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Effect of Gamma Irradiation on the Extraction Yield and Microbial Contamination of Medicinal Plants

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Abstract

Medicinal plants normally carry high bio-burden due to their origin, offering potential hazards to the consumer. In the present study Terminalia chebula, Curcuma longa, Syzygium aromaticum and Mentha piperita were irradiated to gamma rays at doses 2.5, 5.0, 10.0, 20.0 and 25.0 kGy. The microbiological quality of irradiated and un-irradiated medicinal plants was assessed by counting the number of total aerobic mesophilic bacteria, yeast and molds, and coliforms. The gamma irradiation at a dose of 2.5 kGy resulted in 2 log reduction in total aerobic mesophilic bacterial count except Syzygium aromaticum. However, no yeast and molds, and coliforms were found after irradiation at 2.5 kGy. The Gamma irradiation at a dose of 5.0 kGy resulted in 5 log and 4 log reduction in aerobic mesophilic bacterial count of Terminalia chebula and Mentha piperita respectively. Complete sterilization was obtained at 10 kGy. The total extraction yield of the all the medicinal plants was increased by 0.5 to 25% by a dose of 25 kGy. This study indicates that gamma irradiation increases the extraction yield and is an effective treatment for microbial decontamination of medicinal plants.

Key words: medicinal plants, gamma irradiation, microbial contamination

Introduction

Despite tremendous advances in modern medicine, plants continue to make important contribution to health care as witnessed by the increasing interest in alternative therapies like Ayurveda, Unani and Siddha (Rates 2001). The properties of traditionally used drugs have been documented in detail in “Ayurveda”, one of the sacred texts of the Hindu philosophy. It is the foundation stone of ancient medical science of India followed by “Susruta Samhita” and “Charaka Samhita”. According to estimates of World health organization, plants are not just the main component of traditional medicines but they also form up to about 70 per cent of the basis of modern pharmaceutical products. One example is acetylsalicylic acid, the main ingredient in pain killers, which was first extracted from domestic willow almost 150 years ago (Anonymous 2011). The use of medicinal plants as a remedy for various ailments among the tribals in India and world over is evident from various ethnobotanical surveys. As in Ayurveda many medicinal plants viz. Curcuma longa, Azadirachta indica, Emblica officinalis, Ficus benghalensis, Terminalia arjuna, Ocimum sanctum etc. have been used probably as early as 1900 B.C. (Aggarwal et al. 2007). Medicinal plants are precious gifts of nature and are also used to develop characteristic aroma, colour and taste of food.

Medicinal plants are subject to deterioration by chemical and microbial processes during the harvesting, processing, storage and their distribution. Attempts have always been made to decontaminate and preserve these medicinal plants so as to get more safe, natural and potent medicines. The number of methods has been tried for decontamination such as heat treatment, UV irradiation and fumigation. However, volatility and heat sensitivity of the delicate flavor and aroma components of the medicinal plants do not permit the use of heat treatment. Low penetration power of UV radiations makes this irradiation method unsuitable. Fumigation with gaseous ethylene oxide brings down the microbial burden but this method is now prohibited or restricted in many countries due to the carcinogenic nature of one of its residue in treated medicinal plants (Loaharanu 1990; Dickman 1991; Uiji 1992). Consequently, a safe alternative hygienic technology is the need of the day. Various disinfectant technologies have been suggested which include electromagnetic radiations, photo-dynamic pulsing, ultrahigh pressure and CO2 treatment. Among the

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electromagnetic radiations, gamma irradiation is now gaining interest as one of the modern technologies for decontamination with wide range of applications. The source of gamma rays is radioactive elements either Cobalt-60 or Cesium- 137.

Gamma irradiation is now getting recognition throughout the world as a phytosanitary treatment of herbal materials. It improves the hygienic quality of various herbal materials and reduces the losses due to microbial contamination and insect damage (Farkas 1998; IAEA 1992). Besides, it is a fast, safe, convenient, eco-friendly method which reduces the reliance on chemical fumigants and preservatives currently used by industries. The chances of recontamination are also reduced, as it can be done after packaging. However, medicinal plants are still supplied without eliminating pathogens due to the lack of information of various aspects like biological and chemicals effects.

