

Impact of Gamma Radiation Treatments on *Emblica officinalis* Fruit Juice Powder for Physiochemical, Microbial Load and Antioxidant Properties

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KEYWORDS

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ABSTRACT

Introduction: Gamma rays treatment is one of the most common techniques used for microbial control that increases the lifespan of food.

Aim: This study addresses the influence of gamma irradiation on amla fruit juice spray dried powder.

Methods: Amla fruit juice was dried by spray drying technique and further powder was gamma irradiated at different doses i.e., 7-9 kGy, 15-18kGy and 25kGy. Low molecular hydrolysable tannoids, gallic acid, Ascorbic acid were investigated by using HPLC, total tannins and titratable acidity was evaluated by titration method. Identification test was done by TLC and FTIR. Total antioxidant activity was evaluated by DPPH method

Results: There was no change in colour of powder after gamma radiation. At higher dose 15-18 kGy and 25kGy, the powder did not remain in complete free flowing form, some particles of powder agglomerates and form small hard masses. There was no change in FTIR spectra of gamma treated and untreated amla juice powder when analyzed in transmittance mode between 400 and 4000 cm⁻¹. At 25kGy total tannins increased from 33.50 to 35.90%, titratable acidity was decreased from 20.10 to 19.22%, ascorbic acid was also reduced from 4.30 to 4.14%, low hydrolysable tannoids reduced from 21.55 to 21.30% and gallic acid increased from 0.76 to 0.84%. The DPPH activity is slightly increased at 7-9 kGy and 15-18 kGy but decreased at 25kGy. Each of the samples were also tested for total bacterial count. Even after treatment with 7-9 kGy of gamma radiation, the microbial burden decreased significantly.

Conclusion: This study reveals that gamma radiation at 7-9kGy has low effect on phytochemicals, colour, and antioxidant activity, but is extremely significant in reduction of microbial load. Thus 7-9kGy is suitable for treatment to increase shelf life of product.

1. Introduction

There is thousand years history for the use of different part of plant to full fill various requirements i.e. hunger, illness, etc. In fact, medicinal plants have been proved to be very useful for human's health since long times¹. Plants and natural substances are gaining popularity due to their diverse applications in areas such as medicine, nutraceuticals, food, and cosmetics³. Amla (*Emblica officinalis* Gaertn) is a very significant plant in Ayurveda² and a good source of vitamins (Vitamin C, thiamine, riboflavin, niacin and minerals), minerals (calcium, phosphorus, iron) and amino acids. During processing and storage, herbs can become contaminated with germs, insects, and pests. This contamination reduces their shelf life and, in rare situations, causes serious sickness, especially when the plants are combined with food damaging pathogens⁴. Gamma ray treatment for food items was established as one of the techniques for microbial cleaning and food shelf-life extension in the mid of the twentieth century and is currently one of the most exploited approaches^{5,6}. The primary reason for the common use of gamma radiation in food processing is that it can effectively damage DNA, rendering living cells inactive. However, other chemical changes brought on by radiation have little effect on food⁷. Food radiation processing is now an acknowledged and proven technology. Research spanning several decades demonstrates that gamma irradiation is a safe methodology. Treatment of the goods, like fruits, herbs, spices, and nuts, at the rate of 3-10 kGy is also an important alternative to fumigation to prevent microbial deterioration⁹. Treatment above 20 kGy have also been reported to lower the microbial load in dry foods. In a study by Mitra *et al.*, (2004)¹⁰ showed impact of gamma radiation on the dry powders of Haritaki (*Terminalia chebula*) and amla (*Emblica officinalis*) and found that DPPH study increased antioxidant activity, while HPLC investigations demonstrated an increase in the extractability of soluble components. According to Jabin *et al.*, (2023)¹¹, after the treatment, protein content, ash content, and moisture content all increased, but the fat content remained unaltered. The goal of this investigation was to ascertain how various gamma irradiation dosages put impact on chemical composition and

biological activity of fresh amla fruit juice powder.

2. Material and Methods

2.1 Sample collection and preparation of fresh amla juice powder

Fresh amla (*Emblica officinalis*) fruits were purchased from Mumbai (Maharashtra) market. The amla was identified on the basis of macroscopic and microscopic description. The fresh amla fruit was washed in running water and pulverized followed by filtration with muslin cloth to get the juice at room temperature. Further juice was dried by spray dried technique. The temperatures of spray drier at the inlet and outlet were 190–205°C and 85–90°C, respectively¹². After spray drying, the powder was used for further studies.

