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DECONTAMINATION OF *WITHANIA SOMNIFERA* BY GAMMA IRRADIATION AND ITS EFFECT ON ANTIOXIDANT ACTIVITIES

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ABSTRACT

The current investigation was carried out to study the effect of gamma irradiations on decontamination of *Withania somnifera* and its antioxidant activity. *W. somnifera* dry roots were packed and irradiated at doses of 1–10 kGy following the storage at 4C within a week. At the dose of 10 kGy, the microbial contamination was lowered below detection level. Glycowithanolides are known for antioxidant components present in *W. somnifera*. The antioxidant activity of *W. somnifera* extract was investigated by measuring 1,1-diphenyl-2-picrylhydrazyl, hydroxyl and superoxide radical-scavenging activities. These activities were increased in samples extracted from the irradiated roots at 10 kGy. The extraction yield of the glycowithanolides was considered as one of the reasons for the increase of antioxidant activities of the extract.

PRACTICAL APPLICATIONS

Withania somnifera is an important medicinal plant and has been widely used in India and other parts of the world as a home remedy for several diseases. Gamma irradiation has been used as a phytosanitary treatment of food and herbal materials. The present study was conducted to evaluate the effect of gamma irradiation on decontamination of *W. somnifera* and its effect on the antioxidant properties. These results suggest that gamma irradiation can be applied for phytosanitary of other herbal plants.

INTRODUCTION

Withania somnifera L. Dunal, which belongs to the Solanaceae family, is an important medicinal plant and has been widely used in India and other parts of the world as a home remedy for several diseases (Sharma and Dandiya 1991). The major bioactive chemical components of *W. somnifera* appear to be glycowithanolides, comprising of sitoindosides VII–X and withaferin A (Mishra and Singh 2000; Gupta and Rana 2007). Glycowithanolides have been shown to have antioxidant, anti-inflammatory, anti-tumor, immunomodulatory and memory-enhancing properties, and many studies have been conducted to investigate their therapeutic activities (Agarwal *et al.* 1999; Bhattacharya *et al.* 2000, 2001; Davis and Kuttan 2000; Hamza *et al.* 2008; Mote *et al.* 2010). Among them, antioxidant activity has become increasingly popular and the subject of intensive investigation because of increasing demands by food and pharmaceutical industries (Pihlanto *et al.* 2008). Significant environmental pollution in the modern society has led to increasing numbers of free radicals in the human body, resulting in accelerated aging and as a cause of cancer and cardiovascular diseases (Lutsenko *et al.* 2002). Because antioxidants prevent the formation of or scavenge free radicals, the use of antioxidants in the daily diet has become of interest.

Gamma irradiation had been used as a phytosanitary and decontamination treatment of food and herbal materials and has been gaining increasing acceptance in recent years because of its simplicity, nonuse of toxic fumigants and use in the finished packaging (Farkas 1998). For the case of importing Indian mangoes to the United States, irradiation was approved under the 2007 changes as a means to neutralize pests and meet the US regulatory requirements that would not normally be achievable. In near future, other food and herbals including *W. somnifera* will be imported after irradiation treatment.

Because of its strong penetration power, gamma irradiation also influences the cellular structures and active compounds in plants (Khattak *et al.* 2008; Yoon *et al.* 2012). Recently, it has been reported that the extraction yield and the biological activities of medicinal plants changed after gamma irradiation (Jo *et al.* 2003; Khattak *et al.* 2008). There were several reports on the changes of antioxidant activities after gamma irradiation (Choi and Kim 2013; Choi *et al.* 2014). While there are many reports on the biological activities of *W. somnifera*, there are no reports on the use of gamma irradiation for the decontamination.

Therefore, the present study was conducted to decontaminate *W. somnifera* by gamma irradiation and antioxidant activity of *W. somnifera* was measured to examine the biological activity after irradiation. Furthermore, to investigate changes in antioxidant activities of the extract by gamma irradiation, the extraction yields of steroids were measured.

