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Research article

Low-dose electron beam induced changes in phytochemicals and shelf-life of *Desmodium Gangeticum* (L.) DC.

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Key words: *Desmodium gangeticum,* Phenols, Flavonoids, alkaloids, irradiation, shelf-life.

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Abstract

The present study was carried out to study the effect of low-dose electron beam irradiation on phytochemical constituents and shelf-life of *Desmodium gangeticum,* one of the main ingredients of '*dasamula'* in Ayurvda. Plant parts are dried and powdered separately and irradiated with electron beam at dose rate of 0.0 Gy(control), 100Gy, 200Gy and 300 Gy. Control and experimental samples were subjected to extraction using solvents such as acetone, methanol and water by cold maceration method. Total phenolic and flavonoid content in irradiated and non-treated samples were estimated in different solvent-extracts. Total flavonoid and phenolic content was found to be highest in acetone extract of leaves and stem. The results revealed that there is no significant loss of phenolic content after samples have been irradiated. A slight decrease in flavonoid content was observed in irradiated samples. A gradual decline in total alkaloid content is observed in sample irradiated with dose of 300 Gy. Microbiology study was also conducted on 0, 3 and 5th day of storage. The results indicated a gradual decrease in total bacterial and fungal count on radiation processing. The present study suggests that irradiation with higher dose can be an effective method in sterilization of medicinal plant products and thus extending the shelf-life.

Introduction

Food irradiation is a physical means of food processing that involves exposing the pre-packaged or bulk foodstuffs to gamma rays, X-rays, or electrons. Foodstuffs are generally irradiated with gamma radiation from a radioisotope source, or with electrons or X-rays generated using an electron accelerator [1]. Due to the strong desire to reduce the use of chemicals applied to fruits and vegetables, the non-residual feature of ionizing radiation is an important advantage. 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 [2]. Over 42 countries in the world including USA, UK, Canada and France have given clearance for use of radiation in food processing and preservation. Use of biotechnological approaches with low-dose irradiation treatment for enhancing the production of bioactive plant metabolites, such as, phenolic compounds, salicylic acids, coumaric acids, caffeic acids, flavonoids, and anthocyanins has been documented in medicinal plants [3].

Irradiation technology proved to be effective in reducing post-harvest losses, and controlling the stored product insects and the microorganisms [4]. The potential application of ionizing radiation in food processing is based mainly on the fact that ionizing radiations damage very effectively the DNA so that living cells become inactivated, therefore microorganisms, insect gametes, and plant meristems are prevented from reproducing, resulting in various preservative effects as a function of the absorbed radiation dose. At the same time, radiation-induced other chemical changes in food are minimal [5].

Desmodium gangeticum (L.) DC. (Family: Fabaceae) commonly known as Shalaparni is an important species of the genus Desmodium. Due to broad spectrum therapeutic potentiality, it is extensively practiced as traditional medicine in India and other parts of sub-continent over a long period of time [6]. *Desmodium gangeticum* (L.) DC., is a perennial, non-climbing shrub of the Fabaceae family. This family encompasses plants whose characteristics are of high industrial, pharmaceutical, scientific, and cultural importance. Traditionally many *Desmodium* species are used in typhoid, asthma, bronchitis, piles, cough, dysentery, diarrhoea, haemorrhage, biliousness, convulsions etc. and some of them can induce hypotension [7].

Experimental

Materials and Methods

Collection of plant materials and preparation of samples for irradiation

Fresh seedlings of *Desmodium gangeticum* was collected from Govt. Ayurvedic Medical College, Parassinikadavu, Kannur and authenticated by Dr. K.M. Khaleel, Research guide, Kannur University, Kannur, Kerala, India. The plantlets were cultivated in field under greenhouse conditions. Healthy, disease-free plant leaves were collected at maturity and washed thoroughly under running tap water followed by double distilled water and were dried at room temperature (34^{o}C) for one week. Then the dried plants were separated into roots, stem and leaves and were powdered in a laboratory grinder and stored in air tight containers.

