



Effect of Gamma Irradiation on Phytochemical Content and Antimicrobial Activities of Selected Herbs

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Abstract

Seven herbs by irradiating with Cobalt-60 gamma irradiation. The microbial loads i.e., total bacterial count and total fungal count and the phytochemical analysis (total phenol contents, total flavonoids contents and antioxidant activity) of both control and irradiated herbs were determined. The gamma radiation with standard dosages from 1 kGy to 7 kGy decreases bacterial counts of herbal material from 3 log cycles to 6 log cycles. A dosage of 5 kGy decreases the bacterial capacity by 4 log cycles to 7 log cycles in addition a dosage of 7 kGy removed microbial load from the whole herbal medicinal product. The more useful dosages of herbal medicinal product were expected to have in the range from 1 kGy up to 7 kGy. The more effective gamma irradiation between 1 kGy to 7 kGy improved the elimination of microbial load and also improved the quality of the herbal product and also enhanced the shelf life of herbal products for both the local and international marketplaces.

Keywords: Herbal products; Gamma irradiation; Shelf life

Introduction

According to a report of WHO about 80% of world population uses herbal products in their primary form. Even today, herbal medicines have a central role in the medicinal field such as Unani medicine in Pakistan, Ayurvedic medicine in India and

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traditional Chinese medicine in China [1]. In Pakistan approximately there are 3, 600 plant species of which over 2,900 are indigenous has a rich biodiversity. Production of herbal products therefore has a high potential in country in addition to the fact that about 33% medicines worldwide are derivative of lower and higher plants. In Pakistan, herbal medicine has a long history and tradition. The usage of herbal medicinal products is a science based approach for the prevention as well as treatment of diseases the process is called phytotherapy [2].

Plant-derived substances have been getting greater attention due to their multipurpose applications in a number of industries. Aromatic and medicinal plants (AMPs) are considered as the richest reserves for allopathic drugs, folk medicines, food supplements, nutraceuticals, flavors, fragrances, health beverages, cosmeceuticals, pharmaceutical intermediates and chemical entities for synthetic drugs. Therefore, these are now broadly traded in raw as well as in processed forms all over the world [3].

Herbal plant constituents generally carry an excessive amount of molds and bacteria frequently originated from topsoil. Aerobic spore-forming bacteria commonly prevail whereas a wide variety of fungi as well as bacteria naturally present in the micro flora of herbs [4]. Most of the world pharmacopoeias establish different limits for microbial contamination in medicinal plants. In spite of those different groups, one of the common features is that the presence of Salmonella must not be noticed (none in 10 g) [5]. Another recommendation is the detection of aflatoxin, the presence of which can be harmful to health even in small amounts [4].

About medicinal plants, it has been described that exposure to cobalt-60 gamma radiation in the range between 6 kGy to 10 kGys are tolerable to sanitize cardamom, cinnamon, turmeric and fennel etc., which doesn't affect important chemical or sensory changes [6,7]. From Egypt Aziz and coworkers examining 84 medicinal plants collected in different localities of Cairo markets revealed that the significant dosage for removal of actinomycetes as well as fungi was 5 kGy for the whole observed herbal plants.

People have depended on plants as a source of medication as well as food for centuries [8]. Modern research work and practical experience has clearly revealed that treatment using medicinal plant is more valued than using synthetic chemicals for easily available being safe cost effective and for having synergistic effects. Hence, health establishments are looking for nutritional therapies and alternatives approaches. Plants have a wide variety of therapeutic activity such as free radical scavenging, antihypertensive, analgesic, anticholinergic antimicrobial, cardiovascular, anti-fertility antimalarial, stomachic etc., due to the presence of diversity of essential compounds (flavonoids, vitamins, minerals, mucilage's, glycosides, terpenoids, organic acids, alkaloids, tannins, steroids, etc.) [9-12]. In the present work we focused on the evaluation of shelf life of herbal product by irradiating with Co-60.

Materials and Methods

Plant Samples

Plant materials such as *Nigella sativa* (black seed), *Zingiber officinal* (Ginger), *Allium sativa* (Garlic) and *Carulluma tuberculata* (Choonga) were collected from local herbal market Peshawar, Pakistan. Similarly lemon juice, apple cidar and honey were also collected from the same market. Herbs were sorted and washed thoroughly with tap water followed by drying at 40°C in hot air oven. After drying, herbs were cut into small pieces, grounded (Retch Muhle-Germany) and passed through the sieves of mesh size 30 mm. The powdered samples were packed in clear polyethylene pouches and sealed with electric sealer PFS 300 (Ladder, China).

Irradiation

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 1 kGy, 3 kGy, 5 kGy and 7 kGy. The irradiation was carried out at ambient conditions (20°C-35°C, RH 40%-85%). The dose rate was 0.76 kGy per hour, as determined with a Fricke dosimeter. The irradiation time varied from 1 min to 80 min depending upon the dose applied. After irradiation, both radiated and control samples were stored at ambient temperature until the analyses were carried out.

Preparation of extracts

The irradiated and control samples (50 g each) of plant materials were separately extracted in methanol and water 3:150 (v/v) 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 filtrate was concentrated under vacuum at low temperature using a rotary evaporator.

