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Efficacy of e-beam irradiation on shelf-life and accompanied changes in major metabolites of *Desmodium gangeticum* (L.) DC., an Ayurveda medicinal plant

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ABSTRACT

The effects of electron beam irradiation (5, 10 and 15 kGy) on shelf-life and major plant metabolites of an Ayurvedic medicinal plant *Desmodium gangeticum* was evaluated. Its root possesses incredible medicinal effects; hence powdered root sample (*choorna*) was subjected to radiation processing and thereby shelf-life extension. The root sample was found to possess an appreciable amount of phenolic and flavonoid compounds. TPC of untreated sample was completely deteriorated during storage. Flavonoids of untreated sample were depleted almost completely after a shelf-life of 12 months. TFC of all the irradiated samples also decreased significantly ($p < 0.05$) under storage. Irradiated and control samples were found to possess adequate amount amounts of minerals. Electron beam irradiation with dose of 5 kGy and 15 kGy significantly decreased nitrogen and magnesium levels. In contrast, Calcium content was increased significantly ($p < 0.05$) on radiation processing with 5 kGy and 15 kGy. Microbial status revealed that the untreated sample was acceptable only up to 4 months. A dose-dependent reduction in the microbial count resulted in all the radiation processed samples. Treatment with doses of 10 kGy and 15 kGy reduced the colony count to nil immediately after irradiation. Irradiation with 15 kGy kept the sample completely sterilized up to 2 months of storage and was acceptable even 12th month of storage. GC-MS analyses identified compounds with pharmacological importance such as methyl palmitate, mome-inositol, Neophytadiene, etc. Although, a slight change in mineral composition and phytochemicals were observed it could effectively decontaminate the medicinal herb and increase the shelf-life with minimum loss of major metabolites compared to other conventional methods of processing.



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INTRODUCTION

In the traditional system of medicine, the drugs are primarily used as such or an aqueous extract. As the herbs are directly exposed to the outer environment, they are severely contaminated with soil and atmospheric microorganisms. Microbial contamination usually has deleterious impacts on health particularly if the herbs are contaminated with pathogenic organisms. Thus, sanitization of herbal materials is essential to increase the safety of herbal medicines. A medicinal herb should be thoroughly checked for its pharmacognostical, physicochemical, phytochemical, toxicological,

pharmacological and microbiological parameters. Implementation of a good manufacturing practice is a key step towards the improved utilization for medicinal herbs. Adoption of appropriate manufacturing methods could reduce the contamination level markedly. As per the World Health Organization, the herbal medicines should meet some criteria such as its total viable aerobic count should not exceed 10^5 cfu g⁻¹ and the total yeast and mould count should be $<10^3$ g⁻¹.

The traditional methods for decontamination are heat, use of some chemicals, fumigation (ethylene oxide), etc. which can minimize the contaminants markedly, however, affect color, aroma, flavor and overall quality of the herb as a result of loss of aromatic compounds, volatile oils and other active components of the plants. In order to find an alternative method for processing scientists have given considerable importance for the use of high energy ionizing radiations for the sterilization purpose, which include x-rays, gamma rays, electron beams etc. Agencies like WHO, IAEA intensively conducted research on quality and safety of radiation processed foods, and more than 42 countries are approved radiation technology for the commercial purpose. Electron beam irradiation technology uses high energy electrons produced from an electron gun. Compared with gamma rays or X-rays electron beams possess lesser penetrating power and thus their uses limited to treatment of relatively thin packages. Conversely, use of electron beam irradiation is advantageous in that it does not need a radioactive source for its emission and it is cool to use. Internationally, food irradiation has been considered a safe and effective technology by the World Health Organization (WHO), the Food & Agriculture Organization (FAO), and the International Atomic Energy Agency in Vienna (El-Samahy *et al.*, 2000). The joint FAO/IAEA/WHO Experts Committee also confirmed that irradiation at 10 kGy and above does not produce any toxicological hazards or nutritional or microbiological problems in food.

Quite a lot of researchers verified that radiation is an effective, potential preservation tool in food processing and preservation sector. It does not alter the quality parameters of medicinal herbs and spices. Moreover, an improvement in aroma, flavour and other quality attributes were reported. This results in a positive impact on the overall quality of the final herbal preparation and decoctions in Ayurveda. There are only a few studies have explored the impact of this processing technology on the safety and quality of herbal preparations. The present study is to find out the optimum dose required for the shelf-life extension of *D. gangeticum* which brings minor changes in major phytochemical compositions and quality attributes.

