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ORIGINAL ARTICLE

Effect of the Electron Beam Irradiation on Microbial Load and Nutritional Composition of Some Medicinally Important Herbal Raw Materials

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ABSTRACT

Medicinal herbs are involving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Efficacy of many drugs is fading because of the adultered dried raw materials profusely available in indigenous market. Due to this adulteration and altered efficacy the faith in crude drug promotion has declined, affecting the global promotion in Indian herbal products. Present study was carried out to assess the microbiological and biochemical characteristics of six different herbal raw materials namely Terminalia chebula (fruit), Phyllanthus emblica (fruit), Solanum xanthocarpum (root), Citrulus colocynthis (stem), Woodfordia fruticosa (flower) and Withania somnifera (root) after electron beam irradiation. Radiation processing was carried out at dose levels of 8, 12 and 15 kGy. Irradiated and control samples were analysed for microbial load and biochemical compositions like carbohydrates, proteins, phenolics and tannins. Results indicated that the electron beam irradiation significantly reduced microbial load in the samples in a dose dependent manner. Doses selected in the study were sufficient for complete microbial decontamination without affecting the nutritional quality of herbs to a larger extent. Thus in conclusion, electron beam radiation can be considered an effective mean for decontamination of microbial flora of the raw herbal materials and increase their shelf life.

Keywords: *Herbal raw materials, electron beam radiation, microbial load, decontamination*

INTRODUCTION

Plants and their products have been used in prevention, treatment and cure of various disorders since ancient times. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body.

Spices and medicinal plants are widely used as raw material for pharmaceutical preparations and as a supplement for dietetic products, specifically for "self medications". India is sitting on a gold mine of well-recorded and well practiced knowledge of traditional herbal medicine. Medicinal plants may be associated with a broad variety of microbial loading and exert impact on the overall quality of herbal products and preparations [1]. These plants are normally carrying a large number of bacteria and molds, often from soil origin [2]. Generally, herbs are valued for their distinctive aroma, colour and flavour. Most raw materials for pharmaceutical products support some form of microbial growth, depending on the nutritive properties and moisture contents. Microbial contamination is influenced by environmental factors such as temperature, humidity, extent of rainfall during pre-harvesting, harvesting and post-harvesting periods, handling practices, transportation and storage conditions of crude and processed medicinal plant materials.

Microbiological contamination of medicinal herbs is a serious problem in the production of therapeutically preparations. The increasing popularity of natural drugs made their use as a public health problem due to lack of effective surveillance of the use, efficacy, toxicity and quality of these natural products. Indeed, the adverse effects of long-term herbal use adulteration with toxic compounds and contamination by pathogenic microbial or natural toxins like mycotoxins have

been reported for herbal products and medicinal plants [3, 4, 5, 6]. Decontamination treatments should be fast and effective against all microorganisms. The conventional methods of decontamination were fumigation with gaseous ethylene oxide or methyl bromide, which are now prohibited or being increasingly restricted in most advanced countries for health, environmental or occupational safety reasons [7]. Irradiation is an effective technology for resolving technical trade issues for many food and agricultural products. As a disinfection treatment, it offers good broad-spectrum control of many pathogenic and spoilage organisms with minimal change to the herbs [8]. The present study was aimed to determine the types and level of bacterial and fungal load and feasibility of electron beam irradiation to hygenise the commonly used Indian herbal raw materials namely *Terminalia chebula* (fruit), *Phyllanthus emblica* (fruit), *Solanum xanthocarpum* (root), *Citrulus colocynthis* (stem), *Woodfordia fruticosa* (flower) and *Withania somnifera* (root).

MATERIALS AND METHODS

Herbal materials

Six herbal raw materials such as *Terminalia chebula* (fruit), *Phyllanthus emblica* (fruit), *Solanum xanthocarpum* (root), *Citrulus colocynthis* (stem), *Woodfordia fruticosa* (flower) and *Withania somnifera* (root) were selected for present study and were procured from Ayurveda Pharmacy, Moodbidri, Karnataka where they had been stored in plastic containers, jute sacs under warm humid conditions. The samples were irradiated at different dosages and stored at room temperature. Analysis of these samples was carried out once in four months to check the efficiency of electron beam irradiation in microbial decontamination and changes in biochemical parameters like carbohydrates, proteins, tannins, and phenolics.