MATERIALS AND METHODS

Gamma Irradiation of *W. somnifera* Root Powder

W. somnifera roots (100 g) were washed to remove soil and debris and then dried in the shade. Coarsely crushed powder packed in a polyethylene bag was irradiated using a ^{60}cobalt irradiator (cylindrical shape with cobalt 60 pencils, AECL, IR-79, Nordion Inc., Ottawa, ON, Canada) at the absorbed doses of 1, 5 and 10 kGy. The moisture content of the root powder was 21% before gamma irradiation. The source strength was approximately 11.1 PBq with a dose rate on the sample at approximately 10 kGy/h. The containers were continuously rotated during irradiation to obtain a uniform dose. Dosimetry was performed using Alanine-EPR dosimetry system (Bruker Instruments, Rheinstetten, Germany) and the dosimetry showed that the actual dose was within 2% of the target dose. The irradiated volume was 100 mL. The number of samples was four and the treatment was carried out three times for reproducibility. After irradiation, the packed samples were stored at 4C. After irradiation, the samples were analyzed before a week.

Microbiological Analysis

After irradiation, the samples (5 g) were removed from package using a sterile scalpel. The samples were placed in 45 mL of 0.1% peptone water in a sterile stomacher bag. Samples were homogenized using a Stomacher (MIX 2, AES Laboratoire, Combourg, France) at 3,000 rpm for 3 min and filtered through a sterile cheesecloth. For microbial count, serial dilutions were performed in triplicate on selective agar plate.

Total bacterial counts were determined by plating the appropriately diluted samples onto plate count agar (0.5% peptone, 0.25% yeast extract, 0.1% glucose, 1.5% agar, pH adjusted to neutral at 25C; Difco, Detroit, MI). Yeast and mold were plated on potato dextrose agar (20% potatoes, 2% dextrose, 2% agar; Difco) according to the method of Byun *et al.* (1998). Both plates were incubated at 37C for 48 h. For total coliform counts, chromogenic *Escherichia coli*/coliform medium (0.5% sodium chloride, 0.1% sorbitol, 0.49% phosphate buffer, 0.036% chromogenic mixture, 0.3% bacteriological peptone, 0.01% Tergitol-7, 0.1% sodium pyruvate, 1% bacteriological agar, 0.1% tryptophan, pH adjusted to 6.8 at 25C; Oxoid Ltd., Basingstoke, England) was used and plates were incubated at 37C for 24 h. The number of colonies on plate was between 100 and 300 after dilution. The microbial test was carried out immediately after irradiation.

Extraction of Glycowithanolides

Glycowithanolides were extracted as follows: four parts of (1:1) ethanol : water mixture was added to one part of *W. somnifera* root powder and was then cold-percolated for 48 h. Subsequently, the mixture was evaporated under reduced pressure at 55 ± 5C to one-sixth volume and exhaustively extracted with chloroform. The chloroform insoluble aqueous fraction was vacuum-dried below 60C to produce a dry solid and was used for further studies.

The vacuum-dried residue was referred to as glycowithanolides and determined by high-performance liquid chromatography (HPLC; Ganzera *et al.* 2003). The glycowithanolides were dissolved (10 mg/mL) in HPLC-grade methanol and filtered through a 0.45 μm Millipore filter (EMD Millipore, Billerica, MA). HPLC analysis was performed on a Waters Alliance 2690 HPLC system, equipped with a 996 photodiode array detector (Waters, Milford, MA). For all separations, a Synergi MAX-RP 80 Å column (150×4.6 mm, 4 mm particle size) from Phenomenex (Torrance, CA) was used. The mobile phase consisted of water (A) and a mixture of MeOH and reagent alcohol (a mixture of ethanol, methanol and 2-propanol in

the ratio of 90.6:4.5:4.9) in the ratio of 1:1 (B), which were applied in the following gradient elution: from 65 A/35 B in 25 min to 55 A/45 B. All separations were monitored at 230 nm. Standard compound of withaferin A was purchased from Chromadex (Laguna Hills, CA).

The content of total steroids in vacuum-dried residue was determined according to the method by Akbarsha *et al.* (2000). The total steroid content was calculated as percentage of the amount of total steroid to the initial amount of *W. somnifera* root powder.

To measure reducing sugar, extract residue were placed into a centrifuge tube with 80% (v/v) ethanol. The tubes were heated and centrifuged at $1{,}800 \times g$ for 15 min. After adding ethanol, phenol solution and concentrated sulfuric acid, the solution was incubated at room temperature for 15 min. The absorbance was read at 490 nm (Hodge and Hofreiter 1962)

1,1-Diphenyl-2-Picrylhydrazyl Radical-Scavenging Capacity

The free radical-scavenging effect was estimated according to the method of Blois (1958). One milliliter of glycowithanolide solution (4 mg/mL) was added to 1 mL of 0.2 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Sigma-Aldrich Co., St. Louis, MO) and water (1 mL) for a blank absorbance value. The mixture was shaken and left to stand for 30 min at room temperature and measured at 517 nm with a spectrophotometer (UV-1601PC, Shimadzu Co., Tokyo, Japan). The scavenging activity of the DPPH radicals was calculated as a percentage by the following equation:

$$\text{DPPH radical-scavengingactivity (\%)} = \left[1 - \left(A_{\text{sample}} / A_{\text{blank}}\right)\right] \times 100$$

where A_{sample} and A_{blank} are the absorbance with glycowithanolides and blank absorbance, respectively.

