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MICROBIAL LOAD AND STABILITY OF SOME PHYTOCHEMICAL COMPONENTS OF SELECTED SUDANESE MEDICINAL PLANT MATERIALS AS AFFECTED BY GAMMA IRRADIATION

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ABSTRACT
The aim of the present study was to evaluate the effect of gamma irradiation treatment on seeds of pepper cress (*Lepidium sativum* L), seeds of black mustard (*Brassica nigra* L. Koch), leaves of lemon grass (*Cymbopogon citratus*), and calyces of roselle (*Hibiscus sabdariffa* L), pods of senna (*Cassia senna* L) and pods of prickly acacia (*Acacia nilotica* L.). The radiation processing was carried out at dose levels of 0, 5, 10, 15 kGy. The irradiated and control samples were analyzed for microbial load, tannins and total phenol content as well as DPPH scavenging activity. The results indicated that gamma radiation treatment significantly reduced microbial load and showed that this total microbial load decreased linearly with absorbed radiation dose. They, also, indicated maximum reduction in tannin content in lemon grass, prickly acacia and roselle. On the other hand, irradiation with 15 kGy increased the tannin and phenol contents in black mustard, pepper cress and senna and reduced the phenol content of roselle and prickly acacia. The results also revealed that gamma irradiation resulted in a significant decrease of DPPH radical-scavenging activity of the different studied methanolic extracts with exception of pepper cress seeds.

KEYWORDS: Medicinal herbs, irradiation, decontamination, phenols, tannins, antioxidant activity.

INTRODUCTION

Spices and aromatic herbs are well known for their useful phytochemical constituents. However, these plant materials are often highly contaminated by microbes. This contamination can occur from the plants themselves, soil, water, air and dust during post-harvest storage and processing, and it could be a cause of serious food-borne illness (Seo *et al.*, 2007). As utilization of the aromatic herbs in food and bio-industry increases, mass production and the supply of these materials with a high quality are required (Yu *et al*., 2004). Various conventional methods of sterilization and reduction of microbial loads are used; the major methods are fumigation with gaseous ethylene oxide, or propylene oxide, and application of steam (Dickman, 1991). These methods are, however, recognized as less safe, and are now prohibited or being increasingly restricted in most countries (Uijl, 1992). Instead microwave, gamma radiation and ozone are now widely used to reduce microbiological contamination (Emam *et al*., 1995; Farag *et al*., 1995; Zhao and Cranston, 1995).

Many studies dealing with the use of irradiation technology for medicinal and aromatic herbs and spices have concluded that irradiation can be considered a radiologically hygienic and toxicologically safe technology. However, questions focusing on loss of phytochemical constituents, free radicals and radiolytic by-product formation, and changes of antioxidant properties during irradiation are still being debated (Suhaj *et al*, 2006). Research studies showed different results for the effect of gamma irradiation on antioxidant properties of plant materials. Harrison and Were (2007) found that gamma irradiation of almond skins above 4 kGy enhanced the antioxidant activity. Likewise, Variyar *et al.* (2004) reported that the scavenging ability of soybean on DPPH radicals increased with the increase in radiation dose in the range 0.5 to 5 kGy. On the other hand, Lampart-Szcapa *et al.* (2003) found that lupin rhizome extracts showed a decrease in antioxidant activity at doses of 1, 5 and 10 kGy. Ahn *et al.* (2005) also reported that the scavenging ability of Chinese cabbage was reduced after irradiation at 2 kGy. In contrast, Mishra *et al.* (2006) showed that the free radical scavenging activity of tea was not affected due to radiation treatment at 1, 2, 5 and 10 kGy. Murica *et al.* (2004) evaluated the effect of gamma irradiation on antioxidant properties of seven desert spices (anise, cinnamon, ginger, licorice, mint, nutmeg and vanilla) and found that the spices irradiated at 1, 3, 5 and 10 kGy did not show significant differences in antioxidant activity compared with the non irradiated ones. The aim of the present work was to study the microbial load, biochemical characteristics and antioxidant activities of certain commonly used Sudanese herbal products, as influenced by gamma irradiation.