Determination of phenolic contents

Using the method of Velioglu method, the total phenolic contents in the extracts were determined using Folin-Ciocaltaeu reagent [13]. The stock solution of sample was prepared by a deliberation of 1.0 mg/ml of which 40 µl were transferred to a test tube and 0.075 ml of Folin-Ciocaltaeu reagent was added that was diluted 10-fold with deionize H₂O. The mixture was incubated for 5 min at room temperature and then 0.075 ml of 6 % (w/v) of sodium carbonate was added to this mixture. Again the mixture was incubated for 90 min at room temperature and the absorption was measured at 750 nm by a spectrophotometer (UVD-2950, labomed. Inc, USA). Gallic acid (0-50 mg/ml) was used as a standard. The total phenol content was said as gallic acid equals in gram each 100 gram of the samples.

Determination of total flavonoids

For total flavonoids estimation the aluminum chloride (AlCl₃) colorimetric method was used [14]. Stock solutions of each plant extracts were prepared as 5000 µg/ml of methanol. Of these stock solutions 25 µl were separately mixed with 1975 µl of methanol, 100 µl of 10% aluminum chloride, 100 µl (1M) potassium acetate and 2.8 ml of distilled water to have a final

concentration of each sample as, 25 µg/ml. The reaction mixture was then kept at room temperature for 30 min and absorbance was measured at 415 nm with UV-Vis double beam spectrophotometer (UVD-2950, Labomed. inc, USA). The calibration curve was prepared by using quercetin with final concentrations of 0, 2 µg/ml⁻¹, 4 µg/ml⁻¹, 6 µg/ml⁻¹ and 8 µg/ml⁻¹ and total flavonoids contents were determined as quercetin equivalent (g/100 g of the sample).

Determination of DPPH radical scavenging activity

The antioxidant activity of selected herbal ingredients was carried out according to the method of The DRSA of herbal ingredients extracts were determined in terms of hydrogen donating or radical – scavenging ability using the constant radical (1, 1 diphenyl-2-picrylhydrazyl (DPPH) (sigma) [15]. Selected herbal material was extracted with ethanol; therefore DPPH solution was also prepared in similar solvent. Carefully, 1.0 ml of the ethanolic extract (5 mg/20 ml) was mixed with 2.0 ml of DPPH solution (0.159 mg/20 ml × 4). After incubation (30 min) in the dark, the absorption of solution was measured using spectrophotometer at 515 nm at room temperature. The lower absorbance of the reaction indicated lighter radical scavenging activity. Radical scavenging activity was calculated as percentage of DPPH discoloration. The following was used for determination of DPPH radical scavenging activity:

$$\% \text{DPPH} - \text{radical} - \text{scavenging activity (DRSA)} = 100 \times [1 - AE / AD] \quad (1)$$

Whereas AE represented the absorbance of the test sample while AD represented the absorbance of DPPH solution.

Determination of total bacterial count

Using nutrient agar medium according to method of Bergmann total bacterial count of the four samples were find out by dilution plate method [16].

To perform the total bacterial count of both the non-irradiated (control) and irradiated samples the extracts of the four herbal plants i.e. *Carulluma tuberculata* (choonga), *Nigella sativa* (kolonji), *Zingiber officinal* (Ginger) and *Allium sativum* (Garlic) were taken. A saline solution normally 8.5 g/liter was prepared. A dilution of 1:10, 1:100 and 1:1000 was prepared consequently. 1 ml of the sample from each dilution was taken with the help of pipette and poured into purified petri dishes. Sterilized nutrient agar was transferred to each petri dish. On solidification the plates were kept at inverted positions in an incubator at a suitable temperature for bacterial growth is 37°C for 24 h. Colonies of bacterial formed were calculated with the help of a colony counter and bacterial count per gram of sample was calculated.

Determination total fungal count

Total fungal counts of all samples were determined using dilution plate method of Bergmann using potato dextrose media [17]. In order to find total fungal count, the control and radiated extracts, 8.5 g each was dissolved in liter of saline water which give 1:10 dilution. Further required dilutions i.e. 1:100 and 1:1000 were prepared accordingly. 1 ml of each dilution was poured into sterilized petri dish with the help of a sterilized pipette. Sterilized potato dextrose agar (PDA) 15 mL-20 mL was poured into each petri dish. After cooling, oxytetracyclin was added to the medium to control the bacterial growth. The

media plates were incubated at 27°C-30°C for 24 h. In each samples the fungal colonies were counted and also fungal colonies per gram of sample were calculated.

Results and Discussion

The selected herbal plants include *Carulluma tuberculata* (choonga), *Zingiber officinal* (Ginger), *Nigella sativa* (Black seed), *Allium sativum* (Garlic), Lemon Juice, apple cider and honey .Products derived from these herbal plants have great importance owing to their versatile applications in a number of industries. These herbal plants have rich reserves for folk medicines, allopathic drugs, nutraceuticals, fragrances, flavors, health beverages, pharmaceutical intermediates and chemical entities. These medicinal plants have a long history and tradition in Pakistan and are used for thousands of years by culture all over the world. These medicinal plants are still a central part of the medical system, such as Unani medicine in Pakistan, Ayurvedic medicine in India and traditional Chinese medicine in China. Keeping in mind the medicinal importance of above mentioned plants, the selected plants were used for the development of Cardio-NIFA.

Determination of total phenolic contents

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenyl propanoid pathways in living organisms. They act in defense against pathogens, animal mycophage, or fungivores aggression and as response to various abiotic stress conditions, such as rainfall and ultraviolet radiation polyphenols have protective activity which has been previously attributed to free radical scavenging, metal chelating properties, capability of inhibiting or reducing different enzymes, such as telomerase, cyclooxygenase or lipoxygenase and then most importantly as antioxidant compounds with the ability to trap free radicals and thus inhibit the oxidative mechanisms. The total phenolic contents were expressed in milligrams per gram of plant materials. It is evident from the results that irradiating the selected plant materials with gamma radiation of cobalt-60 has greatly affect the total phenol contents compared to control.