Sample irradiation with electron beam radiation

Electron beam irradiation was carried out at Microtron Centre, Mangalore University. Ten gram of samples were packed and sealed in four different high density polythene packets. Samples were exposed to radiation by keeping them at a distance of 30 cm from the beam exit, where uniform beam distribution was very well within in the acceptable limits (samples were kept on a perspex sheet and exposed as dosimetry was also carried out in the same condition) Current integrator calibrated with chemical dosimeters was used to set the doses. The delivered dose uncertainity is very small and it is less than 5%. Irradiation was done in three different doses viz., 8, 12 and 15 kGy with the electron beam energy 8 MeV. Dose uniformity ratio (Dmax/Dmin) was <1.05 (in 6 cm x 6 cm area at 30 cm from the beam exit point). The un-irradiated samples served as controls. Irradiated and control samples were stored at ambient temperature (26 +2^{0}C) until the analysis were carried out.

Biochemical analysis

Spectrometric measurements were performed using UV/VIS Spectrophotometer (ELICO- SL 159) at the wavelength mentioned. All the measurements were carried out in triplicates and the results were analysed and expressed as mean ± standard error.

Total carbohydrate content

Total carbohydrate content was determined by Anthrone method [9]. Using dilute hydrochloric acid 100mg of sample was hydrolysed into simple sugars. It was then neutralised with anhydrous sodium carbonate, centrifuged, supernatant was diluted to a known volume. In a set of experiment 200 l aliquot of sample was mixed with 800 l of cold anthrone reagent and incubated in boiling waterbath for eight minutes. Reaction mixture was rapidly cooled and absorbance was measured at 630nm.

Protein content

Protein content was estimated by Lowry's method [9]. In a set of experiments, 200 l aliquots of aqueous extract of samples was thoroughly mixed with 1000 l of alkaline copper solution and allowed to stand for 10min at room temperature, later 100 l of Folin- Ciocalteau reagent was added and suspension was further incubated in dark for 30min. Absorbance of reaction mixture was measured at 660nm.

Phenolic content

Phenolic content was analysed using Folin- Ciocalteau colorimetric method [9]. Sample was extracted with 80% ethanol, centrifuged and supernatant was evaporated to dryness. The residue was dissolved with known volume of distilled water. In a set of experiment 500 l aliquots of

sample were diluted to 1ml using distilled water. The mixture was further mixed with 125 l of Folin- Ciocalteau reagent. After 3 min 500 l of 20% Na_2CO_3 was added and the reaction mixture was incubated in boiling water bath for 1min, cooled. The absorbance was measured at 650nm and result was expressed as catechol equivalents (mg CAE/g) of the sample.

Tannin content

Tannin content was determined spectrophotometrically by Folin- Denis method [9]. Herbal sample was boiled in distilled water for 30min. Centrifuged and the supernatant as collected. In a set of experiment, 100 l aliquots of sample is mixed with 100 l of Folin- Denis reagent and 200 l of Na_2CO_3 and make the volume to 2ml. Incubate at room temperature for 30min and the absorbance was measured at 700nm and the result was expressed in tannin equivalents.

Microbial enumeration

The microbial study was done to enumerate the type, level of bacterial and fungal load and feasibility of three different doses of irradiation on herbal raw materials. The level of microbial contaminants before and after irradiation was enumerated by following standard methods.

Bacterial count

For bacterial count serial dilution method was followed and serial dilution of 10^{-3} was selected, from which 0.1ml aliquot was spreaded on nutrient agar plates by spread plate technique for assessment of microbial load. The plates were incubated at 30±2°C for 48h. Colony forming units of each sample was calculated for assessing the microbial load.