MATERIALS AND METHODS

The plant materials were collected from the country side of Khartoum state, Sudan, as well as the local markets, where herbs and plants collected from Northern and Western parts of the country was available. Plant parts were selected and studied according to their traditional use/s as medicinal herbs. These were seeds of Pepper

Cress (*Lepidium sativum* L) and Black Mustard (*Brassica nigra* L. Koch), leaves of Lemon Grass (*Cymbopogon citratus*), calyces of Roselle (*Hibiscus sabdariffa* L) and pods of Senna (*Cassia senna* L) and Prickly Acacia (*Acacia nilotica* L.).

Irradiation process

The irradiation process was carried out for the plant samples at different doses (0, 5, 10, 15 kGy) using an experimental cobalt-60 Gamma source (Nordion gamma cell 220-Excell) at the Sudan Atomic Energy Commission, Khartoum. The activity of the source was 6.345 Kci and the energy 1.25 MeV. The irradiation time varied from 1 to 3 hours depending on the dose applied. Irradiated and non irradiated samples of different plant materials were ground to pass through a 0.4 mm screen and kept in glass bottles at room temperature for analysis.

Microbiological analysis

One bag from different plant materials of each treatment was aseptically opened and ten grams of ground plant materials were used to prepare serial dilutions according to standard methods (Association of Official Analytical Chemists (AOAC), 1990). Aerobic plate counts (APCs) were enumerated after plating with double case agar (PCA) and incubated at 37° C for 48 h to determine the total plate counts for bacteria. The plates containing between 30-300 colonies were counted using a colony counter (Scientific and Cook Electronics L.T.D, United Kingdom, London)**,** then the viable count of bacteria in 1 ml of the original sample was calculated from the colony count and the respective dilution. The results were presented as colony forming units per g dry weight (cfu/g).

PREPARATION OF PLANT MATERIAL EXTRACTS

Fifty g from each irradiated plant material were macerated using 500 ml of 80% methanol and allowed to shake for 4 hours, then left over night for 72 hours at room temperature. The extracts were filtered using Whatman no. 1 filter papers.

Total phenol analysis

Determination of total phenols in irradiated and control samples was done with Folin-Ciocalteu reagent, where total phenolic content of the samples was determined as described by Singleton and Rossi (1965) using gallic acid as a standard. The sample (0.5 ml) and 30 ml of HPLC grade water were placed in a 50 ml volumetric flask. To the mixture, 2.5 ml of Folin–Ciocalteu phenol reagent were added. After 5 min 7.5 ml of 20% sodium carbonate were mixed into the solution. The solution was brought up to a volume of 50 ml with HPLC grade water. After closing the flask and inverting it several times, the solution was incubated for 90 min. Absorbance was recorded at 760 nm using a DU 800 UV–VIS spectrophotometer (UNICAM UV/VIS). Gallic acid standard solutions (0–10 mg/ml) were prepared in a similar manner.

Analysis of tannins

According to the protein precipitation method described by Hagerman and Butler (1978), for the preparation of a standard curve 50 to 300 µl of catechin solution were taken and the volume adjusted to 875 µl with buffer C (Resuspension buffer, 5% triethanolamine (v/v), 5% Sodium Dodecyl Sulphate (SDS) (w/v), pH adjusted to 9.4 with HCl) (e.g. 100 µl catechin solutions plus 775 µl of buffer C). One hundred and twenty five micro liters (125 µl) of the ferric chloride reagent was added and mixed. A zero tannin sample was made with 875 µl buffer C and 125 µl of ferric chloride reagent. The absorbance value was recorded at 510 nm using a spectrophotometer (Unicom UV/Vis spectrometer). The standard samples and the zero tannin were incubated at room temperature for 10 minutes then the absorbance was read.