Irradiation was performed at dose level of 1 kGy, 3 kGy, 5 kGy and 7 kGy, respectively. The total phenol contents of *Allium sativa* at 1 kGy, 3 kGy, 5 kGy and 7 kGy were 0.295 ± 0.061 , 0.063 ± 0.063 and 0.069 ± 0.069 mg/g, respectively, while the total phenol contents in control (non-irradiated) was 0.038 ± 0.038 mg/g. The total phenol contents of the gamma irradiated *Zingiber officinal* slightly decreased at dose levels of 1 kGy, 3 kGy, 5 kGy and 7 kGy, respectively. The total phenol contents of *Zingiber officinal* at 1 kGy, 3 kGy, 5 kGy and 7 kGy were 1.623 ± 0.005 , 1.720 ± 0.006 , 1.306 ± 0.008 and 1.532 ± 0.003 mg/g, respectively, while the total phenol contents in control (non-irradiated) was 1.917 ± 0.003 mg/gm. The decrease in total phenol contents of irradiated *Nigella sativa* extracts was observed as compared to that of the control at dose level of 1 kGy, 3 kGy, 5 kGy and 7 kGy, respectively.

The total phenol contents of *Nigella sativa* at 1 kGy, 3 kGy, 5 kGy and 7 kGy were 2.340 ± 0.243 , 2.243 ± 0.267 and 2.042 ± 0.202 mg/gram, respectively, while the total phenol contents in control (non-irradiated) was 2.825 mg/gm. similarly the total phenol contents of *Caralluma tuberculata* was slight increased at dose levels of 1 kGy (2.79 ± 0.20) as compared to control and then decreased at high radiation levels of 3 kGy, 5 kGy and 7 kGy i.e. 2.21 ± 0.24 , 1.5282 ± 0.09 and 2.127 ± 0.19 mg/g, respectively, while the total phenol contents in control (non-irradiated) was 2.50 ± 0.20 mg/gram. The effects of cobalt-60

gamma radiation on total phenolic contents of different herbal medicinal plants are illustrated in FIG. 1. It can be noted that cobalt-60 gamma irradiation with 7.0 kGy caused slight increase in total phenolic contents in *Nigella sativa* and *Carulluma tuberculata*.

However the maximum increase was observed in *Carulluma tuberculata* followed by *Nigella sativa*. On the other hand gamma irradiation caused reduction in the total phenolic content of *Zingiber officinal* followed by *Allium sativum*. The maximum reduction was observed in *Allium sativum*. Mishra and co-authors stated that no significant effect was perceived in total phenolic in radiated tea grasses at 5 kGy [18]. In contrast, Ahn and co-authors found that radiation at 1 kGy or above expressively decreased the phenolic contents in cut Chinese cabbage heads [19]. Huang and Mau reported a higher content of phenolic in irradiated samples compared to non-irradiated mushrooms, similarly Harrison and were found a slight increase in total phenolic content of irradiated almond skin extracts as compared to that of the control at irradiation levels of 7 kGy and above. The dosage of 8 kGy encouraged an increase in the concentration of total phenolic complexes in raw grains from five soybean cultivars, while reduction at dose levels of 2 kGy and 4 kGy was also noticed [20,21]. For irradiated samples of truffles Adamo reported increase in total phenolic content at the dosage leveled of 1 kGy and 7 kGy proposed that through gamma irradiation destructive process of oxidation were capable of breaking the chemical bonds of polyphenols, liberating soluble phenols of lower molecular weight [22].

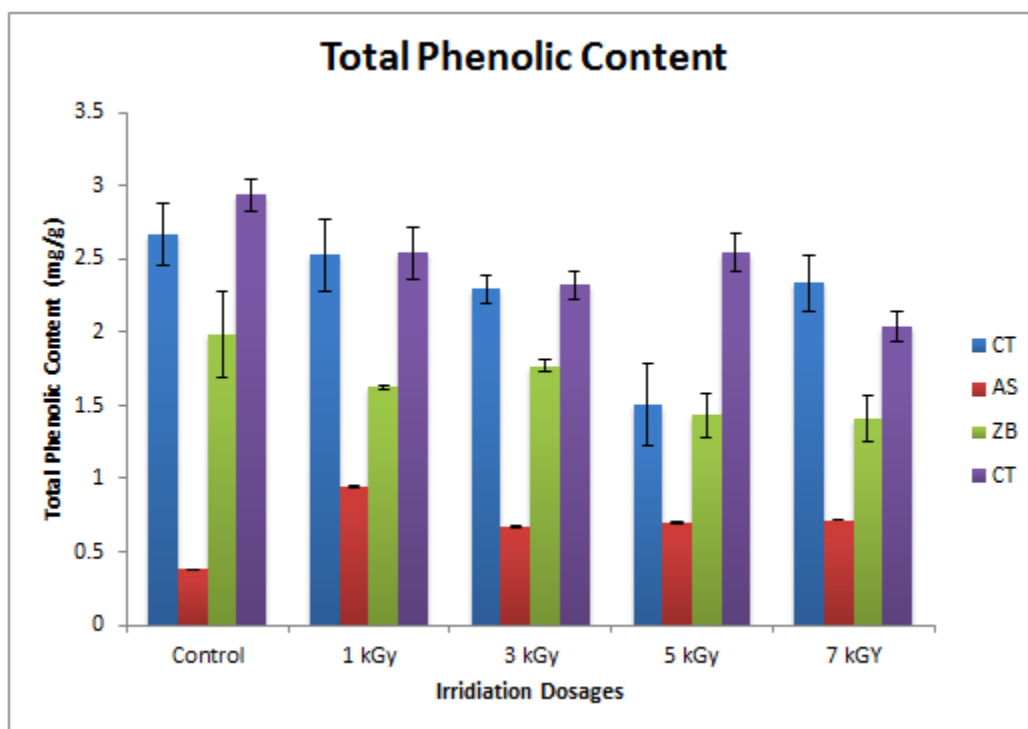


FIG. 1. Effect of cobalt-60 Gamma irradiation on total phenolic content of control and irradiated plants extracts of *Carulluma tuberculata* (CT), *Nigella sativa* (NS), *Zingiber officinal* (ZO), *Allium sativa* (AS). Each data point represents mean \pm SD of triplicate treatments.

