPPH-scavenging activity were 92.0%, 79.4% and 79.1% for methanol, water and acetone extracts, respectively. The high scavenging ability of the methanolic extract can be correlated to the highest phenolic content among the three different extracts of *N. sativa* seed.

After the application of gamma radiation at doses 2, 4, 8, 10, 12 and 16 kGy, the pattern of change in scavenging activity as a function of concentration was similar in all the extracts to that in the control samples (Figs. 2–4). For the control samples, the acetone extract showed a lower scavenging activity than methanol and water extracts for the whole range of concentrations analysed, but, interestingly, following in radiation a significant increase was observed in the free radical-scavenging activity (Fig. 2). At 16 kGy radiation dose and 5 mg/g concentration, the activity was increased by 10.6% (89.7 mg/ml), compared to that of the control. The irradiation-induced increase ($p < 0.01$) in free radical-scavenging activity of acetone extracts (5 mg/g) at doses of 8, 10, 12 and 16 kGy could be the result of high total phenolic accumulation in the acetone extracts (Fig. 1). No significant difference was found in the scavenging activity of control and radiation-processed samples at 2, 4, 8 and 10 kGy (Fig. 3) for the methanol extract at 5 mg/ml concentration, while the activity was found to be increased at radiation doses of 12 (97.3%) and 16 kGy (97.4%). Despite the fact that the phenolic content remained unaffected in the methanolic extract following radiation, surprisingly the free radical-scavenging activity of the extract was increased at higher doses of radiation. This might be due to

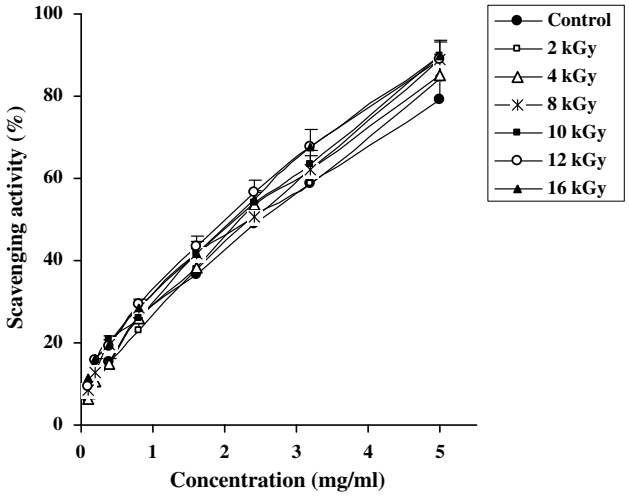

Fig. 2. Effect of gamma irradiation on the scavenging activity of acetone extracts of *Nigella sativa* seed on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Each value is expressed as mean ± standard deviation ($n = 3$). The vertical bars represent the standard deviation for each data point.

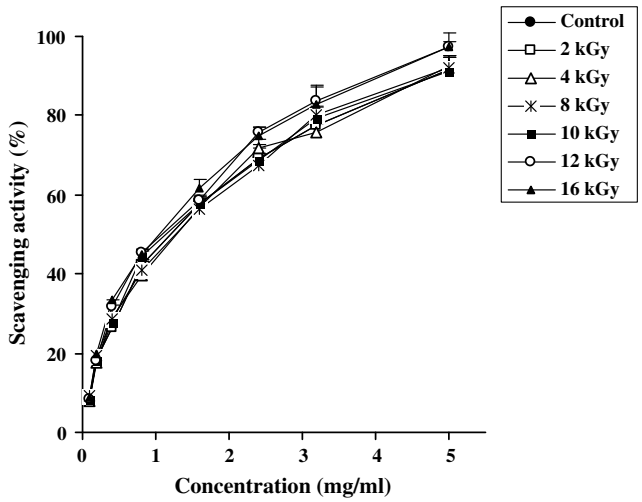

Fig. 3. Effect of gamma irradiation on the scavenging activity of methanol extracts of *Nigella sativa* seed on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Each value is expressed as mean ± standard deviation ($n = 3$). The vertical bars represent the standard deviation for each data point.