The samples were diluted 1:1 with buffer B (5g/L potassium sodium-tartrate tetrahydrate $C_4H_4KNao_6.H_2o$, 12% EtOH, pH adjusted to 3.3 with HCl). For each sample, 1 ml of the protein solution was pipetted into a microfuge tube with 500 µl of the diluted sample, and then incubated for 15 minutes with slow agitation (300 rpm) in thermomixer compact (Eppendorf, Hamburg, Germany).

The samples were centrifuged for 5 minutes in a micro-centrifuge at 14,000 rpm (Eppendorf, Hamburg, Germany). The supernatants were carefully removed, retaining the pellets in the microfuge tubes. Two hundred and fifty micro liters (250 µl) of buffer A (washing buffer, 200 mM acetic acid, 170 mM NaCl, pH adjusted to 4.9 with NaOH) was slowly added to the pelleted samples, and then centrifuged for 1 minute (14,000 rpm). The last 3 steps were repeated to wash the pellet a second time. The supernatants were poured off, 875 µl of buffer C were added, and then the tubes were incubated for 10 min in thermomixer (950-1000 rpm) for complete mixing. Ten min after dissolving the pellet the absorbance was read at 510 nm and the value recorded. Then 125 µl of ferric chloride reagent was added, the sample mixed, incubated for 10 min and the absorbance was re-read at 510 nm.

Antioxidant activity

Free radical scavenging activities were determined using the stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH, Fluka, Germany) as described by (Wang *et al.*, 2002). The flavonol quercetin (1 mM) was analyzed in parallel as positive control. The assays were performed from mixtures containing 100 µl of freshly made 0.1 mM DPPH solution in 96% ethanol and 900 µl of samples at different dilutions (0, 1, 2, 5, 10, 20, 40 and 80 µM). The mixtures were shaken and left to stand for 30 min. at room temperature and the absorbance read at 517 nm using a spectrophotometer (UNICAM UV/Vis spectrometer).

Statistical Analysis

The data were statistically evaluated by the one-way analysis of variance procedure (ANOVA).The least significant difference (LSD) test was applied to compare mean values. All analyses were performed in triplicate (n = 3). The level of significance used was 95 % (Sendecor and Corchan, 1987).

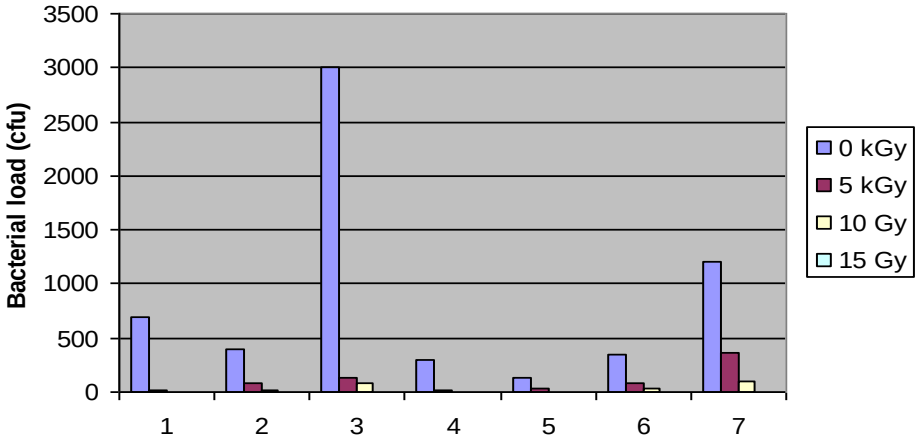
RESULTS AND DISCUSSION

Effect of irradiation on the microbial load

The results of the bacterial count indicated that the non-irradiated samples of Senna (*Cassia senna*) (pods), Senna (leaves), Prickly Acacia (*Acacia nilotica L.)*, Black Mustard (*Brassica nigra* L. Koch), Pepper Cress (*Lepidium sativum* L), Lemon Grass (*Cymbopogon citrates*) and Roselle (*Hibiscus sabdariffa* L.) were highly contaminated with bacteria at the levels of 3.0×10^5, 1.2×10^5, 7.0×10^4, 4.0×10^4, 3.5×10^4, 3.0×10^4, 1.3×10^4 cfu/g, respectively. These values exceeded the level of 1.0×10^4 cfu/g reported by WHO (1998) as the maximum