Estimation of total flavonoids

Flavonoids are a class of secondary plant metabolites with significant antioxidant and chelating properties. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation. The flavonoid content was expressed in terms of quercetin equivalent in milligrams per gram of plant materials. As it is shown, that in case of *Nigella sativa*, the control has total flavonoids 0.030 mg/g. However the total flavonoids contents were decreased when it was irradiated with Co-60 gamma radiation compared to control the flavonoids contents in *N. Sativa* were 0.020 ± 0.005 , 0.016 ± 0.003 , 0.023 ± 0.004 and 0.018 ± 0.001 mg/g at 1 kGy, 3 kGy, 5 kGy and 7 kGy, respectively.

The total flavonoid contents of non-irradiated (control) of *Carulluma tubercullata* was 0.023 ± 0.003 mg/g. The decrease in total flavonoids contents of irradiated *C. tubercullata* extracts was observed as compared to that of the control at irradiation level of 1 kGy ($0.022 \pm$), 3 kGy ($0.023 \pm$), 5 kGy ($0.020 \pm$) and 7 kGy ($0.016 \pm$). The Table also shows the increase in total flavonoids contents of *Zingiber officinal* control was 0.018 mg/g. while the decrease in total flavonoids contents of irradiated *Z. officinal* extracts was observed as compared to that of the control at irradiation level of 1 kGy (0.011 ± 0.004), 3 kGy (0.013 ± 0.004), 5 kGy (0.012 ± 0.004) and 7 kGy (0.014 ± 0.001). Similarly the total flavonoids content of the non-irradiated (control) *Allium sativum* was 0.08 ± 0.003 mg/g. The increase in total flavonoids contents of irradiated *Allium sativum* extracts was observed as compared to that of the control at irradiation level of 1 kGy ($0.09 \pm$), 3 kGy ($0.013 \pm$), 5 kGy ($0.012 \pm$) and 7 kGy ($0.08 \pm$). Effect of gamma irradiation of cobalt-60 on total flavonoids contents of both irradiated and non-irradiated plant materials are shown in FIG. 1 to 4 respectively.

The increase in total flavonoid was observed in *Zingiber officinal* followed by *Allium sativum* and *Nigella sativa* and the lowest (activity) one is located in *Carulluma tubercullata*. The results show that the gamma irradiation has no significant tendency with the increase of total flavonoids. Chattak reported that in herbal medicinal plant soft tissues the phenolic complexes lignin, tannins, flavonoids and antioxidants precursors may conceivably achieved as reactive oxygen species (ROS) scavenging complexes [23-25]. The increased in total flavonoids contents is found useful for antioxidant characteristics of herbal products because of the polymerization of ingredients of phenolic components and along with the fragmentation and cross linking Stajner proved that both the comparatively high scavenging activity and the higher phenolic content can be correlated, which shows good agreement with the reported work of Yang [26]. The Yang verified the existing correlation between phenolic contents on the rhizome samples of *Nelumbo nucifera* and scavenging activity. Unlikely we found a negative correlation that has been verified by both Zielinski and Kozlowska. Huang and Mau reported that the gamma radiation of mushrooms dosages (freeze dried) among 2.6 ~ 20 kGy, doesn't show a substantial changes in their antioxidant activity. Similarly by UN [26] doesn't found major variations in the scavenging abilities of the non-irradiated and radiated Chung kookjang and Doenjang at 5 kGy, 10 kGy and 20 kGy.