Irradiation of plant samples with electron beam accelerators

Twenty five grams of powdered sample was separately packed in polyethylene packets and irradiation was carried out at Microtron Centre, Mangalore University, Mangalore, Karnataka (Microtron accelerator designed by Centre for Advanced Technology, India) at doses of 100 Gy, 200 Gy and 300 Gy at room temperature (28 ± 1 ℃). Irradiation was carried out by exposing both sides of packets for dose uniformity. Irradiation at each dose was done in duplicate. The electron beam irradiation parameters are given as follows:

Beam energy	8.0 MeV (Variable)
Beam current	~20mA
Distance from sample	30cm
Pulse duration	30 sec
Pulse repetition rate	50Hz

Preparation of extract for quantitative analysis

Irradiated and control samples each weighing about 25 gram was successively extracted by cold maceration technique using solvents acetone, methanol and water. The extracts were dried at room temperature and stored at 4^{o}C. The crude extracts thus prepared were used for the estimation of total phenolics and flavonoids.

Determination of total phenolic content [8]

Total phenolic content in each crude extract was estimated using Folin-Ciocalteu method as described by Makkar *et al,* 2000. Hundred milligram of crude extract was dissolved in 100 ml of respective solvent. 1 ml of this solution was transferred to a test tube, added 0.5 ml of 2 N Folin-Ciocalteu reagent followed by 1.5 ml of 20% Na_2CO_3 solution and the volume was made up to 8 ml with distilled water. The mixture was subjected to vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm with a spectrophotometer (Labtronics model LT-290). The total phenolic content was calculated using gallic acid as standard and the results were expressed as gallic acid equivalents (GAE).

Determination of total flavonoids [9]

The total flavonoid content of different crude extracts was estimated using a slightly modified colorimetric assay described by Zhishen*et al*, 1999. 0.5 ml of diluted extract was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes-added 0.15 ml of 10% $AlCl_3$ solution and allowed to stand for 6 minutes, followed by 2 ml of 4% NaOH solution. Finally added distilled water to bring the final volume to 5 ml and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined spectrophotometrically at 510 nm. The analysis was performed in triplicates and the total flavonoid content was estimated using rutin as standard and the results were expressed as rutin equivalents(RE).

Determination of total alkaloids [10]

The total alkaloid contents in different plant samples were quantified according to the method proposed by Harborne, 1973. 5gm of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

Microbial analysis [11]

Microbial quality of irradiated and control samples were screened by total plate count method as described by Zehra., 2009. Two types of media were used for the analysis. Nutrient agar medium is used for bacterial count and potato dextrose agar was used for fungal count. Ten gram sample was dissolved in 90 ml peptone water under hygienic conditions. Samples were homogenate for 5 minutes and serial dilutions were made in peptone water. Appropriate dilutions were plated onto duplicate plates of nutrient agar and potato dextrose agar medium. Plates were incubated at 37 ℃ for 2 days for bacteria and 22 ℃ for 5 days for molds and yeasts. Evaluations of the samples were carried at 3rd and 5th days of storage. The results of total plate count were expressed as log CFU/g

Statistical analysis

All experiments were repeated for a minimum of 3 times and the mean values ± standard error is given in tables.

Results and Discussion

The present study was planned with the prime objective to examine the impact of irradiation on the total phenolic,

flavonoid and alkaloid content of *Desmodium gangeticum*. The impact of irradiation at doses of 100 Gy, 200 Gy and 300 Gy on plant samples were examined through various phytochemical analysis and the results were tabulated as discussed below.

Impacts of irradiation on total phenolic content

Total phenolic content was expressed as mg equivalents of gallic acid/100 mg extracts and is given in table 1. In accordance with the present analysis irradiation did not induce any significant effects on the total phenolic content. Total phenolic content was found to be higher in acetone extract of stem and leaves compared to other extracts. Acetone extract of stem shows 41.05±1.08 mg GAE per 100 mg extract and is decreased to 37.50±4.93 mg on irradiation with 300 Gy. Leaves showed 31.17±1.37 mg GAE per 100 mg acetone extract and is found to be increased to 37.71±2.05 mg on irradiation with 300 Gy. Aqueous root extract shows significant reduction in phenolic content. The control sample possesses 7.72±0.29 mg GAE and is reduced to 3.75±0.76 mg GAE on irradiation with 300 Gy. The changes in phenolic content may be due to some chemical changes happened during radiation processing.