Hydroxyl Radical-Scavenging Activity

Deoxyribose assay was used to determine the hydroxyl radical-scavenging activity (Wang and Jiao 2000). The reaction mixture containing $FeCl_3$ (100 μM), ethylenediaminetetraacetic acid (EDTA) (104 μM), H_2O_2 (1 mM) and 2-deoxy-D-ribose (2.8 mM) was mixed with and without glycowithanolides (4 mg) in 1 mL final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 h at 37C. The mixture was heated at 95C in a water bath for 15 min followed by the addition of 1 mL each of trichloroacetic acid (2.8%) and thiobarbituric acid (TBA) solution (0.5% TBA in 0.025 M NaOH containing 0.02% butylated hydroxyanisole). Absorbance of supernatant was measured at 532 nm. The percentage of hydroxyl radical-scavenging activity of the test sample was determined by the percentage to negative control without glycowithanolides.

Superoxide Radical-Scavenging Activity

The assay is based on the removal rate of xanthine/xanthine oxidase-generated superoxide radical (O_2^{-}) by measuring the reduction of nitro blue tetrazolium (NBT) (Chun *et al.* 2003). Xanthine, 0.1 mM, and 0.1 mM NBT were dissolved in 50 mM potassium phosphate buffer (pH 7.4) with 0.05 mM EDTA (phosphate buffered saline [PBE]) to make tetrazolium blue solution. An aliquot (0.9 mL) of tetrazolium blue solution was added to 0.1 mL of glycowithanolides (4 mg/mL). The reaction was initiated by the addition of 1 mL of xanthine oxidase solution (0.05 units/mL PBE). The mixture was incubated at 37C for 20 min and the reaction was terminated by the addition of 2 mL of 2.0 N HCl. The absorbance of NBT was measured at 560 nm against a blank absorbance value that was treated with 2.0 N HCl in advance of the addition of 1 mL xanthine oxidase solution. The superoxide radical-scavenging activity was expressed as percentage (%) of superoxide quenching, which was calculated as $(1 - B/A) \times 100$, where A is the activity of the enzyme without glycowithanolides and B is the activity of the enzyme with glycowithanolides.

Statistical Analysis

All experiments were carried out three times. The data were analyzed using the Statistical Package for Social Science (SPSS Inc., 10.0, 2000, IBM Corp., Armonk, NY). Statistical test used to check for statistically significant difference was *t*-test. Differences among the mean values were obtained by Least Significant Difference (LSD) multiple comparison tests at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Gamma Irradiation on Microbial Load

Compared with the unirradiated control, all irradiated samples at different doses periods including 1, 5 and 10 kGy had significantly less ($P < 0.05$) population of total aerobic bacteria, yeast and mold and total coliforms (Table 1). However, a dose of 10 kGy reduced the populations to <10 per gram. For the control of microorganisms, dried herbs were allowed to be irradiated to a dose of 10 kGy (Hallman *et al.* 2010).

The aerobic plate count of unirradiated *Withania* roots were 10^5 per gram. However, gamma radiation at a dose of 10 kGy achieved commercial "sterility" of *Withania* roots according to IAEA (1992).

TABLE 1. EFFECT OF GAMMA IRRADIATION ON THE POPULATION OF AEROBIC, YEAST AND MOLD, AND COLIFORM (LOG [COLONY FORMING UNIT/G]) OF *WITHANIA* ROOT

Treatment (kGy)	Aerobic	Yeast and mold	Coliform
0	5.22	2.93	1.31
1	3.18	2.31	1.1
5	1.54	1.71	<1
10	<1	<1	<1

The effect of gamma irradiation on the elimination or control of microorganisms on *Withania* is in agreement with that of Byun *et al.* (1998), who showed that the usage of ionizing radiation (a dose of 7.5 kGy) could obtain satisfactory results (<10 microorganism per gram) pertaining to the microbiological decontamination of Korean medical herb. With the effectiveness of gamma irradiation on decontamination of *Withania* root, the effect on antioxidant activity was further investigated.