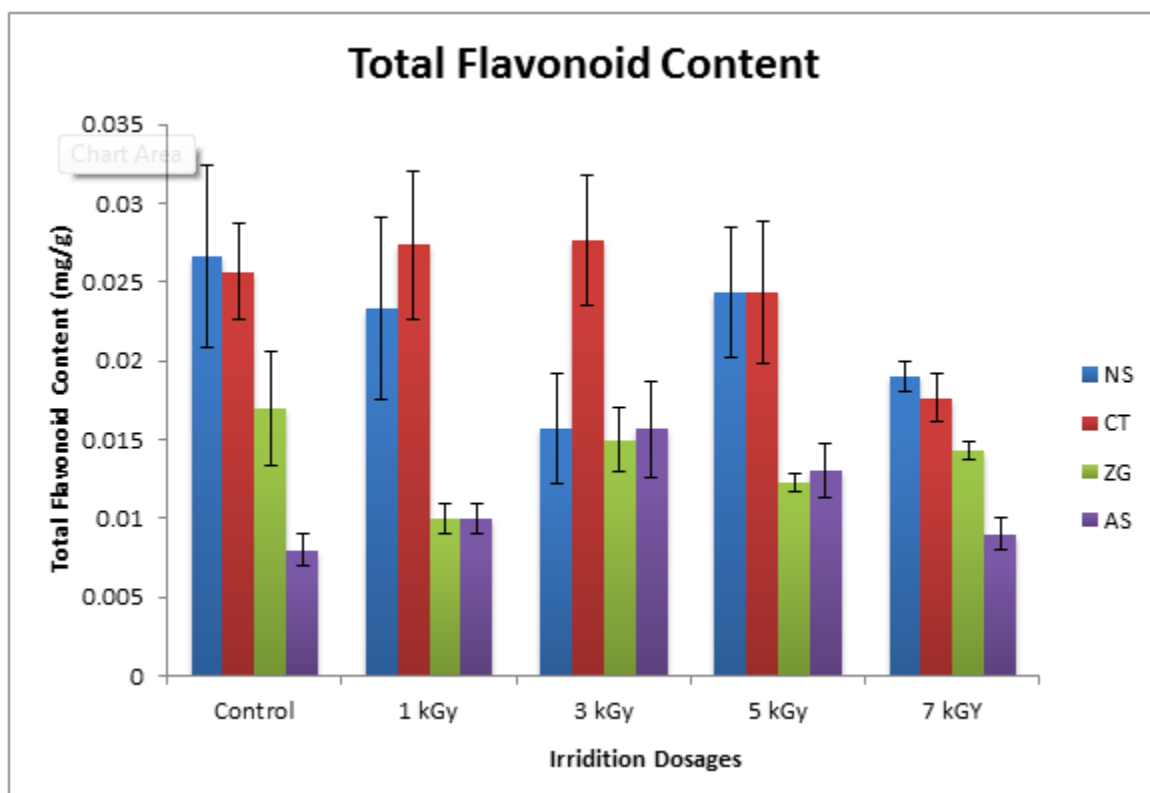


FIG. 2. Effect of cobalt-60 Gamma irradiation on total flavonoids content of control and irradiated plants extracts of *Carulluma tuberculat* (CT), *Nigella sativa* (NS), *Zingiber officinal* (ZO), *Allium sativa* (AS). Each data point represents mean \pm SD of triplicate treatments.

DPPH-Radical-scavenging activity (DRSA)

Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity. The extraction pattern was consistent with total phenolic and flavonoid. The DPPH free radical scavenging activity was expressed in milligrams (mg) per gram (g) of the herbal material. The percent scavenging ability of *Allium sativum* non-irradiated control was 41.67 % \pm 0.73. The increase in scavenging ability of irradiated *A. sativum* extracts was observed as compared to that of the control at irradiation level of 1 kGy (42.42% \pm 0.31) and 3 kGy (45.68% \pm 0.99) respectively. Similarly a slight decrease as compared to the *A. sativum* control at radiation levels of 5 kGy (37.92% \pm 0.25) and 7 kGy (40.67% \pm 1.04) occur, respectively. The anti-oxidant ability of *Zingiber officinal* non-irradiated control was observed (93.11 % \pm 0.61). The scavenging ability of radiated *Z. officinal* at radiation level of 1 kGy and 3 kGy was (94.61% \pm 0.23) and (95.74% \pm 0.79) respectively, which was increased compared to control. Similarly the scavenging percent ability of *Z. officinal* was decreased at radiation level of 3 kGy (92.24% \pm 0.79) as compared to control of *Z. officinal*. It was observed that at 7 kGy (94.24% \pm 0.72) again the increase in percent scavenging of *Z. officinal* as compared to control occurs.

The effects of gamma irradiation on free radical scavenging activity of herbal plants constituents are shown in FIG. 3. respectively. It was observed that *Zingiber officinal*, *Carulluma tuberculata* and *Nigella sativa* have highest anti-oxidative activity whereas the *Allium sativum* has lowest anti-oxidative activity. The gamma irradiation tends to decrease the anti-

oxidant activity of the various deliberate methanolic extracts except the *Carulluma tuberculata*, which is in agreement with reported work of Suhaj et al. (2006), who discovered important reduction in anti-oxidant activity of dark pepper radiated at dosages 5 kGy, 7.5 kGy, 10 kGy, 20 kGy and 30 kGy. Chattak stated that there is increase in the scavenging activity of *N. sativa* seeds when irradiated with gamma radiation [27]. There are various compounds of phenol in plant tissues which are potential antioxidant, flavonoids, tannins and lignin precursor may act as ROS (reactive oxygen species) scavenging compounds. Stajner described that increase in concentration of total phenolic is favorable for antioxidant characteristics of herbal products which is attributed to the polymerization and fragmentation of consistent phenolic compounds [28]. One can correlate the high scavenging activity herbal products to the higher phenolic content, which is in fair agreement with Yang work.

They confirmed a positive correlation between phenolic contents and scavenging activity of the rhizome samples of *Nelumbo nucifera*. On the other hand, Zielinski and Kozłowska reported a negative correlation. Huang and Mau reported that there is no significant modification in scavenging activity of irradiation of freeze dried mushrooms at doses between 2.5 and 20 kGy, respectively [28-30]. Similarly Byun observed that there is no noteworthy variation in the scavenging capabilities of control and irradiated samples at 5.0 kGy, 10 kGy and 20 kGy, respectively [31].