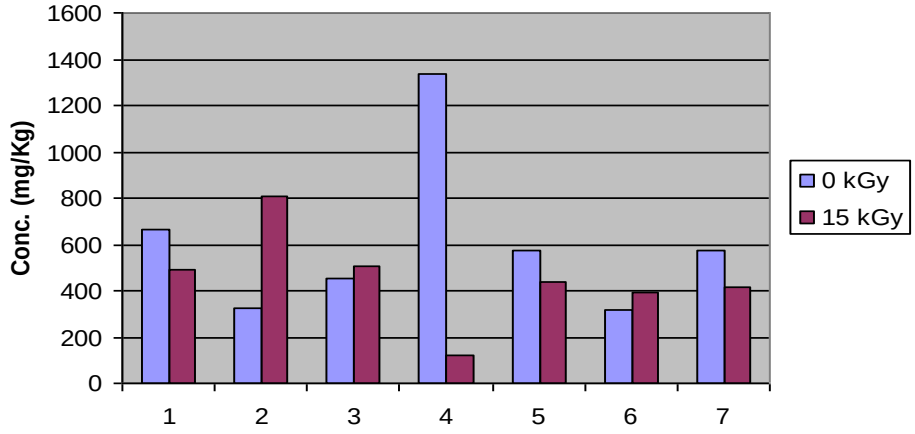
permissible total count level. The high contamination level could be attributed to the high natural micro flora of the herbs as well as the general conditions during their cultivation, harvesting, drying, handling, processing, storage, distribution and sales. However, it was reported that the microbial status of dried herbal material is not so much caused by secondary contamination during processing, but may primarily be due to the fact that plants have their own microbial flora (Abou Donia, 2008). The results given in Fig. 1 showed that this total microbial load decreased linearly with absorbed radiation dose, and this finding is in agreement with the results reported by Nemtanu *et al*. (2009), who reported that gamma irradiation with doses up to 5 kGy led to the desired microbial reduction. The highest sensitivity of microbes to gamma rays at 5 kGy was observed in Prickly Acacia (97.4%), while the lowest sensitivity was in Roselle (69.2%) but the latter showed complete absence of microorganisms at 15 kGy. Abdel-Karem *et al.* (2002) reported that irradiation at a dose level of 5 kGy reduced the total bacterial count of Roselle by 97.5%, while complete elimination was achieved at a dose level of 7 kGy.

Figure 1: Effect of gamma irradiation on microbial load of selected plant materials



1: Prickly Acacia pods 2: Black Mustard seeds 3: Senna pods
4: Lemon Grass leaves 5: Roselle calyces 6: Pepper Cress seeds7: Senna leaves

Figure 2: Effect of gamma irradiation on tannins concentration of selected plant materials



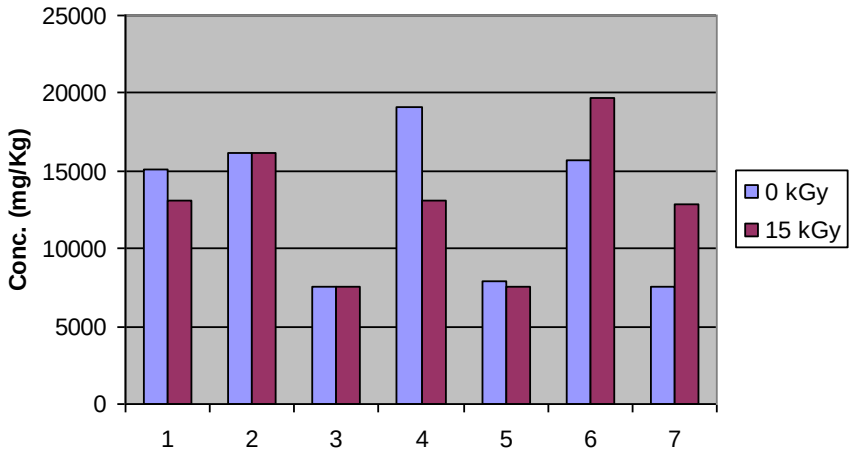
1: Prickly Acacia pods 2: Black Mustard seeds 3: Senna pods
4: Lemon Grass leaves 5: Roselle calyces 6: Pepper Cress seeds 7: Senna leaves