Table 1. Impacts of different doses of irradiation on total phenolic content in different plant parts

Plant parts	Dose (Gy)	Acetone	Methanol	Aqueous
		(mg GAE/100 mg)*		
Leaves	0	31.17±1.37	20.46±1.66	7.54±2.04
	100	38.40±2.17	21.63±0.81	8.48±0.72
	200	37.83±0.90	22.02±0.14	8.21±0.98
	300	37.71±2.05	21.75±1.72	7.67±0.81
Stem	0	41.05±1.08	24.41±1.75	3.20±0.30
	100	40.59±0.90	22.63±2.92	2.30±0.45
	200	39.31±0.55	24.97±1.69	2.69±0.44
	300	37.50±4.93	22.43±10.47	2.93±0.45
Roots	0	16.51±0.45	15.51±0.82	7.72±0.29
	100	20.26±0.51	12.06±2.88	6.71±1.98
	200	18.65±1.64	11.50±0.37	4.83±2.01
	300	18.52±0.89	11.58±0.45	3.75±0.76

Data expressed as mean±SE (n=3)

*Total phenolic content expressed in mg gallic acid equivalents per 100 mg extract

Ghadi *et al.,* 2015 also reported the similar results [12]. He studied the effect of gamma irradiation on the total phenolic content and free radical scavenging activity of Iranian date palm Mazafati (*Phoenix dactylifera* L.). They observed that gamma irradiation dose of 0.5 kGyand 1 kGyon the dates showed no significant effect on antioxidant and total phenolic content. Irradiation of dates at 2.5 k Gy increased antioxidant activity and total phenolic content. Radiation induced chemical changes of phenolic compounds in strawberries was studied by Breitfellner, *et al.,* 2003. They identified four phenolic acids such as gallic acid, p-coumaric acid, caffeic acid and 4-hydroxy benzoic acid. Among these first three were not influenced by irradiation and the concentration of 4-hydroxy benzoic acid increased linear with dose [13].

Study on effects of gamma irradiation on active components, free radicals and toxicity of Cassumunar ginger rhizomes conducted by Thonguphasuk *et al.*, 2014. The study suggested that irradiation at the doses up to 25 k Gy can safely be used to sanitize dried Cassumunar ginger rhizomes and the total volatile oils, phenolic content, antioxidant activity and toxicity were not significantly affected by the irradiation doses [14].

Impacts of irradiation on total flavonoid content

Total flavonoid content of irradiated and non-irradiated samples was analyzed and the results are shown in table 2. Leaves and stem possess highest content of flavonoids compared to roots and among this acetone extract has highest value. Aqueous extract showed least content of flavonoids. Only slight changes were detected among different extracts of each doses of radiation. Acetone extract of leaves showed 62.06±0.58 mg RE per 100 mg extract and is decreased to 58.83±0.84 mg RE on irradiation with dose of 300 Gy. Methanol extract of all the three parts also showed a gradual reduction in flavonoid content on irradiation processing. Aqueous root extract shows noticeable changes in flavonoid composition due to irradiation treatment. It is found to be reduced to 7.25±1.39 mg RE compared to non-irradiated sample which possesses 11.16±2.00 mg RE.

Table 2. Impacts of different doses of irradiation on total flavonoid content in different plant parts

Plant parts	Dose (Gy)	Acetone	Methanol	Aqueous
		(mg RE/100 mg)*		
Leaves	0	62.06±0.58	47.24±0.04	21.56±0.06
	100	60.73±1.11	45.62±0.55	18.15±1.39
	200	60.05±0.96	45.52±1.66	17.90±0.55
	300	58.83±0.84	44.97±1.95	17.26±0.84
Stem	0	58.77±0.84	43.88±0.55	8.27±1.47
	100	50.36±0.96	42.34±0.55	7.76±0.84
	200	55.95±0.32	40.35±0.84	8.14±2.31
	300	53.54 ±0.84	39.23±0.84	7.79±1.11
Roots	0	24.41±2.24	37.53±1.11	11.16±2.00
	100	23.48±2.22	36.06±0.32	10.87±1.11
	200	25.24±0.61	32.46±3.34	9.43±1.47
	300	25.50±0.55	31.31±2.50	7.25±1.39