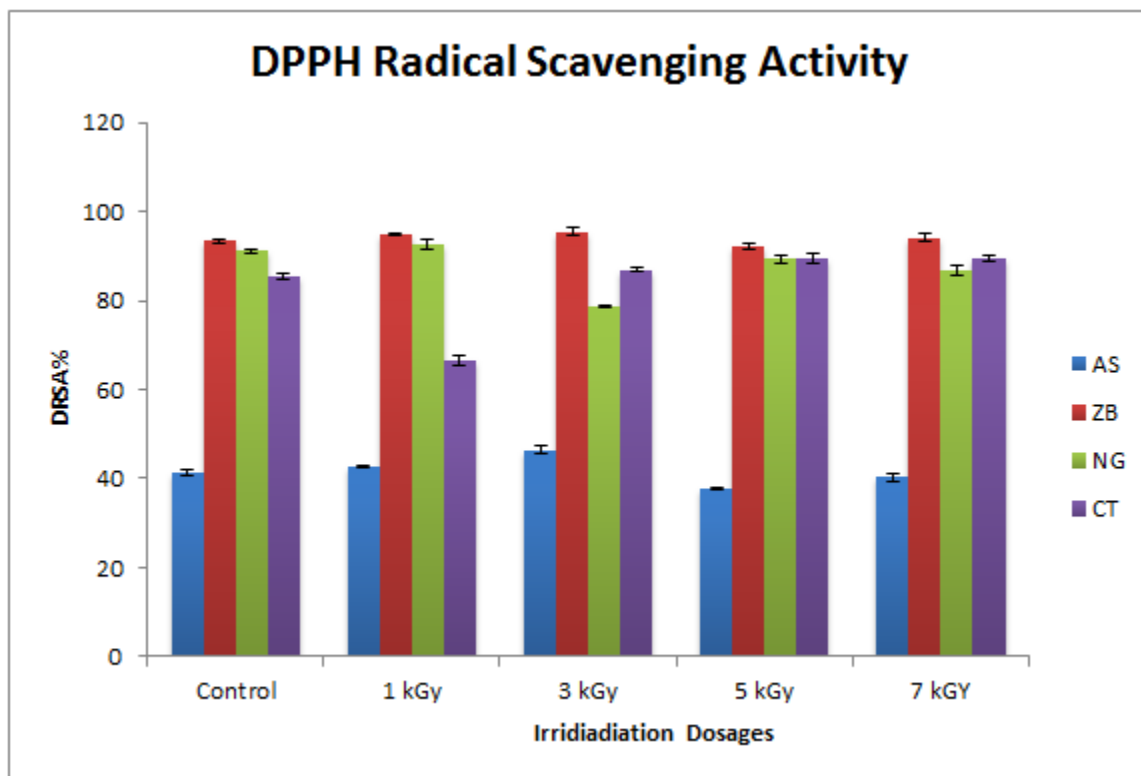


FIG. 3. Effect of cobalt-60 gamma irradiation on DPPH radical scavenging activity of control and irradiated plants extracts of *Carulluma tuberculata* (CT), *Nigella sativa* (NS), *Zingiber officinal* (ZO), *Allium sativa* (AS). Each data point represents mean \pm SD of triplicate treatments.

Total bacterial counts (TBC)

The total bacterial counts (TBC) for the raw or un-irradiated herbal products were extremely high. The log values for *Allium sativum* (control) was 3.9685 which was very high. After the radiation absorbed at the level of 1 kGy the log values decreased to 3.6021 which showed slight decrease in TBC as compared to control *N. sativa*. The log values for *N. sativa* decreased constantly at further radiation levels of 3 kGy, 5 kGy and 7 kGy as $2.3010 \pm$, $1.477 \pm$ and $0.3010 \pm$, respectively which showed that increasing the irradiated dose level could decrease TBC significantly. Similarly in case of *Nigella sativa*, total bacterial count of control was extremely high.

The log value for *N. sativa* (control) was 3.8751 which were very high. After treatment with Co-60 gamma irradiation at dose 1 kGy, 3 kGy, 5 kGy and 7 kGy the total bacterial count was significantly reduced and their log values were $3.3010 \pm$, $2.1761 \pm$, $1.3010 \pm$ and 0.0000 respectively. Similarly in case of *Zingiber officinal*, total bacterial count of control was extremely high. The log value for *Z. officinal* (control) was 3.9081 which were very high. After treatment with Co-60 gamma irradiation at dose 1 kGy, 3 kGy, 5 kGy and 7 kGy the total bacterial count was significantly reduced and their log values were $3.176 \pm$, $3.3973 \pm$, $1.1761 \pm$ and $0.301 \pm$, respectively.