Effect of irradiation on tannins

The levels of total tannins varied after irradiation in the different plant species. Fig. 2 illustrates the results of the effect of gamma irradiation on tannins content of different plant materials. The results indicated that maximum reduction in tannin content (mg/L catechin) due to irradiation with 15 kGy was observed in Lemon Grass, where it has been reduced by 91.1%, followed by Senna leaves (26.7%), Prickly Acacia (26.4%) and Roselle (24%). This reduction in the tannin contents is very favorable, since this anti-nutritional factor has the capacity for decreasing protein digestibility (Toledo *et al.*, 2007). The reduction is probably due to chemical degradation by the action of free radicals produced by the irradiation. Villavicencio *et al.* (2000), Brigide and Canniatti-Brazaca (2006) found that gamma radiation promoted reduction in the tannin contents as the radiation dose increased. On the other hand, irradiation with 15 kGy increased the tannin content in Mustard from 324.1 to 810.4 followed by Pepper Cress from 317.1 to 395.1 and Senna (pods) from 449.2 to 509.2. The differences in effect may be attributed to the different phenolic compounds present in the herbs,

or the part of plant used (in seeds the tannin seems to increase, where as in vegetative tissues the tannin decreased). Hydrolysable tannins, which may be more susceptible to gamma-irradiation compared to the condensed tannin, may explain the increase in tannin contents. Harrison and Were (2007) reported an increase in tannin contents of irradiated clove and nutmeg that have appreciable amounts of hydrolysable tannins compared to the condensed tannins present in cinnamon and other spices.

Figure 3: Effect of gamma irradiation on total phenol concentration of selected plant materials

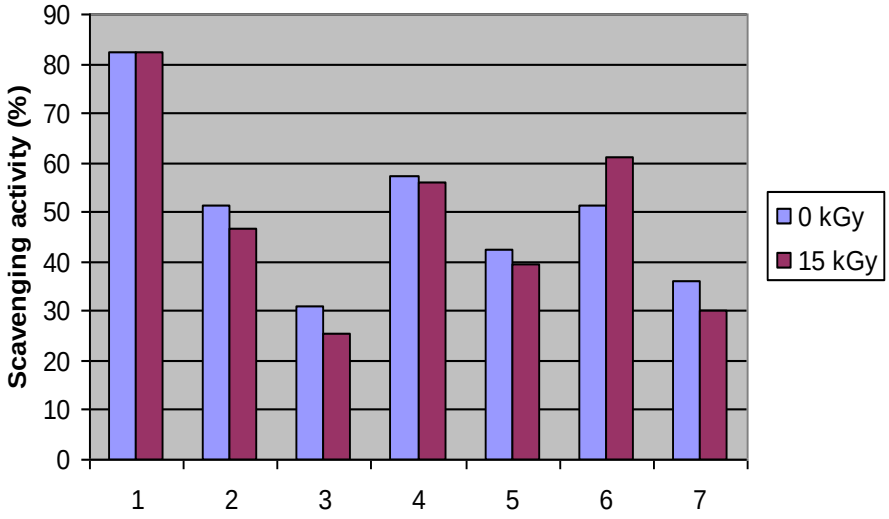


1: Prickly Acacia pods 2: Black Mustard seeds 3: Senna pods
4: Lemon Grass leaves 5: Roselle calyces 6: Pepper Cress seeds 7: Senna leaves