Since its roots are highly medicinal and well explored, it should be carefully subjected to toxicological and microbiological evaluations.

Desmodium gangeticum (L.) DC. is a well-explored medicinal plant being a potential source of various modern herbal medicines. The plant is one among the *Dasamoola* of Ayurveda system of medicines, commonly known as 'orila' in Malayalam. It is an endangered ethnomedicinal plant known as 'Shalparni' or 'Prishiparni' in Hindi and Sanskrit. It is abundantly distributed throughout India. It is regarded as the master of medicinal plants in Ayurveda since it is an ingredient of various Ayurvedic formulations. The roots of this plant constitute as one of the ingredients of the Ayurvedic medicine, *Dasamoola*, a dietary supplement, which has marked its use in treating diseases like rheumatism, jaundice, paralysis, filaria and edema. (Sagar & Upadhyay, 2013). Cardioprotective action of methanol extract of its roots has been demonstrated by Kurian *et al.* (2010). The ethanolic extract contains potential anti-inflammatory and antinociceptive principles (Sagar *et al.*, 2010).

MATERIALS AND METHODS

Reagents and chemicals

The following chemicals were used in the experiments, and the producer and the purity grade are mentioned in brackets. Methanol, Acetone, Ethanol (Merck, HPLC grade), 2.5 N Hydrochloric acid (Merck, 37 %), Sodium carbonate anhydrous (Merck), 2N Folin-Ciocalteu's phenol reagent (Merck), Sodium nitrate, Sodium hydroxide pellets pure (Merck), and Acetic acid glacial (Merck, 100 %), Nutrient agar (Hi-Media), Rutin (Hi-Media), Gallic acid (Aldrich, 97 %).

Collection and preparation of plant samples

Fresh plantlets of *D. gangeticum* were collected from Ayurveda Medical College, Parassinikadavu, Kannur, Kerala, India and cultivated under greenhouse conditions. Healthy, disease-free plants were collected at maturity and washed thoroughly under running tap water to remove surface contaminants and followed by double distilled water, dried at room temperature (34 °C) for one week. Plant roots are selected for the study because of its pronounced medicinal values. The dried plant materials were powdered in a laboratory grinder to get 'choorna'. The samples weighing about 15 grams were packed in polyethylene packets for irradiation processing. A non-irradiated sample was kept as control.

Sample irradiation with an electron beam

Electron beam irradiation was carried out from Industrial accelerators division, Raja Ramanna Centre for Advanced Technology, Department of

atomic energy, Government of India, Indore, too absorbed doses of 5 kGy, 10 kGy and 15 kGy with beam energy of 8.2 MeV and beam current of ~20 mA at room temperature ($28 \pm 1^\circ\text{C}$). Irradiation was carried out by exposing both sides of packets for dose uniformity. Irradiation at each dose was done in triplicate. The absorbed dose was measured by Fricke and Hart dosimetry (1996).

Extraction

Irradiated and control samples each weighing about 20 gram was packed in small thimbles separately and successively extracted with solvents acetone and methanol using Soxhlet extractor for 10-12 hours. The thimble was air dried each time before extracting with the next solvent. The aqueous extract was prepared by cold percolation method. The extract yield with respect to dried plant material was calculated for each sample. The different solvent extracts were concentrated by rotary vacuum evaporator, and the crude extracts were dried and stored at 4°C . The crude extracts thus prepared were used for further analyses.

Determination of Total phenolic content

Total phenolic content in experimental and control samples was estimated by the method described by Makkar *et al.*, 2000. Gallic acid was used as the standard, and the results were expressed in milligram gallic acid equivalents (GAE).

Determination of Total flavonoid content

The total flavonoid content of experimental and control samples was quantified according to a slightly modified calorimetry method described by Zhishen *et al.*, 1999. Rutin was used as the standard, and the results were expressed in milligram rutin equivalents (RE).

Determination of Total alkaloid content

The total alkaloid contents in different plant samples were quantified according to the method proposed by Harborne, 1973.

Mineral analysis

Macro and micronutrients in non-irradiated and samples irradiated with 15 kGy were analysed using Atomic Absorption Spectrometer (Perkin Elmer AA Analyst 700). Concentrations of N, P, K, Ca, Mg, S, Fe, Mn, B, Cu and Zn were determined in each sample. The results were obtained while using a working standard of 1000 ppm for each of the species (Sadasivam and Manikam, 1992).