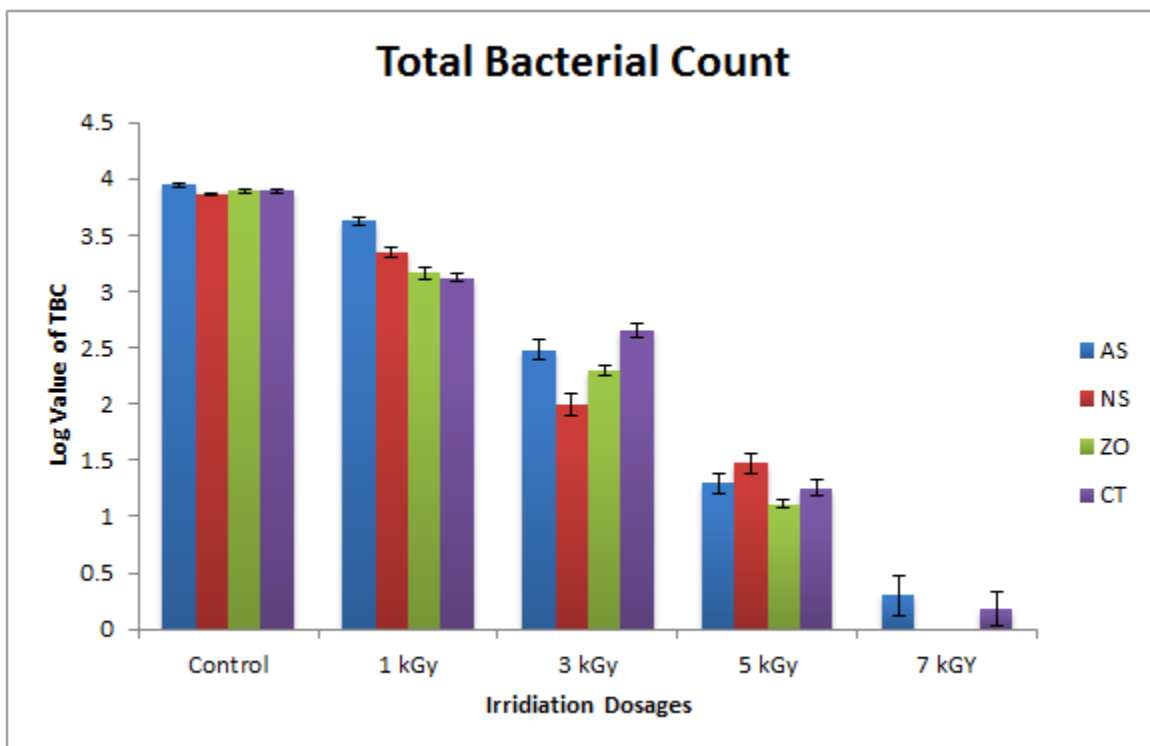


FIG. 4. Effect of cobalt-60 Gamma irradiation on total fungal count (TBC) of *Carulluma tubercullat* (CT), *Nigella sativa* (NS), *Zingiber officinal* (ZO), *Allium sativa* (AS). Each data point represents mean \pm SD of triplicate treatments.

Total fungal count (TFC)

Spoilage of herbal products by various types of fungi is also a serious storage issue which needs proper consideration. Gamma radiations of Co-60 origin are used to reduce the total fungal count in herbal products. The total fungal count was extremely high for un-irradiated sample. In case of *Allium sativum* the log value of TFC was 3.4771, which was extremely high. After irradiating with Co-60 gamma radiation the TFC values were significantly decreased. The log value at 1 kGy *A. sativum* was 2.6020 ± 0.001 which were constantly decreased with increase in radiation dosage. At 3 kGy and 5 kGy the TFC of *A. sativum* were 2.0000 ± 0.003 and 1.761 ± 0.001 , respectively, while at 7 kGy the log value was 0.000. These results showed that irradiating with gamma radiation can significantly decrease the TFC of sample.

Similarly in case of *N. sativa*, total fungal count of control was extremely high. The log value for *N. sativa* (control) was 3.6021 ± 0.005 which were very high. After treatment with Co-60 gamma irradiation at dose 1 kGy, 3 kGy, 5 kGy and 7 kGy the total fungal count was significantly reduced and their log values were 2.4771 ± 0.005 , 2.310 ± 0.003 , 1.3010 ± 0.004 and 0.3010 ± 0.001 respectively. These results showed that irradiating with gamma radiation can significantly decrease the TFC of sample.

The total fungal count (TFC) for the raw or un-irradiated herbal products was extremely high. The log value for *Zingiber officinal* control was 3.5441 ± 0.003 which were very high. After the radiation absorbed at the level of 1 kGy the log values decreased to 2.9031 ± 0.001 which showed slight decrease in TFC as compared to control *Z. officinal*. The log values for *Z. officinal* decreased constantly at further radiation levels of 3 kGy, 5 kGy and 7 kGy as 2.5441 ± 0.002 , 1.379 ± 0.002 and 0.3010 ± 0.005 , respectively, which showed that increasing the irradiated dose level could decrease TFC significantly.

Similarly in case of *Carulluma tuberculata*, total fungal count of control was extremely high. The log value for *C. tuberculata* (control) was 3.5798 which were very high. After treatment with Co-60 gamma irradiation at dose 1 kGy, 3 kGy, 5 kGy and 7 kGy the total fungal count was significantly reduced and their log values were $2.9542 \pm$, $2.3979 \pm$, $1.3010 \pm$ and 0.0000, respectively. These results showed that irradiating with gamma radiation can significantly decrease the TFC of sample shown in FIG. 5.

Effect of irradiation on the microbial load

The control samples of *Nigella sativa* (black seed), *Zingiber officinal*, (Ginger), *Allium sativa* (Garlic) and *Carulluma tuberculata* (Choonga) were contaminated by microbes. In 1998 the WHO reported the maximum permissible total count level of 1.0×10^4 cfu/g. This high contamination level is because of great expected micro vegetation of the aromatic plant along with the common circumstances throughout their agronomy, processing, drying, harvesting, handling, storage, sales and supply. However, it has been testified that during processing the bacterial position of the herbal medicinal plants constituents is not so far affected by lesser impurity, however possibly will mostly owing to the microbial flora which herbal

medicinal plants ensure [32]. This total microscopic load decreased linearly with absorbed radiation dose as shown in figures closed agreement with the reported results of Nemtanu who described that 5 kGy radiation leads to decrease the desired