To our knowledge, adequate literature is not available on the effect of gamma irradiation on extraction yield and various microbiological parameters for Terminalia chebula, Curcuma longa, Syzygium aromaticum and Mentha piperita. Therefore, the present study is aimed at evaluation of the effect of various gamma irradiation dose levels on the extraction yield and microbial contamination of the medicinal plants.

Material and Methods

Plant materials. The Medicinal plants viz. Terminalia chebula Retz. (fruit), Curcuma longa Linn. (Rhizome), Syzygium aromaticum Linn. (Flower buds), and Mentha piperita Linn. (Leaves) were purchased from the local market in Ghaziabad, Uttar Pradesh, India and identified by botanists. The collected plant materials were air- dried for 5-7 days under shade at room temperature, powdered with a grinder and packed in polythene bags.

Gamma irradiation. The polythene packed pulverized medicinal plants (150 g each) were irradiated using gamma rays from a cobalt-60 source at room temperature. The dose levels applied were 2.5, 5.0, 10.0, 20.0 and 25 kGy. Un-irradiated medicinal plants were used as a control.

Enumeration of microorganisms. The microbiological methods were used from 'protocol for testing Ayurvedic, Siddha and Unani medicines' (Anonymous 2007). Using pour plate method, samples (10g) were decimal diluted serially with saline solution and added to Nutrient Agar for total mesophilic bacterial count, Sabouraud chloramphenicol agar for total yeast and molds count and Mac-Conkey agar for total coliforms count. All these inoculated medium were incubated aerobically and respectively at 30 ± 2°C for 72h, 25 ± 2°C for 5 days and 30 ± 2°C for 48h and colony-forming units (CFU) were counted.

Sterility test. The sterility test was performed using Sabouraud dextrose broth medium for fungi and fluid thioglycollate medium for bacteria and incubated at 25 ± 2°C and 32 ± 2°C respectively for 14 days.

Extraction yield. The irradiated and un-irradiated medicinal plants (50 g each) were separately extracted in methanol using a soxhlet extractor. All the extracts were filtered through Whatman No. 1 filter paper and concentrated under vacuum at 45°C. Extraction yields were calculated using standard method (Khanzadi et al. 2008).

Statistical analysis. All determinations were obtained from triplicate measurements and results were expressed as mean ± standard deviation. The data were analyzed using one-way ANOVA and least significant difference tests for the mean differences between controls and irradiated samples for all the parameters. Statistical significance was declared at $p < 0.05$, or mentioned otherwise.

Results and Discussion

The total aerobic mesophilic bacterial count in the un-irradiated medicinal plants were found to be in the range of 103 -106 CFU g-1 (Table1). Irradiation at a dose of 2.5 kGy resulted in 2 log reduction however, 3 log reduction was found in Syzygium aromaticum. The Gamma irradiation at a dose of 5.0 kGy resulted in 5 log and 4 log reductions in case of Terminalia chebula and Mentha piperita respectively, however no mesophilic bacterial count was recorded in Curcuma longa and Syzygium aromaticum.

Table 1. Effects of various doses of gamma irradiation on the total mesophilic bacterial count (CFU g^{-1})

Medicinal plant	0kGy	2.5 kGy	5 kGy	10kGy	20kGy	25kGy
Terminalia chebula	3.5×10^6	2.5×10^4	6.7×10^1	ND	ND	ND
Curcuma longa	2.1×10^3	1.8×10^1	ND[a]	ND	ND	ND
Syzygium aromaticum	1.6×10^5	5.2×10^2	ND	ND	ND	ND
Mentha piperita	2.8×10^5	3.8×10^3	3.5×10^1	ND	ND	ND

[a] ND- Not detected (Detection limit > 10 CFU $g^{-1)}$

Data represent mean standard ±deviation of three measurements.

Significant different $p< 0.05$

The differences in sensitivity to irradiation among microorganisms might be due to the differences in their chemical and physical structure, antiseptic properties of different plants and their ability to recover from the radiation injury (Jana Sádec ká 2007). The dose of irradiation required to control microorganisms in medicinal plants, therefore, varies depending on the resistance of the particular species and the initial number of organisms present. The actual dose employed is a balance between what is needed and that what can be tolerated by the product without objectionable changes (e.g. off-flavours, texture changes, flavour alterations).Furthermore, in the sterility test, at a dose of 5.0 kGy, Terminalia chebula and Mentha piperita showed bacterial growth on 5th and 7th day respectively whereas Curcuma longa, Syzygium aromaticum showed it on 11th day of incubation. A dose of 10 kGy or higher resulted in complete sterilization of medicinal plants and the sterility was maintained for 14 days. Our previous study has also indicated that a dose of 10 kGy was required to obtain a product of good microbiological quality (Prakash et al. 2009).Table 2 showed that the total coliform counts varied between 102 and 104 CFUg-1 in the un-irradiated medicinal plants.