Fungal count

For fungal count serial dilution of 10^{-3} was selected. 0.1ml aliquot from 10^{-3} serial dilution was spreaded on Potato dextrose agar (PDA) plates supplemented with streptomycin for assessment of fungal load. Plates were incubated at room temperature for five days. The fungal colonies were counted, identified using standard manuals Illustrated genera of Imperfect fungi [10] and An Illustrated Manual on identification of some seed borne Aspergilli, Fusaria, Penicillia and their mycotoxins [11].

RESULTS

In the present study bacterial population was drastically reduced in the irradiated raw materials at 12kGy and there was no bacterial count noted at 15kGy in all the plant species over the control (Fig.1). Among bacterial invaders *E. coli* and species of *Staphylococcus* occurred in all the raw materials. The figure 2 represents the fungal colonies recorded in different dosages in six herbal raw materials. *Withania somnifera* had higher fungal mycoflora followed by *Solanum xanthocarpum* and *Phyllanthus emblica.* In general at 8kGy of radiation reduces the contaminants, whereas 12kGy fully removed the fungal flora from *Withania somnifera, Phyllanthus emblica* and *Terminalia chebula.* The fungi enumerated from raw materials includes species of *Penicillium, Rhizopus, Mucor* and *Aspergillus*. Among the genus *Aspergillus*, *A. niger* and *A. flavus* observed as predominant.

Biochemical analysis of raw materials showed a variation with respect of irradiation dosage and storage periods in different herbal raw materials (Figs 3-6). In some raw materials the phenolic and protein content decreased with increase in dosage, while in others the variation is not consistent.

DISCUSSION

The effect of electron beam irradiation on the microbial contamination of herbs is dependent on dose and the type of microorganism present. Its effect on bacterial reduction is relatively dose dependent manner, so the end result depends on the initial contamination level. In the present study the microbial load was proportionately reduced with three different doses of irradiation on viz, 8, 12 and 15kGy in raw materials of six plants namely, *Terminalia chebula, Phyllanthus emblica, Solanum xanthocarpum, Citrulus colocynthis, Woodfordia fruticosa* and *Withania somnifera* compared to non-irradiated one. The complete decontamination was observed in different plants at different dosages of irradiation. Among the six herbal raw materials *Terminalia chebula* recorded highest bacterial count which was assumed due to its high nutritive contents. In *Phyllanthus emblica* and *Woodfordia fruticosa* complete microbial decontamination was achieved at 8kGy. Dose

of 12kGy was sufficient to completely hygenise the *Solanum xanthocarpum* and *Citrulus colocynthis* samples. Dose of 15kGy was necessary to completely hygenise the *Terminalia chebula* and *Withania somnifera* samples.