2.2 Gamma irradiation of amla juice powder

Three packets of fresh amla juice powder (1 kg) were packed in light density polyethylene bag and sent to Universal Medicare Centre, Vadodra, Gujarat for gamma irradiation at different doses i.e. 7-9 kGy, 15-18kGy and 25kGy. 1Kg of freshly made amla juice powder, sealed in a polybag, was preserved as a non-irradiation sample. Gamma radiation was performed in a cobalt-60 source chamber¹³. Further gamma irradiated samples were used for the evaluation of many physiochemical, phytochemical, microbiological and antioxidant properties.

2.3 Thin layer chromatography analysis

As per the Indian Pharmacopoeia procedure (2018), TLC was carried out using a mixture of ethyl acetate, toluene, glacial acetic acid, and formic acid (45:20:20:5) on silica gel 254 coated plates. The sample solution of both untreated and gamma irradiated was prepared by refluxing 0.5 g amla juice powder (treated and untreated) with 25 ml methanol for 15 minutes, the mixture was allowed to cool and was filtered using Whatman filter paper (No. 40). 25 ml sample solution was concentrated to 5 ml for TLC analysis. The 0.01% gallic acid was prepared in methanol to use as reference solution. 10µl of each solution was applied as spot and was allowed to rise up to 8 cm. The TLC plate was dried and examined under UV light (254nm) and sprayed with anisaldehyde sulphuric acid reagent. The plate was then heated to 100°C for five to ten minutes and observed under day light¹⁴.

2.4 Organoleptic analysis

The organoleptic analysis was carried out to observe the difference in appearance, taste, aroma and texture of fresh amla fruit juice powder before and after gamma radiation treatment¹⁵. The result was interpreted with plus (+) rating, 5+ rating describes very much similar, 1+ describes very much dissimilar.

2.5 Color Spectrophotometer

The color of powder was also measured by color spectrophotometer (make Konica Minolta) using JAYPAK: quality control system [Ver:01.08.001] software in LAB unit. According to Kusumiyati and Putri (2023), where L* stands for luminosity or brightness, a* represents greenness (-)/redness (+) and b* represents blueness (-)/yellowness (+)¹⁶.

2.6 Phytochemical evaluation

2.6.1 Low hydrolysable tannoids

To investigate the low molecular hydrolysable tannoids, Natreon Inc. HPLC method was followed with slight modification and CAPROS chromatogram of *Emblica officinalis* fruit extract was taken as reference. The powdered amla juice was mixed in 50 ml of distilled water and then passed through a 0.45µm syringe filter. The following parameters were applied to the filtered solution during HPLC analysis. Reversed phase column C18, 150mm × 3.9mm, 4mm, with a wavelength of 280nm and a mobile phase of 0.1 M sodium acetate buffer (pH 3.9). The HPLC analysis of amla juice powder was performed using Shimadzu make machine equipped with solvent delivery system, an autosampler, a DAD (diod array detector) and LC solution data acquisition system. The individual peak of tannoids was calculated against gallic acid standard and all four peaks constituted total hydrolysable tannoids¹⁷.

2.6.2 Gallic acid

Gallic acid was analyzed by using HPLC having mobile phase A, 0.136g potassium dihydrogen phosphate and 0.5 ml orthophosphoric acid in 1000ml water, mobile phase B, 100% acetonitrile, column C18, 4.6 mm X 250 mm, particle size of 5 μ and UV detection at 270 nm. Sample was dissolved in water at conc. of 0.5mg/ml and gallic acid (0.1mg/ml) was used as reference standard (Sigma Aldrich) ¹⁸.

2.6.3 Ascorbic acid

Ascorbic acid was evaluated by HPLC method. The sample and standard (sigma aldrich) were prepared in mobile phase. The composition of mobile phase is methanol: water: glacial acetic acid in the ratio of 30:69:1. The sample were analysed with following conditions, column C18-250 mm X 4.6mm, 5 μ m, flow 0.5ml/min, oven temperature 30°C, injection volume 10 μ l, diode array detector wavelength 230nm ¹⁹.