Table 2 . Effects of various doses of gamma irradiation on different types of microorganisms.

Type of Microorganisms	Initial Count (CFU g^{-1})	Dose of Gamma Irradiation (kGy) for complete decontamination
Coliforms	10^2-10^4	2.5
Yeast and Molds	10^2-10^3	2.5
Mesophilic bacteria	10^3-10^6	10.0

Data represent mean standard ±deviation of three measurements.
Significant different p< 0.05

However, no coliform was observed at even a lower dose of 2.5 kGy. Our results are in accordance with Owczarczyk et al. (2000) and Yu et al.(2004) who reported that gamma irradiation is an effective method for microbial decontamination. The total yeast and molds count was in the range of 102 – 103 CFU g-1 in the un-irradiated medicinal plants. The majority were yeasts, which are reported to be common flora on plant surfaces. However, no yeast and molds was found after irradiation at 2.5 kGy. This might be due to the fact that fungus is known to be relatively sensitive to gamma irradiation (Wills 1983) hence; a dose of 2.5 kGy completely eliminated the yeast and molds. All the air dried samples of medicinal plants in their native state were found to be contaminated with different types of microorganisms suggesting the need of decontamination of medicinal plants to obtain safe products for medicinal usage.

The extraction yields of all the four medicinal plants in methanol were determined at various doses of gamma irradiation and results are given in Table 3.

Table 3. Effect of various doses of gamma irradiation on extraction yield (%, w/w) of medicinal plants.

Medicinal plant	0 kGy	2.5 kGy	5 kGy	10 kGy	20 kGy	25 kGy
Terminalia chebula	59.91	60.2	63.5	66.4	72.8	74.9
Curcuma longa	35.64	35.94	36.8	38.8	39.4	40.9
Syzygium aromaticum	27.32	28.1	28.9	29.5	31.5	32.2
Mentha piperita	13.45	13.9	14.7	15.2	15.8	16.4

Data represent mean standard ±deviation of three measurements.
Significant different p< 0.05

Terminalia chebula showed the highest extraction yield (59.91%), followed by Curcuma longa, Syzygium aromaticum and Mentha piperita with extraction yields of 35.64 %, 27.32% and 13.45%, respectively in unirradiated samples. After irradiation to gamma rays, subsequent and significant increase in the level of extraction yields was recorded in all the medicinal plants. The extraction yield in Terminalia chebula, Curcuma longa, Syzygium aromaticum and Mentha piperita methanol extract was increased from 0.5% to 25.0%, 0.8 % to 14.7 %, 2.8 % to 17.8 % and 3.3 % to 21.9 % at 25 kGy gamma irradiation. The increase in the dry weights of extracts following irradiation, might be due to degradation of some high molecular weight components or solubilization of some of the non-soluble components of the plant material which are now detected in the extract. The measured effect of irradiation was enhanced in the methanol extracts, which is able to extract both polar and semi-polar components. These results confirm findings of our previous study (Prakash et al. 2010) and those of Huang and Mau (2007) and Kim et. al. (2000) who have also reported an increase of 5-30% in the extraction yields of Korean medicinal plants, using various solvents, with a dose of10kGy gamma irradiation; however the difference in increase in extraction yields, as compared to that reported in the literature might be due to different chemical composition of the plants.

This study indicates that gamma irradiation technology has potential to improve microbial decontamination and extraction yield of the medicinal plants. Moreover, gamma irradiation is an effective technology for resolving technical trade issues for many food and agricultural products (WHO 1994; FDA 2001). As a disinfestation treatment also, it is very efficient and has broad-spectrum control of many organisms including pathogenic and spoilage organisms to the acceptable microbial contamination limits in herbal materials and is one of few methods to control internal pests

(FDA, 2001). Therefore, in the interest of the human welfare and for improvement in the quality of herbal medicines, gamma irradiation of the medicinal plants at a dose of 5kGy is very effective treatment prior to manufacture of medicines.

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