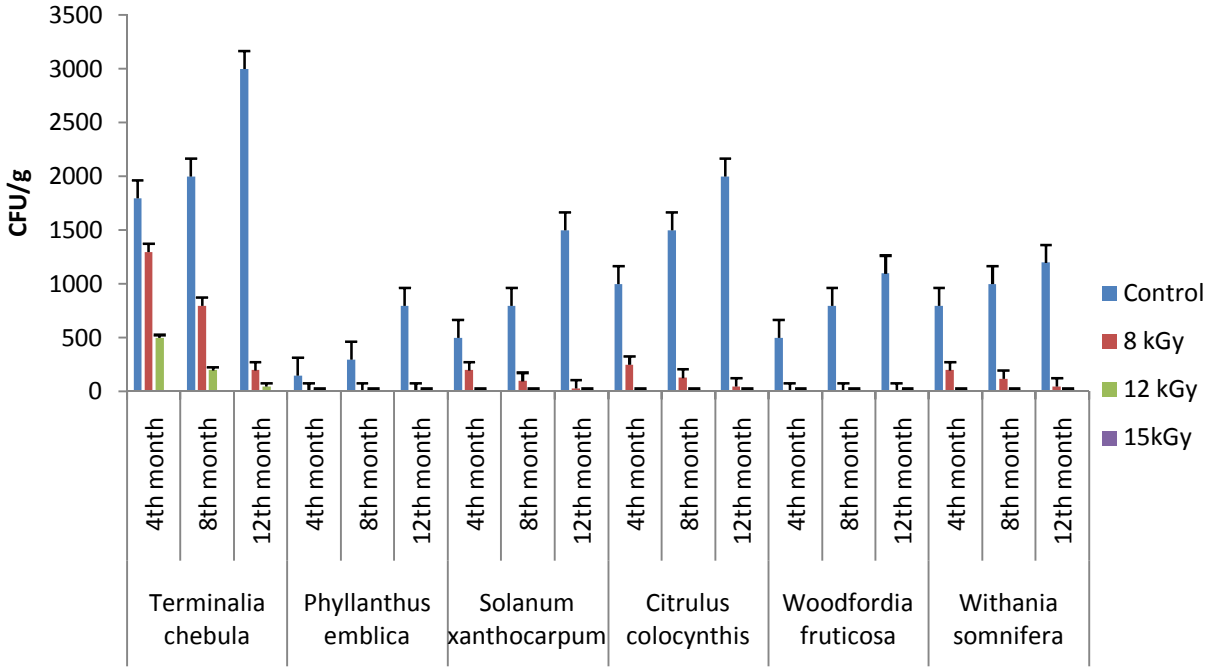

Fig. 1: The variations in bacterial count (CFU/g) in herbal raw materials after irradiation and storage

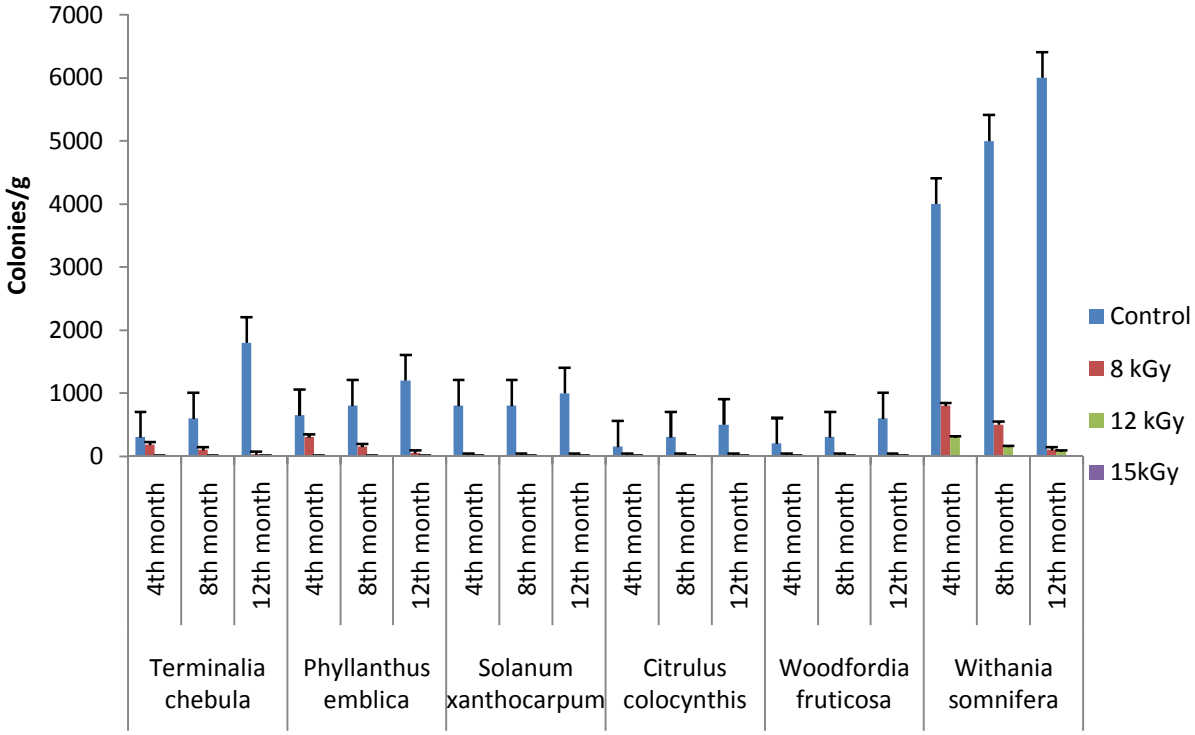

Fig. 2: The variations in fungal count in herbal raw materials after irradiation and storage