2.7 Titration methods

2.7.1 Titrable acidity

A.O.A.C. (1980) and Ranganna (1986) proposed a method for determining the titratable acidity. 0.2 g of fresh amla juice powder was taken and added 30 ml of distilled water followed by 10-minute sonication. A small amount of 1% phenolphthalein solution was added. The sample solution was titrated against N/10 NaOH. Total Titrable acidity was calculated as Citric acid in percentage ^{20,21}.

$$\text{Titratable acidity (\%)} = \frac{(0.0064 \times \text{Volume of NaOH used}) \times 100}{\text{Weight of sample (g)}}$$

2.7.2 Tannin

Tannin contents in amla fruit juice powder was performed according to AOAC (1980) and Poltanov *et al.*, (2009)²² method. 0.2g sample was taken in 1 L conical flask, then 750 ml deionized water was added, sonicated for 10 minutes and then 25 ml indigo sulphonic acid was added. This was titrated using a 0.1 N aq. KMnO₄ solution till blue-colored solution turned green, and then golden yellow. Titrating 25 ml of indigo sulphonic acid was done concurrently for the blank. Each ml of 0.1N KMnO₄ = 0.004157 g of tannins. Indigo sulphonic acid solution was prepared by adding 0.2g Indigo carmine in 100 ml of mixture. The mixture consisted of 10 ml hydrochloric acid and 990 ml (20% w/v) solution of sulphuric acid. The tannins of the juice were expressed in terms of in percentage ^{20,22}.

$$\text{Tannins \%} = \frac{\text{Volume of 0.1 N KMnO}_4 \text{ consumed} \times 0.004157 \times \text{Normality of KMnO}_4 \times 100}{\text{Weight of sample (g)} \times 0.1}$$

2.8 Fourier Transformed Infrared (FT-IR) analysis

For the identification of functional groups present in any compound, Fourier transform infrared (FTIR) spectroscopy has shown to be a useful method ²³. Functional groups like N-H, O-H, C-H, C = O, C = C, C = N and simply C = N are detected by FTIR spectroscopy ²⁴. Each extract's powdered sample was placed onto an FT-IR spectroscope (Shimadzu, Japan) with resolution of 4 cm⁻¹ and scan range of 400 to 4000 cm⁻¹. Using FTIR equipment, the untreated and gamma-irradiated sample was scanned in the transmittance mode between 400 and 4000 cm⁻¹ ²⁵.

2.9 Total antioxidant activity

The effect of varying gamma irradiation dosages on antioxidant capacity of powdered fresh amla fruit juice was assessed using DPPH, slightly altering the Luo *et al.*, (2009) methodology ²⁶. DPPH activity was carried out in radiated and irradiated amla fruit juice powder. Five different concentrations 40 μ g/ml, 80 μ g/ml, 120 μ g/ml, 160 μ g/ml and 200 μ g/ml of amla juice powder was prepared in methanol. 3 ml DPPH solution was added in each concentration and further volume was made to 10 ml with methanol²⁷. Following a vigorous shaking, the mixture was allowed to stand in the dark for 30 minutes. Using a Shimadzu 1800 UV spectrophotometer, the OD was taken at 517 nm. Methanol was used in place of the sample to achieve the absorbance of the control. Standard of ascorbic acid was prepared in different concentration 10 μ g/ml, 20 μ g/ml, 30 μ g/ml, 40 μ g/ml, 50

µg/ml and further 3 ml DPPH solution was added and made to 10 ml with methanol. Total antioxidant activity was calculated

$$\text{Total antioxidant activity (\%)} = \frac{(\text{Absorbance of control} - \text{absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

2.10 Analysis of microbial load

The total bacterial count (TBC) of all fresh amla juice powder samples-both non-irradiated and gamma-irradiated-was determined using the pour plate count method. 10 g sample was dissolved in Ninety millilitres of sodium chloride peptone buffer solution (pH 7). To ascertain the bacterial count, successive dilutions were made and plated on soyabean casein digest agar plates. For TBC, microbial counting was carried out 24–48 hours after incubation at 37°C²⁸. In case of yeast and mould count plating was done on sabouraud dextrose agar plate and incubation was done at 25°C. Yeast and moulds counting were performed after 5 days. The result of microbial load is shown in Table 3.

3. Result and Discussion

Gamma irradiation is often an inexpensive method of sterilizing and decontaminating food. Nevertheless, radiation has a detrimental effect on the foods' functional and nutritional qualities in addition to its beneficial effects. The research conducted by Charoenteeraboon et al. (2010) provides solid evidence for this effect of the received irradiation dose²⁹. Food quality is directly impacted by variations in constituent levels because it is based on its chemical composition. Thus, to ascertain whether or not the gamma-irradiation processing led to the alterations in the phytochemical contents of the amla powder, fresh amla juice powder was thoroughly examined in the current study. Thus, the gamma radiation is useful if it brings the decontamination of amla juice powder and simultaneously enhancing the shelf life without affecting much in physiochemical and biological properties of the powder.