Radical-Scavenging Activity of Gamma-Irradiated *W. somnifera*

Gamma irradiation can also affect the physiological activities of herbal plant. Therefore, the antioxidant of gamma-irradiated *W. somnifera* was measured. Antioxidant activity is considered to be one of the most important biological activities of glycowithanolides and has been known to reduce the risk for chronic diseases including cancer and heart disease (Willcox *et al.* 2004). The mechanisms of antioxidant activity are very diverse and different methods are often used to measure them.

Glycowithanolides from nonirradiated *W. somnifera* displayed DPPH radical-scavenging activity of 53% (Fig. 1)

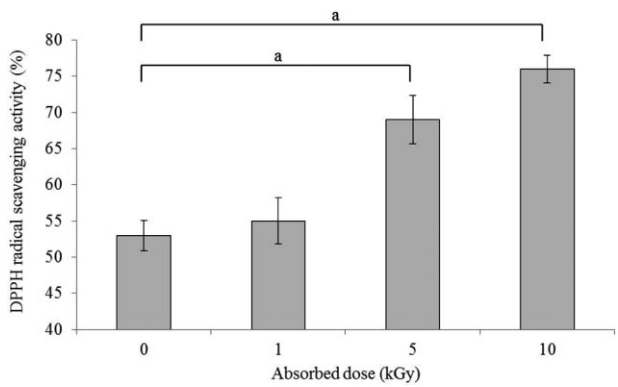

FIG. 1. 1,1-DIPHENYL-2-PICRYLHYDRAZYL (DPPH) RADICAL-SCAVENGING ACTIVITY OF *WITHANIA SOMNIFERA* EXTRACT AFTER IRRADIATION

[a] Statistically significant difference was indicated by lower case letter ($P < 0.05$).

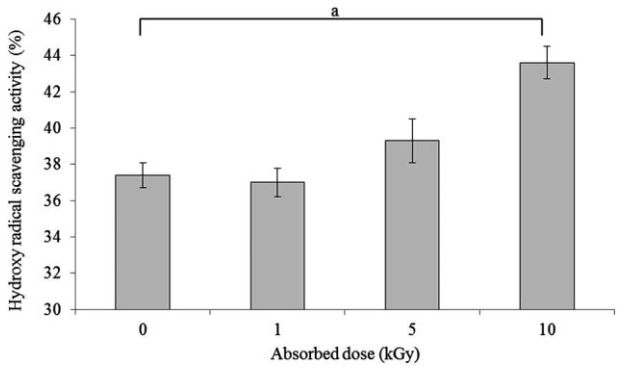

FIG. 2. HYDROXY RADICAL-SCAVENGING ACTIVITY OF *WITHANIA SOMNIFERA* EXTRACT AFTER IRRADIATION

[a] Statistically significant difference was indicated by lower case letter ($P < 0.05$).

and the activity (55%) was not changed ($P > 0.05$) by irradiation at a dose of 1 kGy. However, on irradiation at higher doses, the extracted glycowithanolides exhibited higher DPPH radical-scavenging activities at the same concentration. Irradiation at a dose of 5 kGy resulted in an increase of up to 69% ($P < 0.05$), while at 10 kGy, the extracted glycowithanolides showed a higher DPPH radical-scavenging activity of 76% ($P < 0.05$).

Hydroxyl radicals are highly reactive and therefore damaging to cells, particularly, by initiating lipid peroxidation (Aikens and Dix 1993). In plants, superoxide and hydrogen peroxide are generated in illuminated chloroplasts and it is likely that hydroxyl radicals are also formed (Asada 1992). Therefore, some protective compounds are produced in plants to maintain a low radical concentration (Kandaswami and Middleton 1994). In the present study, the extracted glycowithanolides had a hydroxyl radical-scavenging capacity of about 37.4%, which increased up to 43.6% on gamma irradiation. Extracted glycowithanolides after irradiation at doses of 1 and 5 kGy showed hydroxyl radical-scavenging activities of 37% ($P > 0.05$) and 39.3% ($P > 0.05$), respectively. The increase by the irradiation up to 5 kGy had no statistical significance, but the activity was significantly increased to 43.6% ($P < 0.05$) at 10 kGy (Fig. 2).