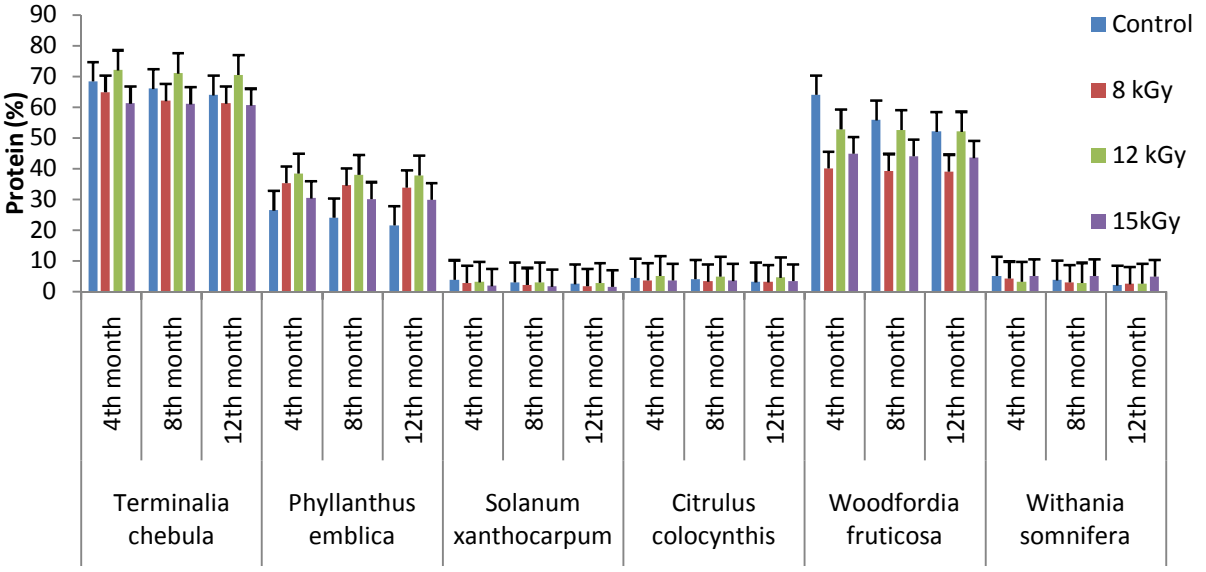

Fig. 3: The variations in total protein (%) in herbal raw materials after irradiation and storage