3.1 Thin Layer chromatography (TLC)

The qualitative screening of phytochemicals present in fresh amla juice was studied by thin layer chromatography for both gamma treated at 7-9 kGy, 15-18 kGy and 25 kGy and untreated sample of amla juice powder. The spots on TLC plate (Figure 1) of gamma irradiated sample observed under UV light 254nm and after spray with anisaldehyde sulphuric acid followed by heating 110°C showed same spots as untreated sample of fruit juice amla powder. Gallic acid presence was also observed in all gamma radiated and non-radiated samples. The bands observed in untreated sample is same as present in gamma radiation treated samples. Therefore, there is no overall impact of gamma irradiation on major phytochemicals.

3.2 Organoleptic Analysis

Organoleptic characteristics of fresh amla juice powder were evaluated for appearance, color, taste, aroma and texture because these characteristics are important for end user acceptance. Table 1 represent the influence of gamma radiation on organoleptic characteristics, where no significant changes were observed on color, taste, and aroma but there was slight change in texture of powder. The color of powder was observed with naked eye and found that color of gamma irradiated amla fruit juice powder (Figure 2. b, c, d) was similar to untreated amla fruit juice powder (Figure 2a). The L*A*B value (Figure 3) of fresh amla juice powder and gamma irradiated powder remain same. The untreated and gamma-irradiated (7-9 kGy, 15-18 kGy, and 25 kGy) samples' L*A*B values did not alter. The free-flowing powder gain moisture and solidifies to some extent and converted in multiple solid mass after gamma radiation treatment at 15-18kGy and 25kGy dose but remains unchanged after 7-9 kGy gamma radiation. Following gamma irradiation at doses of 7-9 kgy, 15-18 kGy, and 25 kGy, the colour of amla juice powder does not change³⁰.

3.3 Analysis of Phytochemicals

The low hydrolysable tannoids are slightly reduced (Table 2) after gamma irradiation as compared with untreated amla juice powder. In this study the total tannin in untreated sample was 33.5±0.80% but it increased to 34.3±0.36 at 7-9 kGy dose, 34.7±0.56 at 15-18 kGy and 35.9±0.33% at 25kGy. Although there is sharp increase, other studies also reported increase in tannins content after gamma irradiation³¹. The impact of gamma irradiation on the profile of organic acids is shown in Table 2. Organic acids are an essential part of food and

an important factor in flavor perception. Non-irradiated amla fruit juice powder contained organic acid as citric acid ($20.1\% \pm 0.53$) but reduced to $19.15 \pm 0.14\%$ at 7-9 kGy, $19.59 \pm 0.49\%$ at 15-18kGy and $19.22 \pm 0.28\%$ (w/w) at 25kGy gamma treatment. The gallic acid content was increased slightly from untreated 0.76 ± 0.02 to 0.82 ± 0.01 , 0.83 ± 0.0047 and 0.84 ± 0.0047 at gamma irradiation dose 7-9 kgy, 15-18kGy and 25kGy respectively. The ascorbic acid content in untreated amla juice powder was $4.3 \pm 0.016\%$ whereas $4.27 \pm 0.016\%$ at 7-9kGy, $4.19 \pm 0.017\%$ at 15-18kGy and 4.14 ± 0.012 at 25kGy. Figure 4 shows the graphical representation of phytoconstituent changes in gamma irradiated and untreated amla juice powder. There is very low effect of gamma irradiation on major phytochemicals.

The ascorbic acid content is reduced from $4.3 \pm 0.016\%$ in untreated sample to $4.27 \pm 0.016\%$, $4.19 \pm 0.017\%$ and 4.14 ± 0.012 at 7-9kGy, 15-18kGy and 25kGy treated sample respectively. As per the earlier study done by Khattak, (2013) ascorbic acid decreased considerably at 3, 6, 9 and 12 kGy radiation doses ², same is also reported by Jabin *et al.*, (2023)³².

According to Maraei *et al.* (2017), gamma irradiation at various dose levels promotes the manufacture of several phenolic compounds, including ellagic acid, gallic acid, pyrogallol, and catechol ³⁴. In our study the gallic acid is also showing the same trend, increased in gamma irradiated amla juice powder.