Similar results were obtained on assaying the superoxide scavenging capacity (Fig. 3) of glycowithanolides: the nonirradiated and irradiated samples at 1 kGy displayed activities of 20.1% and 23% ($P > 0.05$), respectively. When doses of 5 and 10 kGy were applied on the roots, the extracted glycowithanolides showed increased activities of 27.5% ($P < 0.05$) and 41.9% ($P < 0.05$), respectively.

Extraction Yield of Glycowithanolides with Gamma Irradiation

To investigate the reason for the increased radical-scavenging activities of glycowithanolides from gamma-

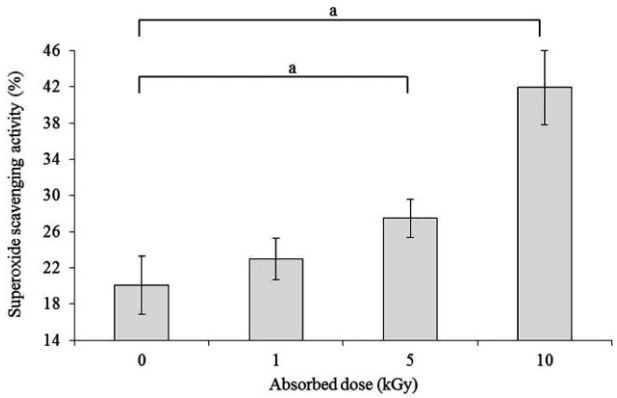

FIG. 3. SUPEROXIDE RADICAL-SCAVENGING ACTIVITY OF *WITHANIA SOMNIFERA* EXTRACT AFTER IRRADIATION
[a] Statistically significant difference was indicated by lower case letter ($P < 0.05$).

irradiated *W. somnifera*, the total steroid content of extract was compared between nonirradiated and irradiated samples (Fig. 4). The total steroid content after nonirradiated extraction was 1.08% of *W. somnifera* root powder, which was the same as (1.19%, $P > 0.05$) with gamma irradiation at a dose of 1 kGy. However, on gamma irradiation of the root powder at a dose of 5 kGy, the total steroid content extracted significantly increased to 1.63% ($P < 0.05$). At a higher dose of 10 kGy, the steroid content further increased up to 1.91% ($P < 0.05$). From this result, an increased yield to 1.91% of total steroid from gamma-irradiated *W. somnifera* resulted in the higher antioxidant activities of glycowithanolide extract.

Bhattacharya *et al.* (2002) reported that the water-soluble fraction of *W. somnifera* extract contained sitoindosides VII–X and withaferin A as the major bioactive entities, besides these, oligosaccharides (polyglucans, molecular weight <2,000 Da) with relative abundance of 12–15% constituted the carriers of the bioactive compounds. In addition, a recent article has showed that the antioxidant activity of κ-, ι-, λ-carrageenans increased by irradiation (Abad *et al.* 2013). The increase in antioxidant activity potential of gamma-irradiated carrageenan can be attributed mainly to the depolymerization of the carrageenans with corresponding increase in reducing sugar (Abad *et al.* 2013). Thus, this could have also occurred with inclusion complex of oligosaccharides presents in vacuum-dried fraction of *W. somnifera* under gamma radiation. Therefore, the content of the reducing sugar in extract residue was determined. The reducing sugar of vacuum-dried residue was about 0.337% and was not significantly changed in other gamma-irradiated powder residues. This result means the oligosaccharides in vacuum-dried residue is not degraded considerably. The extent of depolymerization of polysaccharides was dependent on the content of water, its native molecular weight and irradiation dose (Choi and Kim 2013). But, because of low moisture content of *W. somnifera* root powder and small size of oligosaccharide at relatively low dose (less than 10 kGy), oligosaccharides in root powder was not affected by irradiation.

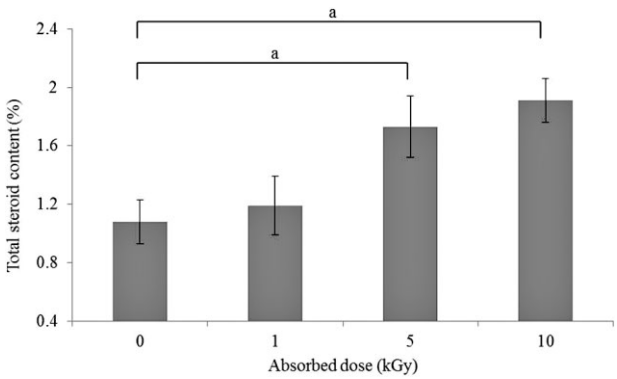

FIG. 4. CONTENT OF EXTRACTED TOTAL STEROID (%) AFTER IRRADIATION
[a] Statistically significant difference was indicated by lower case letter ($P < 0.05$).