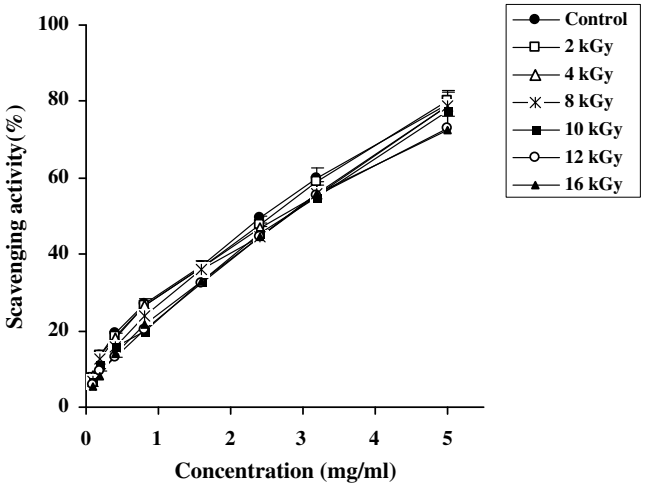

Fig. 4. Effect of gamma irradiation on the scavenging activity of water extracts of *Nigella sativa* seed on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Each value is expressed as mean ± standard deviation ($n = 3$). The vertical bars represent the standard deviation for each data point.

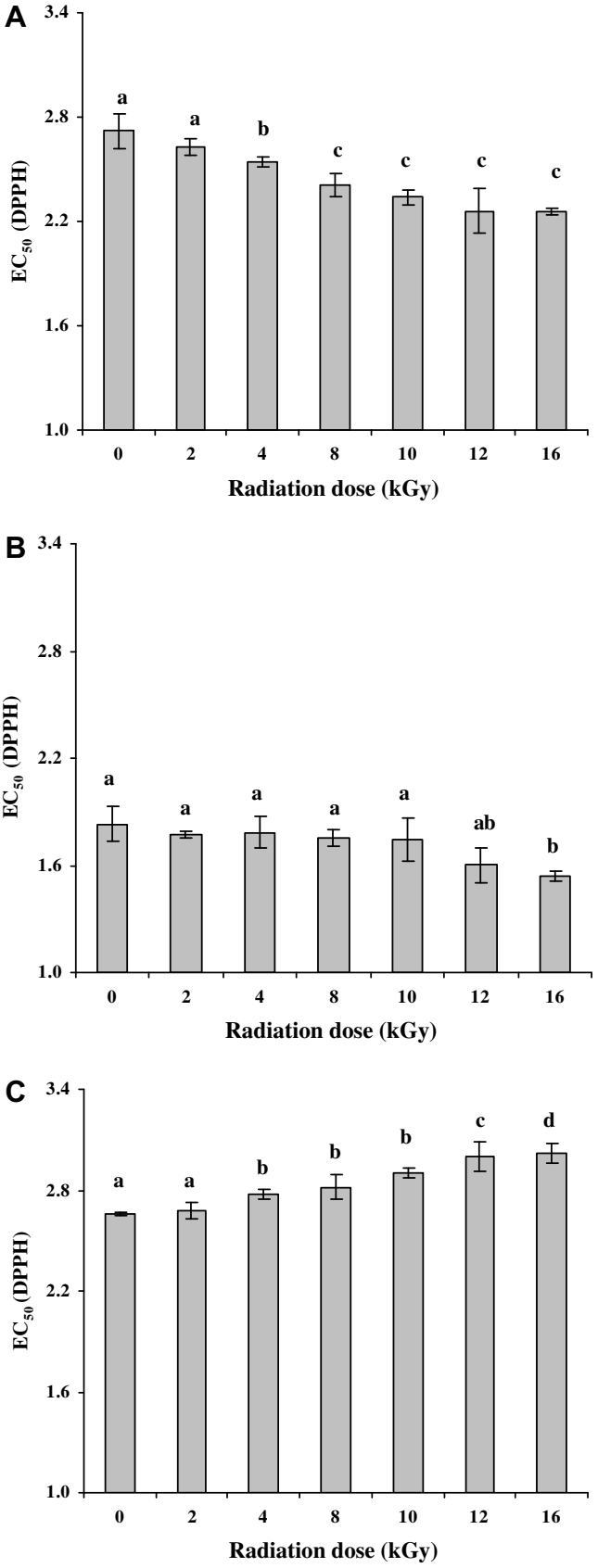

Fig. 5. EC_{50} values (mg/ml) for acetone (A), methanol (B) and water (C) extracts of *N. sativa* seed, according to the DPPH radical-scavenging test with various doses of gamma irradiation. Values expressed as means ± standard deviation ($n = 3$). The vertical bars represent the standard deviation. Values with different superscript letters are significantly different ($p < 0.05$).