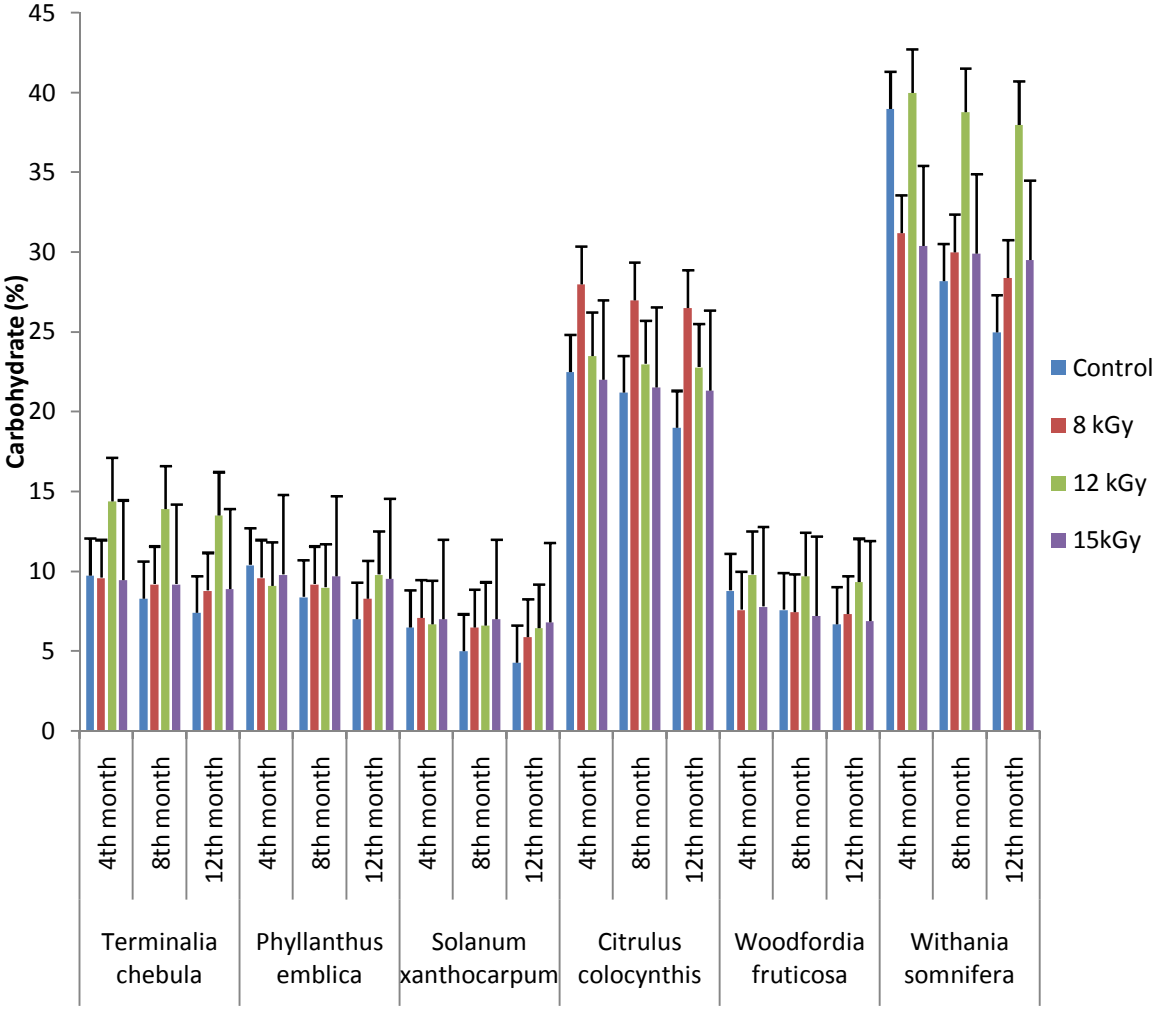

Fig. 4: The variations in total carbohydrate content (%) in herbal raw materials after irradiation and storage