GC-MS analysis

GC-MS analyses were conducted with methanol extract of irradiated and control samples using a Shimadzu GC-MS (Model Number: QP2010S) equipped with Rxi-5Sil MS column (30 m \times 0.25

mm ID \times 0.25 μm). Helium (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/minute, and an injection volume of 1 μl was employed. An injection port temperature of 280°C and an ion-source temperature of 200°C were set. The oven temperature was programmed from 80°C for 2 minutes with an increase of 5°C /minute to 280°C with a hold time of 5 minutes. Scan interval was programmed for 0.50 seconds with a mass range of 50-500 amu. Total GC running time was 45 minutes. The peaks were identified by comparing their mass fragmentation pattern with that of NIST 11 & WILEY 8 library data.

Storage studies

Microbial analysis was conducted according to the method described in the Bacteriological Analytical Manual, US, FDA. Radiation processed and untreated samples were subjected to microbial evaluation. 25-gram sample was aseptically weighed and added to 225 ml of phosphate buffer solution (pH 7.2) to obtain 10^{-1} dilution and shake well. Appropriate dilutions were made till 10^{-3} dilution. Aseptically pipetted 1 ml sample from each dilution into triplicate Petri dishes and pour 18-20 ml of nutrient agar medium. Cooled to $40-50^\circ\text{C}$. Gently rotated the Petri dishes clockwise and anti-clockwise for uniform distribution of the inoculum with molten agar medium and allowed to solidify. The plates were incubated at $36 \pm 1^\circ\text{C}$ for 24-48 hours. Colonies were counted in each dilution and recorded and calculated. The results were expressed in Colony forming units per ml (cfu/ml).

Statistical analysis

All the experiments were conducted in three different batches, and triplicates of each parameter were taken for each batch of experiments. Data are expressed in terms of mean and standard error (SE) values. The mean values are compared using one-way ANOVA (Analysis of variance) test for the significance of their difference ($P < 0.05$). The data were analysed using the GraphPad Prism software version 6.01.

RESULTS AND DISCUSSION

Impacts of irradiation on total phenolic content (TPC)

TPC of irradiated and untreated control samples of *D. gangeticum* analysed in different solvent extracts are given in Table. 1. Methanol and acetone extracts had an appreciable amount of phenolics compared to aqueous extract. A slight enhancement in TPC was found in all the extracts of samples immediately after irradiation. A significant reduction ($p < 0.05$) in phenols was observed in acetone extract of untreated sample. In contrast, a sig-

Table 1: Total phenolic contents (mg gallic acid equivalents/gram) of non-irradiated and irradiated samples analysed in acetone, methanol and aqueous extracts

Dose (kGy)	Phenolic content (mg GAE/gram extract)					
	Within 10 days of irradiation			Storage of 12 months		
	Acetone	Methanol	Water	Acetone	Methanol	Water
0	13.95±1.57	22.31±0.55	1.39±1.16	-	-	-
5	23.44±2.4*	26.9±0.36*	1.68±0.72	21.45±1.12	22.34±1.07	-
10	23.98±1.04*	28.47±1.3*	1.52±0.55	23.13±0.05	27.29±0.65	-
15	24.94±0.41*	29.03±1.65*	2.04±0.9	23.86±0.43	28.79±0.21	-

Data are expressed in mean± standard deviation; n=5; *Significantly different from control (p<0.05)

Table 2: Total flavonoid contents (mg rutin equivalents/gram) of non-irradiated and irradiated samples analysed in acetone, methanol and aqueous extracts

Dose (kGy)	Total flavonoid content (mg RE/g extract)					
	Within 10 days of irradiation			Storage of 12 months		
	Acetone	Methanol	Water	Acetone	Methanol	Water
0	23.25±0.84	37.31±2.62	9.94±1.95	-	-	-
5	25.98±2.88	37.40±0.64	9.56±0.84	20.68±1.72	28.76±1.41	-
10	28.13±0.84*	38.49±1.47	9.46±1.69	22.33±2.01	31.69±0.99	-
15	28.74±1.39*	39.04±0.32	10.07±0.83	24.16±0.51	34.52±1.23	-

Data are expressed in mean± standard deviation; n=5; *Significantly different from control (p<0.05)

Table 3: Total alkaloid content (mg alkaloid/gram powder) of non-irradiated and irradiated samples during storage