Data expressed as mean±SE (n=3)

*Total flavonoid content expressed in mg rutin equivalents per 100 mg extract

Wang *et al.,* 2009 studied the changes of flavonoid content and antioxidant capacity in blueberries after illumination with UV-C. The study reported that the levels of flavonoids in blueberries were found to increase after illumination with

UV-C [15]. Koseki, *et al.*, 2002 studied the effects of irradiation in medicinal and eatable herbs. The results indicated that no significant changes in chemical composition on increasing dose of irradiation [16].

Impacts of irradiation on total alkaloid content

Irradiated and control samples were analyzed for total alkaloid content and the results are shown in table 3. Root possesses 3.84 mg alkaloid per gram of powdered sample and is reduced to 2.76 mg on irradiation with dose of 300 Gy.

Table 3. Impacts of different doses of irradiation on total alkaloid content in different plant parts.

Plant parts	Total alkaloid content (mg g^{-1})			
	Dose of radiation (Gy)			
	0	100	200	300
Leaves	2.86	2.93	2.52	2.35
Stem	3.11	2.89	2.70	2.68
Roots	3.84	3.16	3.18	2.76

Data expressed in milligram alkaloid per gram of powdered sample

Stem possess 3.11 mg alkaloids and is decreased to 2.68 mg on radiation treatment with 300 Gy. Leaves also showed a gradual decline in alkaloid content on radiation processing. From the results it can be assumed that slight reduction in alkaloid content is due chemical changes occur during radiation processing.

Microbial analysis

Microbial contamination of irradiated and control samples are shown in table 4. Results showed that low dose electron beam irradiation was ineffective in prolonging shelf life. On fifth day of storage both experimental and control samples were heavily loaded with microbial contamination. Only slight decrease in bacterial colonies was observed in samples treated with 300 Gydose rate and fungal contamination was persistent. Total bacterial count was shown to be increased on storage and it also shows a gradual reduction on irradiation. Total molds and yeast colonies were reduced from 4.90×10^3cfu/g to 6.21×10^2cfu/g on irradiation with 300 Gy on first day of storage. Both control and irradiated samples showed gradual increase in colonies on fifth day of storage. To remove bacterial contamination completely higher dose of radiation treatment is needed. Kirthy Reddy., 2012 studied the effect of irradiation on storage quality of preserved tomato crush. Their study reported that total bacterial count is decreased on irradiation and is increased on storage [17].

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 irradiation dose of 5 kGy is effective to reduce the content of microorganisms without lowering curcuminoid contents [18].

Table 4.Microbial contamination of irradiated and non-irradiated plant samples.

Sample	Dose (Gy)	Microbial contaminations (cfu/g)					
		Storage (days)					
		Total bacterial count			Total molds and yeasts		
		0	3	5	0	3	5
D. gangeticum	0	5.89×10^5	7.14×10^8	9.78×10^9	4.90×10^3	6.12×10^3	2.66×10^4
	100	6.05×10^5	4.69×10^7	3.56×10^9	2.77×10^3	6.37×10^3	5.85×10^4
	200	5.57×10^5	9.15×10^7	5.90×10^8	8.35×10^2	2.80×10^3	2.57×10^4
	300	5.18×10^5	7.00×10^6	9.83×10^7	6.21×10^2	9.94×10^2	7.95×10^3

Conclusion

Food irradiation is a physical means of food processing that involves exposing the pre-packaged or bulk foodstuffs to gamma rays, X-rays, or electrons. Several studies have reported that there is no significant loss of any nutrients after food has been irradiated. The present study evaluated the effects of low dose electron beam irradiation on the phenolic, flavonoid and alkaloid content of *D. gangeticum*. The present investigation revealed that radiation processing does not show any predominant changes in the total content of tested metabolites. Microbiology studies suggest that low dose electron beam irradiation up to 300 Gy is ineffective in decontamination purpose. The present study can recommend that irradiation with higher dose will be an effective method for decontamination of medicinal herbs and thereby increasing the shelf-life.

Conflict of Interest

The authors declare no known conflict of interest.

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