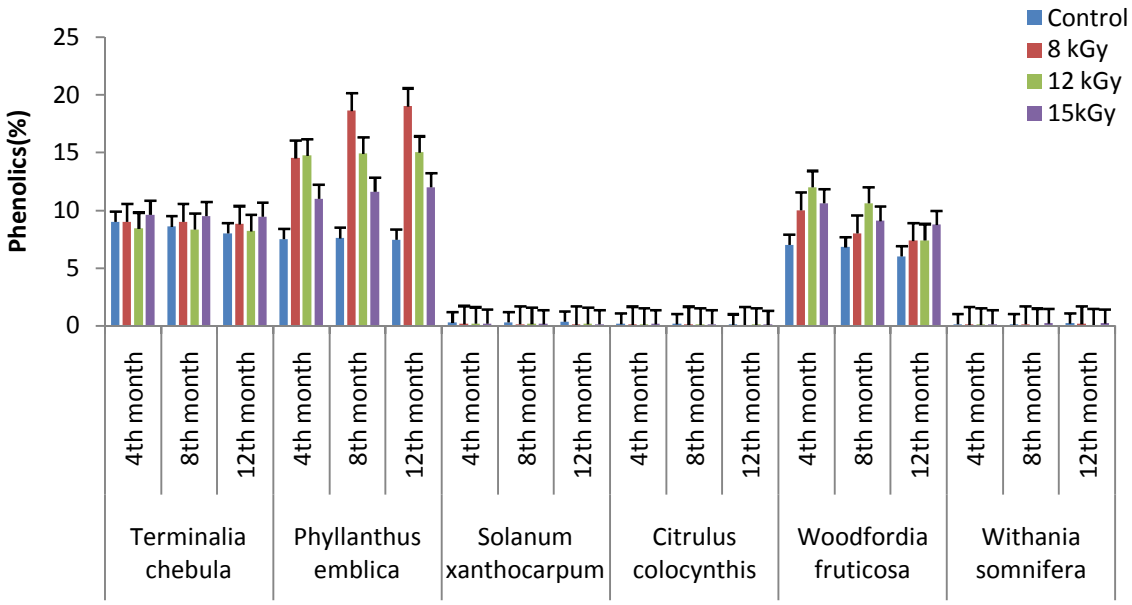

Fig. 5: The variations in phenolic content (%) in herbal raw materials after irradiation and storage

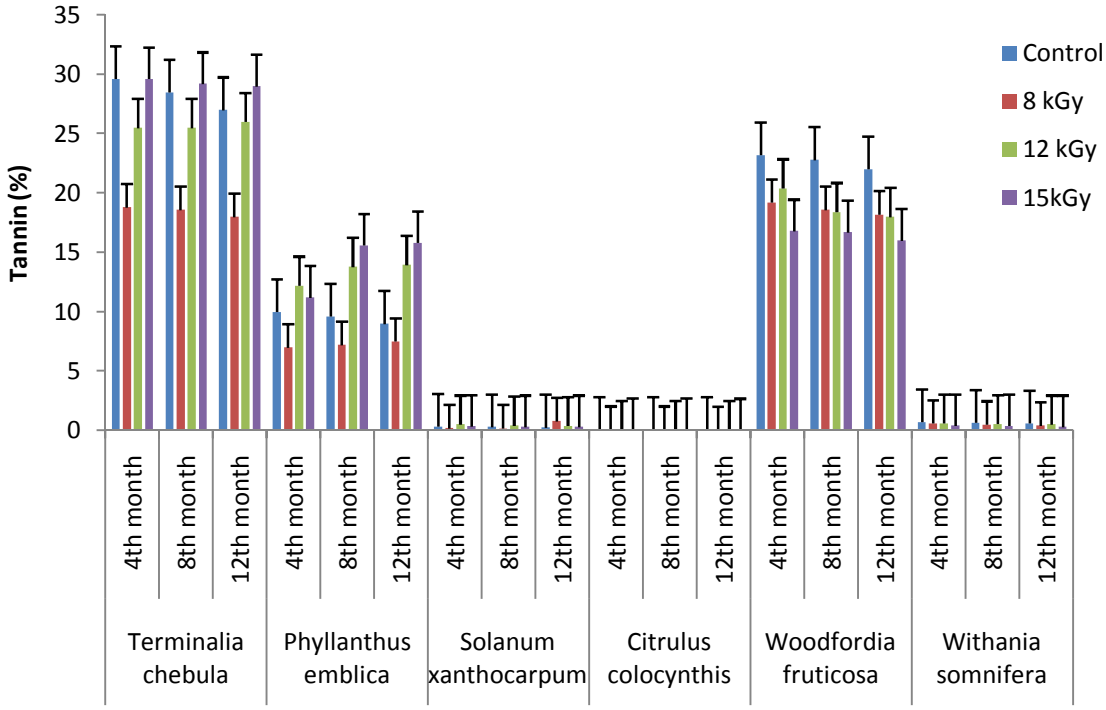

Fig. 6: The variations in Tannin content (%) in herbal raw materials after irradiation and storage