Storage periods (Months)	Total alkaloid content (mg per 1 g powder)			
	0 kGy	5 kGy	10 kGy	15 kGy
0	2.48±0.05	2.09±0.32	2.05±0.29	2.01±0.11
12	0.62±0.09*	1.89±0.22	1.76±0.16	1.69±0.20

Data are expressed in mean± standard deviation; n=5; *Significantly different from control (p<0.05)

Table 4: Mineral composition of non-irradiated and irradiated samples

Mineral	Radiation dose (kGy)		
	0	5	15
Nitrogen	2170±5.56	2162±4.00*	2140±13.85*
Phosphorus	40.07±2.30	40.59±2.28	40.25±3.35
Potassium	400±3.60	398.6±0.57	400±1.00
Calcium	800.33±10.01	862.53±2.27*	960.16±6.50*
Magnesium	340.33±5.13	318±1.18*	240.66±9.29*
Sulfur	50.27±3.95	51.89±2.93	50.97±3.63
Iron	28.93±3.94	29.63±1.03	29.86±2.98
Manganese	4.12±0.07	4.02±0.09	3.91±0.19
Zinc	1.51±0.10	1.47±0.19	1.12±0.27
Copper	0.71±0.21	0.69±0.10	0.65±0.16
Boron	1.70±0.18	1.71±0.04	1.74±0.16

Data are expressed in mg 100 g-1 on a dry weight basis (n=3, mean ± SD); *Significantly different from control (p<0.05)

nificant increase was observed in methanol extract. Aqueous extract showed a slight enrichment in irradiated samples. However, it was insignificant ($p>0.05$). Up to 12 months of storage, processing with radiation did not affect the phenolic content significantly. TPC of the non-irradiated sample had a negative correlation with increasing the period of storage. Phenolics of the untreated sample were completely deteriorated during a storage period of 12 months. Whereas, all the irradiated samples retained a substantial amount of phenolics in acetone and methanol extracts even

up to 12 months of storage. Sample processed with a dose of 15 kGy showed the highest content of phenolics both instantaneously after processing and also after the mentioned storage period. Aqueous extract does not exhibit the presence of phenolics under storage. Sample irradiated with 5 kGy and 10 kGy also well retained the TPC under storage. The results revealed that a dose of 5 kGy might be adequate for the retention of TPC of powdered roots of *D. gangeticum*. It was obvious that processing with e-beam did not have a significant impact on the phenolic content of the selected sample

Table 5: Major compounds identified in GC-MS analyses of non-irradiated and irradiated samples

No.	Compound name	Peak area %	
		0 kGy	10 kGy
1	Hexadecanoic acid, methyl ester	13.48	7.64
2	Mome-inositol	11.92	11.15
3	9-octadecenoic acid (z)-, methyl ester	8.89	4.81
4	(E)-Phytol	7.4	4.75
5	(-)-Beta-sitosterol	6.63	NA
6	Neophytidiene	6.55	4.03
7	Methyl stearate	5.55	NA
8	Trolamine	5.15	NA
9	Docosanoic acid, methyl ester	3.91	2.7
10	Palmitic acid, beta-monoglyceride	3.61	10.58
11	4-Methylhexanoic acid	3.64	NA
12	Methyl tetracosanoate	3.19	1.45
13	1,4-di-O-Acetyl-2,3,5-Tri-O-Methylpentitol	3.11	NA
14	Phytol, acetate	2.15	1.32
15	9,12-Octadecadienoic acid, methyl ester	2.87	1.48
16	Loliolide	NA	6.37
17	Tetradecanoic Acid, Methyl Ester	1.59	1.39
18	Phytene-2	1.27	4.816
19	Phenol, 2,4-Bis(1,1-Dimethyl Ethyl)-	1.12	0.7

and further it may be an efficient method of processing with minimal loss of phenolic compounds.

A similar result was reported by Kavitha *et al.*, 2015, who studied the effect of gamma irradiation (0.25 to 1.0 kGy) on antioxidant. The study

revealed that irradiation treatment induced a significant enhancement in flavonoid content and superoxide anion radical activity and DPPH radical activity. A negligible decrease in total phenolic content and reducing power activity was also reported. Thongphasuk *et al.*, 2014 evaluated the effects of gamma irradiation on active components, free radicals and toxicity of Cassumunar Ginger rhizomes. The results of the study showed that irradiation at the doses of 10 and 25 kGy significantly increased free radicals in Cassumunar ginger rhizomes. However, the volatile oils, total phenolic content, antioxidant activity and toxicity were not significantly affected by the irradiation doses.