The antioxidant activity of glycowithanolides is found to be closely associated with the content of sitoindosides and withaferin. Thus, the extracted amount of withaferin A was determined by HPLC analysis (Fig. 5). The HPLC chromatogram of vacuum-dried residue of *W. somnifera* is shown in Fig. 5A. The content of withaferin A was significantly higher in samples from the irradiated root at 5 and 10 kGy ($P < 0.05$), while from the root irradiated at 1 kGy, although the extracted amount of withaferin A increased, it was not statistically significant ($P > 0.05$).

Many previous studies have substantiated the assumption that irradiation increases the radical-scavenging ability of natural compounds. Khattak *et al.* (2008) reported that gamma irradiation increased the extraction yield of phenolic compounds from *Nigella sativa* seeds and its free radical-scavenging activities. Analogous findings have also been reported for rosemary, in which the antioxidant activity of the extract increased on exposure to radiation because of the increased content of phenolic compounds (Pérez *et al.* 2007). Harrison and Were (2007) reported that the phenolic content in gamma-irradiated almond skin extract increased and was attributed to the phenolic compounds released from glycosidic components. Similar results have been reported by Huang and Mau (2007) and Kim *et al.* (2000). Gupta *et al.* (2011) also reported that the extraction yield of several medicinal plants was increased on gamma irradiation, depending on the absorbed dose. The plausible reason for the increased yield by gamma irradiation could be attributed to the breakage or loosening of structural

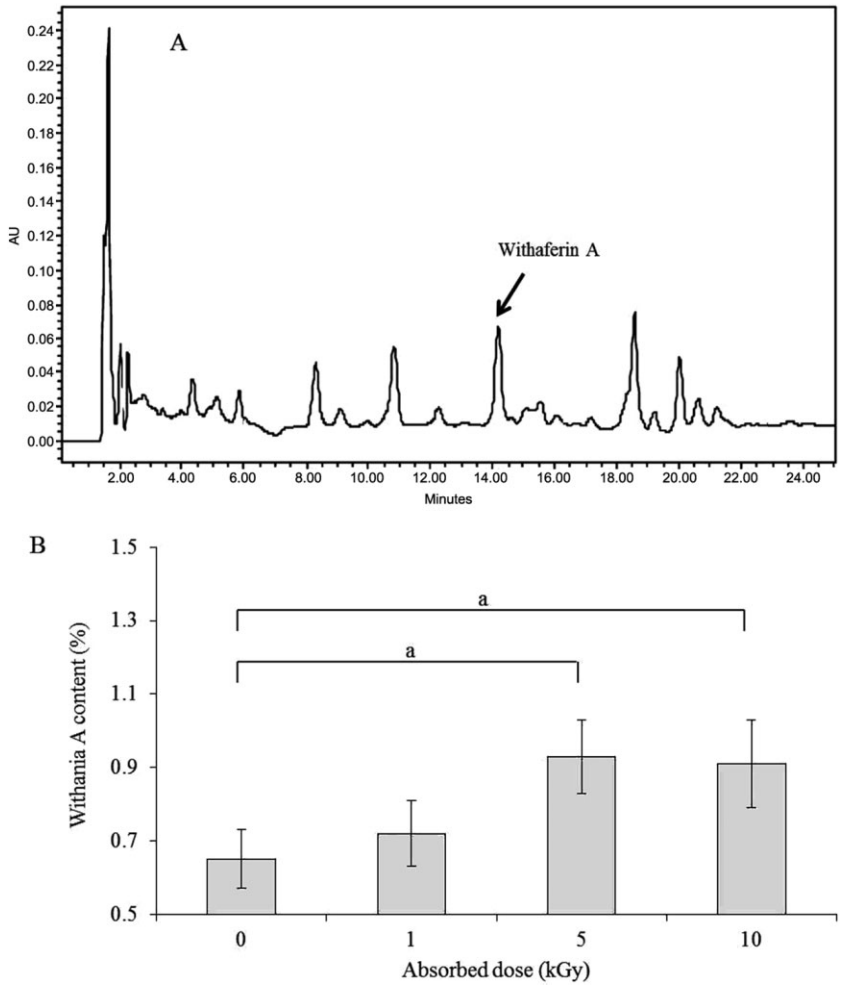

FIG. 5. (A) HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF *WITHANIA SOMNIFERA* VACUUM-DRIED RESIDUE AND (B) CONCENTRATION OF WITHAFERIN A FROM *W. SOMNIFERA* IRRADIATED AT DIFFERENT DOSES [a] Implies that the differences between values were statistically significant ($P < 0.05$).

materials such as cellulose and lignin. Yoon *et al.* (2012) presented that the cell wall of *Undaria* was broken by irradiation, thus making the compounds easily accessible to the solvent.