Eschericha coli and *Staphylococcus* spp. were predominant among the contaminants at varying levels. A similar investigation was made Warude [12] and recorded the existence of pathogenic bacteria like *E. coli, Salmonella typhi, Pseudomonas aeroginosa* and *Staphylococcus aureus* and also the gastrointestinal illness effect of *Staphylococcus* spp. [13,14]. Contamination by microorganisms was influenced by environmental conditions, improper handling and storage of medicinal plants. Gamma irradiation of 7.7 kGy and 8.8 kGy was effective in decontaminating *Salmonella* spp., *Staphylococcus* spp., and coliform bacteria that occured in 17 species of herbs used in Thai traditional remedies [8].

Among the six herbal samples selected in the present study *Withania somnifera* encountered highest fungal load followed by *Solanum xanthocarpum* and *Phyllanthus emblica*. A dosage of 8kGy was sufficient to destroy all fungal contaminants, whereas dosage of 12kGy was needed to completely hygenise *Withania somnifera, Phyllanthus emblica* and *Terminalia chebula.* The herbs were contaminated with *Penicillium* spp., *Rhizopus*, *Mucor* and *Aspergillus* spp., of which *Aspergillus niger* and *Aspergillus flavus* were found to be dominant. Fungal contamination has been reported to affect the chemical composition of the raw materials and thereby decrease the medicinal potency of herbal drugs as reported in one of the earlier studies [15]. Mold and aflatoxin contamination was seen in stored raw materials of six medicinal plants, *viz*. *Adhatoda vasica*, *A. racemosus*, *Evolvulus alsinoides*, *Glycyrrhiza glabra*, *P. zeylanica* and *T. chebula* [16]. The fungal species commonly enumerated in herbal materials and finished products are *Fusarium, Aspergillus, Penicillium, Mucor, Rhizopus, Absidia, Alternaria, Cladosporium and Trichoderma* [12]. Contamination of rhizomes of *Asparagus racemosus, Atropa belladona, Withania somnifera, Plumbago zeylanica*, fruits of *Terminalia chebula* and seeds of *Mucuna pruriens* with fungal aflatoxins was reorted [17]. Fungal contaminated herbal plants are a health hazard to people, particularly in the case of *A. flavus, A. ochraceus* and species of *Fusarium* and *Penicillium* [18]. Soil and air are the main inoculum source for causing contamination in crude spices and other field practices like harvesting, handling and packing may cause additional contamination [19]. In the present study there was no uniformity in association of fungal species with raw materials. It may be due to the presence of specific secondary metabolites in different raw materials which may be fungitoxic in nature to some of the fungal species and provide chemical resistance against them. If left untreated, mold can produce aflatoxins, which destroy the flavour and colour of the ingredients and make them unsuitable for use. Microbial contamination levels in irradiated herbs continued to decline during storage because radiation-damaged microorganisms cannot reproduce. Radiation treatment has been found to control pathogens and other microorganisms [20]. US Food and Drug Administration (FDA) regulations allow the irradiation of herbs, spice and vegetable seasonings for microbial disinfection up to 30 kGy .

In the present study minimum variation was recorded in the carbohydrate, protein, phenolic and tannin content of the herbal samples with irradiation treatment. Phenolic content of herbal samples increased with irradiation treatment. It may be attributed to degradation of tannins by irradiation and the interruption of the phenolic compound's structure resulting in increased extraction of phenolic content [21]. The extent of chemical changes that occurs in dehydrated state is less than the change that occurs in fully hydrated state [22]. Chemical degradation depends on chemical structure, physical state, and chemical composition of substrate [23]. It is well known that the nutritive values of the macronutrients in the diet (proteins, fats, carbohydrates) are not significantly altered by irradiation at the petitioned doses. Irradiation of carbohydrates at doses up to 10kGy has minimal effect on carbohydrate functionality [22]. Irradiation of herbal materials with electron beam with suitable radiation doses decontaminate microorganisms and did not alter the biochemical constituents of the tested materials and preserved the nutritive quality of herb.

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