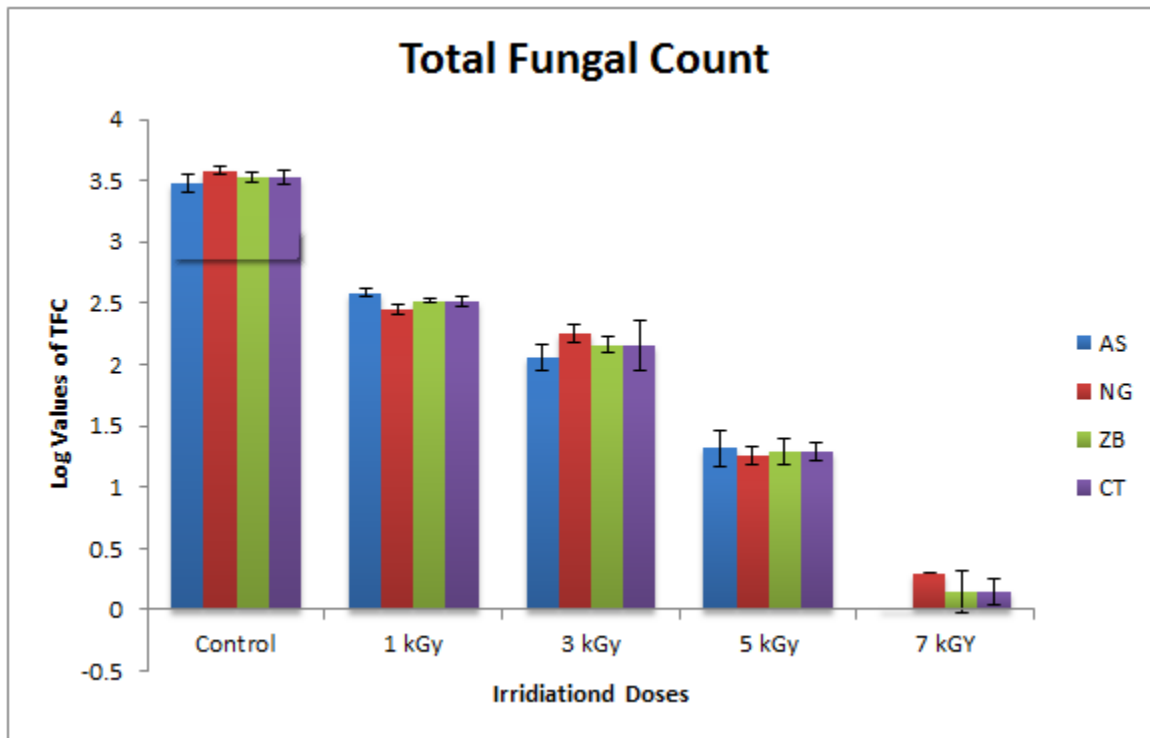


FIG. 5. Effect of cobalt-60 Gamma irradiation on total fungal count (TFC) of *Carulluma tubercullat* (CT), *Nigella sativa* (NS), *Zingiber officinal* (ZO), *Allium sativa* (AS). Each data point represents mean \pm SD of triplicate treatments.

microbial reduction [33]. At 5 kGy the uppermost compassion of microorganisms to cobalt-60 gamma irradiation were detected in *Carulluma tubercullata*, whereas the lowest sensitivity was detected in *Allium sativum*. However at 7 kGy the micro-organisms were absent.

The gamma radiation at a dosage level of 5 kGy decreased the total microbial load of herbal extracts by 98.5% and it was totally removed at a dosage level of 7 kGy [34]. To completely decontaminate the product and to obtain the required standard the use of gamma irradiation helped it by chance. To achieve the highest standard of medicinal herbal product and food through the destruction of microorganism gamma radiation is one of the hardly any processes that allow it. Gamma irradiation kills the microbes by excruciating the molecules of water into hydrogen, hydroxyl and oxygen radicals is the primary mechanism involve these radicals combine with the microbial components like DNA and destroy or deactivate them so a result they stop their function [35]. Gamma irradiation has the ability to extend shelf life and improve the hygienic quality of the product and also increase their effectiveness on both export markets and on domestic levels. Consequences of this study has revealed that gamma radiation of the herbal products with medium to high doses significantly decrease their

bacteriological loads to standard domestic and worldwide values in contrast to control or un radiated samples. To completely decontaminate the herbal product to acceptable standard effective gamma irradiation dosage from 3 kGy to 7 kGy was required. This observation might be due to non-uniformity of the microbial loads and that underlines the need for good manufacturing practices (GMPs) in production protocols to ensure low microbial load of products and subsequently the use of low (effective) irradiation doses. A dose of 7 kGy was significant to remove all polluting micro flora in all the herbal products.

Further deep study at 7 kGy of all the samples indicate that microbial load reduced to the level of $<10^3$ cfu/g and the examination at 5 kGy reduced the microbial load the level of $<10^4$ cfu/g. in other hand in a related examination gamma radiation amongst 1 kGy-7 kGy decrease the microbial load of the herbal medicinal product less than 10^3 cfu/g without causing any useful variations in composition and quantity of the product [36]. Further study of the raw herbal products at 7 kGy decreases the microbial load from 6 log cycles to 8.6×10 cfu/g. In the same way the microbial load of some local herbal teas decreases by 4 to 5 log cycles at 2 kGy to 3 kGy.

Conclusion

In Pakistan approximately there are 3,600 plant species of which over 2, 900 are indigenous has a rich biodiversity. Production of herbal products therefore has a high potential in country in addition to the fact that about 33% medicines worldwide are derivative of lower and higher plants. It is important to improve and modernize the herbal medicine industry for the country to break into the global market. Through the use of gamma radiation herbal product production of high quality assurance the state a vast trade marketplace like in most Asian countries. It is suggested that upcoming educations should examine the radio sensitivities of the various fungal and bacterial isolates of medicinal herbal yields with the view to their removal with low gamma radiation doses.

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