Therefore, in this study, the gamma irradiation was successfully applied to decontaminate *W. somnifera* and antioxidant activities were observed to be enhanced at dose range of 5 to 10 kGy. The enhanced radical-scavenging activities of glycowithanolides were attributed to the heightened release of active compounds, including sitoindosides and withaferin, on gamma irradiation.

CONCLUSIONS

W. somnifera was decontaminated by gamma irradiation. However, antioxidant activities were observed to be enhanced at a dose range of 5 to 10 kGy. By assessing extraction yield, it was confirmed that the increased radical-scavenging activities of glycowithanolides under gamma irradiation owed to the better release of active compounds. This could be another benefit of the application of irradiation for phytosanitary purposes and disinfection. This result suggests that irradiation treatment could be a safe and efficient phytosanitary treatment for *W. somnifera* and there is post-irradiation enhancement of the antioxidant.

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REFERENCES

ABAD, L.V., RELLEVE, L.S., RACADIO, C.D.T., ARANILLA, C.T. and DE LA ROSA, A.M. 2013. Antioxidant activity potential of gamma irradiated carrageenan. Appl. Radiat. Isotopes *79*, 73–79.

AGARWAL, R., DIWANAY, S., PATKI, P. and PATWARDHAN, B. 1999. Studies on immunomodulatory activity of *Withania somnifera* (ashwagandha) extracts in experimental immune inflammation. J. Ethanopharmocol. *67*, 27–35.

AIKENS, J. and DIX, T.A. 1993. Hydrodioxyl (perhydroxyl), peroxyl, and hydroxyl radical-initiated lipid peroxidation of large unilamellar vesicles (liposomes): Comparative and mechanistic studies. Arch. Biochem. Biophysics *305*, 516–525.

AKBARSHA, M.A., VIJENDRAKUMAR, S., KADALMANI, B., GIRIJA, R. and FARIDHA, A. 2000. Curative property of *Withania somnifera* dunal root in the context of carbendazim-induced histopathological changes in the liver and kidney of rat. Phytomedicine *7*, 499–507.

ASADA, K. 1992. Ascorbate peroxidase – a hydrogen peroxide-scavenging enzyme in plants. Physiol. Plant. *85*, 235–241.

BHATTACHARYA, A., GHOSAL, S. and BHATTACHARYA, S.K. 2001. Anti-oxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. J. Ethnopharmacol. *74*, 1–6.

BHATTACHARYA, S.K., BHATTACHARYA, A., SAIRAM, K. and GHOSAL, S. 2000. Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. Phytomedicine *7*, 463–469.

BHATTACHARYA, S.K., BHATTACHARYA, D., SAIRAM, K. and GHOSAL, S. 2002. Effect of *Withania somnifera* glycowithanolides on a rat model of tardive dyskinesia. Phytomedicine *9*, 167–170.

BLOIS, M.S. 1958. Antioxidant determinations by the use of a stable free radical. Nature *181*, 1199–1201.

BYUN, M.W., YOOK, H.S., KANG, I.J., CHUNG, C.K., KWON, J.H. and CHOI, K.J. 1998. Comparative effects of gamma irradiation and ozone treatment on hygienic quality of Korean red ginseng powder. Radiat. Physics Chem. *52*, 95–99.

CHOI, J. and KIM, H.J. 2013. Preparation of low molecular weight fucoidan by gamma-irradiation and its anticancer activity. Carbohydr. Polymers *97*, 358–362.

CHOI, J., LEE, S.G., HAN, S.J., CHO, M. and LEE, P.C. 2014. Effect of gamma irradiation on the structure of fucoidan. Radiat. Physics Chem. *100*, 54–58.

CHUN, O.K., KIM, D.O. and LEE, C.Y. 2003. Superoxide radical scavenging activity of the major polyphenols in fresh plums. J. Agric. Food Chem. *51*, 8067–8072.

DAVIS, L. and KUTTAN, G. 2000. Effect of *Withania somnifera* on cyclophosphamide induced urotoxicity. Cancer Lett. *148*, 4–17.