Impacts of irradiation on total flavonoid content

The flavonoid content of different extracts obtained from radiation processed and untreated samples analysed immediately after irradiation and after a storage period of 12 months are given in Table. 2. Flavonoids were found to be rich in methanol extract followed by acetone and water. A slight increase in flavonoids of all the three extracts was observed. The methanol extract of sample processed with a radiation dose of 15 kGy showed the highest content of flavonoids, *i.e.*,

39.04±0.32 mgRE immediately after irradiation. At the same time, the methanol extract of untreated sample possesses 37.31±2.62 mgRE. However, a slight increase was found to be insignificant ($p>0.05$). A similar result was obtained in case of flavonoids of acetone extract also. At doses of 10 kGy and 15 kGy flavonoid content of acetone extract was found to be increased significantly ($p<0.05$). The aqueous extract exhibited almost equivalent levels of flavonoids in the entire irradiated and non-irradiated sample. Flavonoids of the untreated sample were depleted almost completely after a shelf-life of 12 months. TFC of all the irradiated samples also decreased significantly ($p<0.05$) under storage. Even though the flavonoid content was reduced, all the irradiated samples have fairly retained flavonoids in acetone and methanol extracts compared to the untreated sample. Flavonoid of aqueous extract was lost to beyond the level of detection in all the irradiated and non-irradiated samples under storage. Based on the results, it was clear that radiation processing can preserve the flavonoids effectively without major deterioration compared to the untreated sample.

Radiation-induced changes in phenolic compounds in Strawberries was evaluated by Breittfeller *et al.*, 2003. They reported that among the four phenolic acids identified one increases with dose and the three were not affected by irradiation. Among the four flavonoids identified three decreases with dose one was not affected by irradiation. Studies of Koseki *et al.*, (2002) on the effects of irradiation in medicinal and edible herbs also

revealed somewhat similar results. The study aimed at reducing the contamination of medicinal herbs by irradiation and revealed that no significant changes in therapeutic actions of irradiated herbs, since it does not affect the bioactive components like alkaloids, phenolics, flavonoids, tannins, oils etc. which actually offers the pharmacological properties.

Effects of radiation processing on total alkaloid content

The alkaloid content of radiation processed and non-irradiated powdered roots of *D. gangeticum* are presented in Table. 3. The plant was fairly rich in alkaloids with a magnitude of 2.48 ± 0.05 mg per 1-gram root powder without any processing. An insignificant ($p > 0.05$) reduction in alkaloid was observed in all radiation processed samples analysed immediately after irradiation. Sample processed with 15 kGy exhibited 2.01 ± 0.11 mg which was a little lesser than untreated sample. Intermediate levels of alkaloids are displayed by the samples treated with 5 kGy and 10 kGy. Gradual reduction of alkaloid content was shown in all the analysed samples after the storage period of 12 months. A significant ($p < 0.05$) reduction in alkaloid was found in the control sample compared to irradiated samples. However, the extent of reduction was insignificant in all the irradiated samples. After the shelf-life of 12 months, the non-irradiated sample retained only 0.62 ± 0.09 mg alkaloids. Whereas, the sample processed with 5 kGy has retained the alkaloids more efficiently with a magnitude of 1.89 ± 0.22 mg. Samples processed with 10 and 15 kGy also well-retained alkaloids compared to control sample. 1.76 ± 0.16 mg and 1.69 ± 0.20 mg alkaloids are upheld by samples irradiated with 10 kGy and 15 kGy respectively.

Changes in mineral composition

Mineral composition of irradiated and non-irradiated powdered roots of *D. gangeticum* is shown in Table. 4. All the irradiated and control samples were found to possess an adequate number of amounts of minerals. Electron beam irradiation with a dose of 5 kGy and 15 kGy significantly decreased nitrogen and magnesium levels. At the same time, Calcium content was increased significantly ($p < 0.05$) on radiation processing with 5 kGy and 15 kGy. Calcium level was noted as 800.33 ± 10.01 mg/100 g of untreated powdered sample, and it showed enrichment in the sample treated with a dose of 15 kGy with a magnitude of 960.16 ± 6.50 mg/100 g. Untreated sample was found to possess 340.33 ± 5.13 mg of magnesium per 100 g and processing with radiation of 15 kGy significantly reduced the amount to 240.66 ± 9.29 mg. Other minerals such as phospho-

rus, potassium, sulfur, iron, manganese, zinc, copper and boron were not significantly altered by radiation processing with an electron beam at 5 kGy and 15 kGy.