Effect of irradiation on total phenols

The results of the effect of gamma irradiation on total phenol content of different plant materials are illustrated in Fig 3. It was observed that irradiation with 15 kGy caused slight increase in phenol content in Mustard (0.1%) followed by Senna (pods) (1.3%). However, the maximum increase was observed in Senna (leaves) followed by Pepper Cress. On the other hand irradiation with 15 kGy reduced the phenol content of Roselle (5.1%) and Prickly Acacia. The maximum reduction was observed in Lemon Grass. Adamo *et al.* (2004) reported increase in phenol content for irradiated samples of truffles at the dose level in the 1.0-1.5 kGy and proposed that the destructive process of oxidation and gamma radiation were capable of breaking the chemical bonds of polyphenols, thereby releasing soluble phenols of low molecular weight. Harrison and Were (2007) found an increase in total phenolic content of irradiated almond skin extracts as compared to that of the control at irradiation levels of 4 kGy and above. Similarly, Huang and Mau (2007) reported a higher content of phenolics in irradiated compared to non-irradiated mushrooms. The dose of 8 kGy promoted an increase in the content of total phenolic compounds in raw grains from five soybean cultivars, while a decrease at doses of 2 and 4 kGy was observed (Toledo *et al.*, 2007). Ahn *et al.* (2005) found that gamma irradiation at 1 kGy or above significantly reduced the phenolic contents in cut Chinese cabbage heads. In contrast, Mishra *et al.* (2006) described that no significant effect was observed in total phenolics in irradiated tea leaves at 5 kGy.

Figure 4: Effect of gamma irradiation on DPPH radical- scavenging activity of selected plant materials extracts



1: Prickly Acacia pods 2: Black Mustard seeds 3: Senna pods

4: Lemon Grass leaves 5: Roselle calyces 6: Pepper Cress seeds7: Senna leaves

Effect of irradiation on antioxidant activity

In Fig. 4 the results of DPPH-scavenging activity changes of irradiated plant materials are shown. The highest antioxidative activity was found in Prickly Acacia followed by Lemon Grass and Pepper Cress while the lowest activity was found in Senna pods. The results also revealed that gamma irradiation resulted in a significant tendency to decreasing of DPPH radical-scavenging activity of the different studied methanolic extracts with exception of Pepper Cress seeds. This finding is in agreement with that reported by Suhaj *et al.* (2007) who detected significant decrease in DPPH radical scavenging activity of black pepper irradiated at doses 5, 7.5, 10, 20 and 30 kGy. However, Chattak *et al.* (2008) reported that gamma irradiation enhanced the scavenging activity of *Nigella sativa* seed samples. In plant tissues many phenolic compounds are potential antioxidants, flavonoids, tannins and lignin precursors may act as ROS (reactive oxygen species) scavenging compounds. It has been reported that an increase in total phenolics was beneficial for antioxidant properties of soybean seeds due to polymerization of phenolic constituents and also cross-linking and fragmentation (Stajner *et al.*, 2007). As observed, the comparatively high scavenging activity can be correlated to the higher phenolic content. This agrees with that reported by Yang *et al.* (2007), who demonstrated a positive correlation between scavenging activity and phenolic contents on the rhizome samples of *Nelumbo nucifera*. In contrast, Zielinski and Kozlowska (2000) indicated a negative correlation.

According to the report of Huang and Mau (2006) irradiation of freeze-dried mushrooms at doses between 2.5 and 20 kGy did not show significant modifications in their scavenging activity. Byun *et al.* (2002) observed no significant changes in the scavenging abilities of non-irradiated and irradiated Chungkookjang and Doenjang at 5, 10 and 20 kGy.

CONCLUSION

The reported results show that gamma irradiation can be considered effective to improve the quality of plant materials in terms of reduction of microbial contamination. Moreover, after irradiation up to 15 kGy the content of tannins and total phenol was modified. The results also revealed that gamma irradiation resulted in a significant tendency to decreasing of DPPH radical-scavenging activity of the different studied methanolic extracts with exception of Pepper Cress seeds.

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