FARKAS, J. 1998. Irradiation as a method for decontaminating food: A review. Int. J. Food Microbiol. *44*, 189–204.

GANZERA, M., CHOUDHARY, M.I. and KHAN, I.A. 2003. Quantitative HPLC analysis of withanolides in *Withania somnifera*. Fitoterapia *74*, 68–76.

GUPTA, G.L. and RANA, A.C. 2007. *Withania somnifera* (Ashwagandha): A review. Pharmacogn. Rev. *1*, 129–136.

GUPTA, P.L., GARG, N. and JOSHI, P. 2011. Effect of gamma irradiation on the extraction yield and microbial contamination of medicinal plants. Internet J. Food Safety *13*, 351–354.

HALLMAN, G.J., LEVANG-BRILZ, N.M., ZETTLER, J.L. and WINBORNE, I.C. 2010. Factors affecting ionizing radiation phytosanitary treatments, and implications for research and generic treatments. J. Econ. Entomol. *103*, 1950–1963.

HAMZA, A., AMIN, A. and DAOUD, S. 2008. The protective effect of a purified extract of *Withania somnifera* against doxorubicin-induced cardiac toxicity in rats. Cell Biol. Toxicol. *24*, 63–73.

HARRISON, K. and WERE, L.M. 2007. Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of Almond skin extracts. Food Chem. *102*, 932–937.

HODGE, J.E. and HOFREITER, B.T. 1962. Determination of reducing sugars and carbohydrates. In *Methods in Carbohydrate Chemistry*, Vol. *1* (R.L. Whistler and M.L. Wolfrom, eds.) pp. 380–394, Academic Press, New York, NY.

HUANG, S.J. and MAU, J.L. 2007. Antioxidant properties of methanolic extract from *Antrodia camphorate* with various doses of γ-radiation. Food Chem. *105*, 1702–1710.

IAEA. 1992. *Irradiation of Spices, Herbs and Other Vegetable Seasonings*, International Atomic Energy Agency, Vienna. TECDOC-639.

JO, C., SON, J.H., LEE, H.J. and BYUN, M.W. 2003. Irradiation application for color removal and purification of green tea leaves extract. Radiat. Physics Chem. *66*, 179–184.

KANDASWAMI, C. and MIDDLETON, J.R.E. 1994. Free radical scavenging and antioxidant activity of plant flavonoids. Adv. Exp. Med. Biol. *366*, 351–376.

KHATTAK, K.F., SIMPSON, T.J. and IHASNULLAH. 2008. Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella sativa* seed. Food Chem. *110*, 967–972.

KIM, M.J., YOOK, H.S. and BYUN, M.W. 2000. Effects of gamma irradiation on microbial contamination and extraction yields of Korean medicinal herbs. Radiat. Physics Chem. *57*, 55–58.

LUTSENKO, E.A., CARCAMO, J.M. and GOLDE, D.W. 2002. Vitamin C prevents DNA mutation induced by oxidative stress. J. Biol. Chem. *277*, 16895–16899.

MISHRA, L.C. and SINGH, B.B. 2000. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. Alt. Med. Rev. *5*, 334–346.

MOTE, R.N., PILLAI, M.M. and PAWAR, B.K. 2010. Antioxidant effect of glycowithanolides on esterase activity in salivary glands of D-galactose stressed mice. Int. J. Biol. Med. Res. *1*, 193–201.

PÉREZ, M.B., CALDERÓN, N.L. and CROCI, C.A. 2007. Radiation-induced enhancement of antioxidant activity in extracts of rosemary (*Rosmarinus officinalis* L.). Food Chem. *104*, 585–592.

PIHLANTO, A., AKKANEN, S. and KORHONEN, H.J. 2008. ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*). Food Chem. *109*, 104–112.

SHARMA, K. and DANDIYA, P.C. 1991. *Withania somnifera* (Dunal) – Present status. Indian Drugs *29*, 247–250.

WANG, S.Y. and JIAO, H. 2000. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J. Agric. Food Chem. *48*, 5677–5684.

WILLCOX, J.K., ASH, S.L. and CATIGNANI, G.L. 2004. Antioxidants and prevention of chronic disease. Crit. Rev. Food Sci. Nutr. *44*, 275–295.

YOON, M., CHOI, J., LEE, J.W. and PARK, D.H. 2012. Improvement of saccharification process for bioethanol production from *Undaria* sp. by gamma irradiation. Radiat. Physics Chem. *81*, 999–1002.