The results of the present study is in agreement with the earlier studies conducted by Rahman *et al.*, 2015, analysed the effects of gamma irradiation processing on the mineral components of *Cucumis sativus* suggested that some minerals are positively correlated with irradiation, whereas some other minerals were sensitive to radiation processing. Bhat *et al.*, in 2008 studied the effects of electron beam irradiation on Nelumbo seeds and revealed that radiation decreased the content of potassium at 10 kGy onwards and zinc from 5 kGy onwards and remaining all the minerals were not affected significantly. The study suggested the use of radiation technology which effectively retains the mineral composition after processing.

Changes in various phytochemicals identified through GC-MS analysis

The compounds present in the methanolic extract of irradiated and untreated samples were separately evaluated and given in Table. 5. with reference to peak area percentage. GC-MS chromatograms showed the presence of various phytochemical constituents and are identified and characterised by comparing with the mass spectra of the constituents of NIST& Wiley library data. One of the major compounds identified in the untreated sample was Hexadecanoic acid, methyl ester (methyl palmitate) and its peak area percentage were found to be 13.48. Sample treated with 10 kGy also shown the presence of methyl palmitate with an area percentage of 7.64. Mome-inositol was also identified in both samples almost in a similar range in terms of its peak area. Compounds such as 9-octadecenoic acid (z)-, methyl ester (methyl oleate), phytol, neophytidiene, docosanoic acid, methyl ester, Palmitic acid, beta-monoglyceride, methyl tetracosanoate, Phytol, acetate, 9,12-Octadecadienoic acid, methyl ester (Methyl linoleate), phytene-2, etc. were detected in both untreated and radiation processed samples. Compounds such as (-)-Beta-sitosterol, Methyl stearate, Trolamine, 4-Methylhexanoic acid, 1,4-di-O-Acetyl-2,3,5-Tri-O-Methylpentitol, etc. were not detected in sample processed with an electron beam. Palmitic acid, beta. -monoglyceride was detected in both samples with area percentages of 3.61 and 10.58 respectively.

The major compound identified was Methyl palmitate, belongs to the class of organic compounds known as fatty acid methyl esters. These are compounds containing a fatty acid that is esterified with a methyl group (HMDB). The antioxidant and

antifungal potential of methyl palmitate was reported by Pinto *et al.*, 2017. Its uses as a flavouring agent in food were also reported. In 2012, Saeed *et al.* demonstrated the anti-inflammatory activity of methyl palmitate in several experimental models. The study reported that methyl palmitate possesses powerful anti-inflammatory activity by reducing carrageenan-induced rat paw oedema and also decreased the levels of prostaglandin in inflammatory exudates. Another major compound was mome-inositol, which was reported to possess Antiallopecic, Anticirrhotic, Antineuropathic, Cholesterolytic, Lipotropic, and sweetener (Kumar *et al.*, 2012). One of the identified compounds Neophydiene was found in both control and irradiated samples, is a well-known enzyme inhibitor found to be effective in inhibition of cyclooxygenase or lipoxygenase leads to decreased production of prostaglandins and leukotriene's (James *et al.*, 2000).

The effectiveness of electron beam irradiation on shelf-life extension

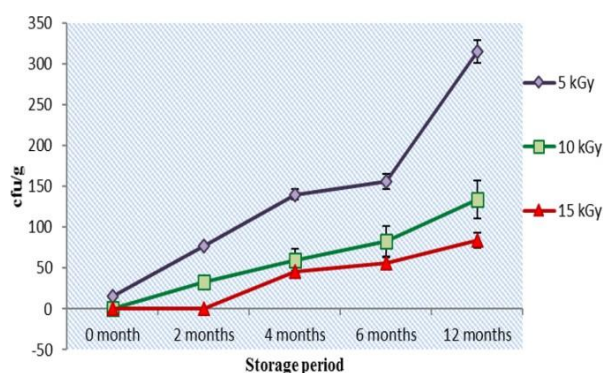


Figure 1: Total bacterial count of *D. gangeticum* samples treated with different doses, i.e., 5 kGy, 10 kGy and 15 kGy during storage