some conformational changes, induced by radiation in the structure of phenolic contents. The differences in free radical-scavenging activity of the water extract of control and radiation-processed (2, 4 8 and 10 kGy) seed samples were statistically insignificant at 5 mg/ml concentration. However, a significant decrease in scavenging activity was observed at gamma irradiation dose levels 12 and 16 kGy (Fig. 4).

For *N. sativa* samples, the EC_{50} (DPPH) values were clearly affected by the type of extraction solvent (Fig. 5) and showed the following order: methanol (1.83 mg/ml) < water (2.64 mg/ml) < acetone (2.69 mg/ml). Irradiation significantly affected the EC_{50} values, which decreased by 16% and 15% ($p < 0.05$) in acetone and methanol extracts, respectively at 16 kGy radiation dose. The EC_{50} value was increased in aqueous extract following exposure to gamma radiation.

Though various researchers have worked on the antioxidant activities of the plant seed (Burits & Bucar, 2000; Machmudah, Shiramizu, Goto, Sasaki, & Hirose, 2005; Thippeswamy & Naidu, 2005; Yu, Zhou, & Parry, 2005), nothing is reported on the effect of gamma irradiation on the free radical-scavenging activity of *N. sativa*. However, earlier research studies showed different results for the effect of gamma irradiation on the antioxidant properties of plant materials. A research study conducted by Jo, Son, Lee, and Byun (2003) indicated that the scavenging ability on DPPH radicals was increased in green tea extracts following irradiation at 10 and 20 kGy. Variyar, Limaye, and Sharma (2004) also found that the soybean scavenging ability on DPPH radicals increased with gamma irradiation doses from 0.5 to 5 kGy. On the other hand, Lampart-Szczapa, Korczak, Nogala-Kalucka, and Zawirska-Wojtasiak (2003) reported that increased doses of irradiation decreased the antioxidant effects of lupin seed extracts. Ahn et al. (2005) found that, immediately after irradiation at 2 kGy, the scavenging ability of Chinese cabbage was reduced. According to a Huang and Mau (2006) report, methanol extracts of irradiated freeze-dried mushrooms did not show significant modifications in their scavenging activity as a result of irradiation doses between 2.5 and 20 kGy. Byun, Son, Yook, Jo, and Kim (2002) observed no significant changes in the scavenging abilities of non-irradiated and 5, 10 and 20 kGy-irradiated Chungkookjang and Doenjang.

4. Conclusion

Post-irradiation effect at dose levels 2–16 kGy on the total phenolic content, free radical-scavenging activity and extraction yield was studied for the pharmacologically and culinary important plant *N. sativa*. The study showed that the solvents used in the extraction process played a key role in assessing the effect of irradiation on scavenging activity and total phenolic content of the seed extracts. The methanol extracts exhibited a higher level of scavenging activity and a higher phenolic content, whether they were irradiated or not. From the analysis of our results it can be inferred that gamma radiation doses applied to the *N. sativa* seed increased the extraction yields. The total phenolic content of *N. sativa* seed in acetone extracts was increased following the radiation process. The free radical-scavenging activities of the methanol and acetone extracts using DPPH free radical were found to be enhanced with increased gamma radiation treatment. The decrease in the phenolic content and scavenging activity in water extract might be acceptable, when we consider the increasing demand and benefit of the high quality of the plant materials. Contaminated materials shorten the shelf-life of the seed samples and hence of end-products to which they are added. They also impose a direct health hazard to the consumers.

It is concluded that radiation dose up to 16 kGy can improve the quality of *N. sativa* seed samples, in addition to the enhancement of

scavenging activity, extraction yield and increase in the phenolic content. This study therefore, supports the use of gamma radiation as a phytosanitary treatment for *N. sativa* and calls for further investigations to elucidate its effect on the other biological activities and constituents of the plant.

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