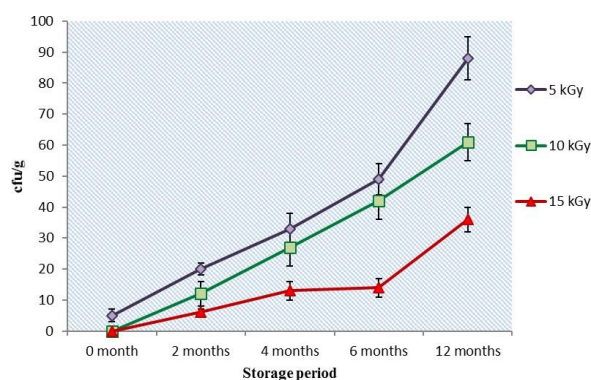


Figure 2: Total yeast and mould count of *D. gangeticum* samples treated with different doses, i.e., 5 kGy, 10 kGy and 15 kGy during storage

Radiation processed and non-irradiated samples were subjected to microbiological studies to detect the status of contamination, and the results are represented in fig. 1 & 2. The colony count in untreated sample was too numerous from second

month onwards, and it was exceeded the permitted level of contamination after 4 months of storage. Counts were significantly ($p < 0.05$) higher during the storage beyond this period and almost spoiled. Contamination levels were significantly reduced in all the radiation processed samples. Treatment with doses of 10 kGy and 15 kGy reduced the colony count to nil immediately after irradiation. Irradiation with 15 kGy kept the sample completely sterilised up to 2 months of storage and was acceptable even 12th month of storage. A positive correlation was found in colony count of all samples with storage months. Colony count reduced with increasing dose of radiation. A similar result was obtained with yeast and mould count also. No colonies were observed immediately after irradiation with 10 kGy and 15 kGy. A non-irradiated sample was heavily contaminated with yeast and mould and became undesirable beyond 6 months of storage. Both bacterial count and yeast and mould count remained low in all the samples processed with radiations and acceptable during storage of 12 months also. At the same time, colonies in the untreated sample increased greatly and almost spoiled during 12 months of storage.

The effect of gamma irradiation on microbial content and curcuminoids of *Curcuma Amada* rhizomes was studied by Rahayu *et al.* (2016). Their results suggested that an irradiation dose of 5 kGy is effective to reduce the content of microorganisms without lowering curcuminoid contents. Food irradiation with doses between 1 kGy and 5 kGy was very efficient in insect disinfection and several fold reduction of spoilage microorganisms and thereby shelf-life extension (Urbain, 1983).

Radiation processing of Tomato (*Lycopersicon esculentum* Mill.) as a measure to reduce post-harvest losses was proved through the study of Akter & Khan (2012). The study has evaluated the effects of different doses of gamma irradiation on the overall quality parameters such as colour, total soluble solids and firmness and suggested that dose of 750 Gy was ideal dose for maintaining the quality and shelf-life improvement.

CONCLUSION

The present study was conducted with the main objective to evaluate the effects of electron beam irradiation on the shelf-life and major secondary metabolites such as phenols, flavonoids, alkaloids and mineral composition of *Desmodium gangeticum* root samples. The impacts of electron beam irradiation at doses of 5, 10 and 15 kGy on the samples were analysed and compared to that of non-irradiated samples. The study revealed that radiation processing does not produce significant changes in the analysed metabolites and mineral composition. Processing with a dose rate of 10 kGy

was found to be sufficient in the shelf-life extension of powdered root sample of *D. gangeticum*. Since its roots are highly medicinal and at the same time they are heavily contaminated with microorganisms due to its direct exposure to soil, it cannot be stored for a long time without an effective sterilisation procedure. So the present study recommends the use of electron beam irradiation for the shelf-life extension with minimal changes in phytochemical composition compared to other traditional methods of processing.

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REFERENCES

- Akter, H., and Khan, SA. 2012. Effect of gamma irradiation on the quality (Colour, firmness and total soluble solids) of Tomato (*Lycopersicon esculentum* Mill.) stored at a different temperature. *Asian Journal of Agricultural Research*, 6(1), 12-20.
- Bhat, R., Sridhar, KR. 2008. Nutritional quality evaluation of electron beam irradiated lotus (*Nelumbo nucifera*) seeds. *Food Chemistry*, 107, 174-184.
- Breitfellner, F., Solar, S., Sontag, G. 2003. Radiation-induced chemical changes of phenolic compounds in strawberries. *Radiation Physics and Chemistry*, 67, 497-499.
- El-Samahy, SK., Youssef, BM., Aaskar, AA., Swailam, HMM. 2000. Microbiological and chemical properties of irradiated mango. *Journal of Food Safety*, 20, 139-156.
- FAO/IAEA/WHO. Report of a Joint FAO IAEA WHO Study Group. Rome: Food and Agriculture Organization of the United Nations. 1999.
- Fricke, H., Hart, EJ. *Radiation dosimetry*, Academic press. 1996.
- Hagerman, A., Muller, I., & Makkar, H. Quantification of tannins in tree foliage. *A laboratory manual*. Vienna: FAO/IAEA. 2000, 4-7.
- Harborne J: *Phytochemical methods*. Chapman and Hill, Ltd London. 1973, 49-188.
- James, MJ., Gibson, RA. 2000. Dietary polyunsaturated fatty acids and inflammatory mediator production. *American Journal of Clinical Nutrition*, 71,343-348.
- Kavitha, C, Kuna, A., Supraja, T., Sagar, SB., Padmavathi, TVN., Prabhakar, N. 2015. Effect of gamma irradiation on antioxidant properties of ber (*Zizyphus mauritiana*) fruit. *Journal of Food Science and Technology*, 52(5), 3123-3128.
- Koseki, PM., Villavicencio, ALCH., Brito, MS., Nahme, LC., Sebastiao, KI., Rela, PR., Almeida-Mradian, LB., Mancini-Filho, J., Freitas, PCD. 2002. Effects of irradiation in medicinal and edible herbs. *Radiation Physics and Chemistry*, 63, 681-684.
- Kumar, NR., Reddy, JS., Gopikrishna, G., Solomon, KA. 2012. GC-MS determination of bioactive constituents of *Cycas beddomei* cones. *International Journal of Pharma and Bio Sciences*, 3(3), 344-350.
- Kurian, GA., Suryanarayanan, S., Raman, A., Padikala, J. 2010. Antioxidant effects of ethyl acetate extract of *Desmodium gangeticum* root on myocardial ischemia-reperfusion injury in rat hearts, *Chinese medicine*, 5(3)
- Pinto, MEA, Araujo, SG., Morais, M., Sa, NP., Lima, CM., Rosa, CA., Siqueira, EP., Johann, S., Lima, LARS. 2017. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. *Annals of Brazilian Academy of Sciences*, 89(3), 1671-1681.
- Rahayu, DP., Saputri, SP., Darwis, D. 2016. The effect of gamma radiation on microbial content and curcuminoids of *Curcuma amada* Roxb. Rhizomes. *Atom Indonesia*, 42(2), 53-58.
- Rahman, MM., Roy, M., Sajib, MAM., Sarkar, A., Hussain, MS. 2015. Radiation effects on essential minerals content of Cucumber (*Cucumis sativus*). *American Journal of Food and Nutrition*, 3(3): 69-74.
- Sadasivam, S., Manikam, A. *Biochemical Methods*. New Age International Publishers. Chennai, India. 1992.
- Saeed, NM., El-Demerdash, E., Abdel-Rahman, HM., Algendaby, MM., Al-Abbasi, FA., Abdel-Naim, AB. 2012. Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models. *Toxicology and Applied Pharmacology*, 264(1), 84-93.
- Sagar, MK., & Upadhyay, K. 2013. Evaluation of in vitro antioxidant, antinociceptive and anti-inflammatory properties of *Desmodium gangeticum* (L.) in experimental animal models. *American Journal of Phytomedicine and Clinical Therapeutics*, 1(3), 256-265.
- Sagar, MK., Upadhyay, A., Kalpana., Upadhyay, K. 2010. Evaluation of in vitro antioxidant, antinociceptive and anti-inflammatory properties of

Desmodium gangeticum (L.) in experimental animal models. *Archives of Applied Science Research*, 2(4), 33-43.

Thongphasuk, W., Thongphasuk, J., Bavovada, R., Chamulitrat, W. 2014. Effects of gamma irradiation on the active components, free radicals and toxicity of Cassumunar ginger rhizomes. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(7), 432-436.

Urbain, JH. 1983. *Radurization and radication: fruits and vegetables*. Preservation of foods by iodine radiation. 3, edited by Josephson, E.S., & Peterson, M. Boca Raton, Fl: Crc Press.

Zhishen, J., Mengecheng-Taxonomy., Jianming, W. 1999. The determination of flavonoid content on mulberry and their scavenging effects on superoxide radical. *Food Chemistry*, 64, 555-559.