3.4 Fourier Transformed Infrared (FT-IR) analysis

In FTIR analysis fresh amla juice powder (Figure 5) contains major 13 peaks at wavelength of 3270.91, 2922.85, 2346.55, 1704.64, 1609.07, 1446.45, 1316.64, 1205.64, 1019.93, 918.65, 760.31, 663.31 and 506.4. At first sight, according to FTIR spectra (A, B, C and D), there is no difference observed in gamma treated and untreated amla juice powder. Thus, gamma irradiation at 7-9kGy, 15-18kGy and 25kGy has no negative impact on product chemical groups. The gallic acid FTIR spectrum was also matching with major peaks of amla juice powder spectrum. Thus, study also supports the presence of gallic acid. It was clear that neither the distinctive infrared bands nor any new ones appeared or disappeared from the spectrum.

3.5 DPPH Activity

Ghosal *et al.* (1996) used detailed chemical analysis techniques like chromatography, spectroscopy, and critical analysis to show that fresh amla juice gets its antioxidant properties from low-molecular-weight tannins, not just its high vitamin C content [36]. Figure 6 shows the calibration curve (the standard curve: $y = 1.9621x + 0.929$, $r^2 = 0.9944$) of ascorbic acid. In this experiment amla spray dried untreated sample showed antioxidant activities 42.7%–92.7% at 40 ug/ml to 200 ug/ml concentration as compared to gamma irradiated sample. The gamma irradiation sample showed 45.6% to 94.7% at 7-9 kgy, 48.52% to 94.7% at 15-18kGy and 40.3% to 93.60%. There is some increment observed in antioxidant activity of amla juice powder after gamma irradiation at 7-9 kGy and 15-18 kGy, whereas clear reduction is seen at 25kGy.

Polyphenol content in amla fruit has also been linked to strong antioxidant activity in in-vitro studies ^{22,37,38}. This is particularly evident in the fruit's capacity to neutralize harmful molecules called free radicals, including the DPPH radical ³⁹. The antioxidant properties of lupin seed extracts were lessened by the increasing radiation dosage ^{40,27}. The scavenging capacities of control and 5, 10, and 20 kGy irradiation soybean samples have likewise not shown any discernible modifications ²⁷. According to Ahn *et al.* (2005), low and medium concentrations often have little effect on phytochemical antioxidants; nevertheless, the antioxidant activity of anise, cinnamon, mint, liquorice, nutmeg, vanilla, and ginger was unaffected by doses up to 10 kGy ⁴¹. This study also reports that fruit juice amla powder is not having any significant reduction in antioxidant activity and slightly increased at 7-9 kGy and 15-18 kGy but clear reduction is observed at 25kGy (Figure 7).

3.6 Microbiological Analysis

When gamma irradiated samples were exposed to 7-9 kGy, 15-18 kGy and 25kGy there was no bacterial load was noticed. In untreated sample the total bacterial count was $1.3 \times 10^3 \pm 3.8 \times 10^2$ cfu/g and total fungal count was $8.7 \times 10^2 \pm 2.0 \times 10^2$ cfu/g but after gamma treatment the microbial load becomes nil. Gamma radiation treatment disrupts the structure of DNA in microorganisms within the food. This damage prevents them from reproducing and contributing to spoilage. Additionally, gamma rays can weaken enzymes that degrade food components, further extending shelf life⁴². Increasing the absorbed radiation dose can achieve two goals: inactivating or killing harmful microbes in food and preventing the reproduction of parasitic insect eggs, both leading to extended preservation. In our study, at lowest dose 7-9kGy drastic reduction in total bacterial and yeast and moulds count has been observed immediately after irradiation. Thus, radiation treatment at 7-9 kGy

has been found effective in reducing the microbial load.

4. Conclusion

Gamma irradiation appears to have minimal impact on the concentration of phytochemicals within irradiated fresh amla juice powder. Some minor effects have been observed i.e. tannins and gallic acid are slightly increased but total titrable acidity, vitamins C and hydrolysable tannoids are slightly reduced after gamma treatment. DPPH activity slightly increased after irradiation at 7-9 kGy and 15-18kGy but some reduction observed at 25 kGy. The TLC and FTIR spectra of non-irradiated and irradiated sample remains same. There is no microbial load observed after gamma radiation even at 7-9 kGy dose. Thus, gamma irradiation is a safe and effective technique for preserving food quality, with an optimal treatment dose of 7-9 kGy

Conflict of Interest: The research team has no competing interests to report.

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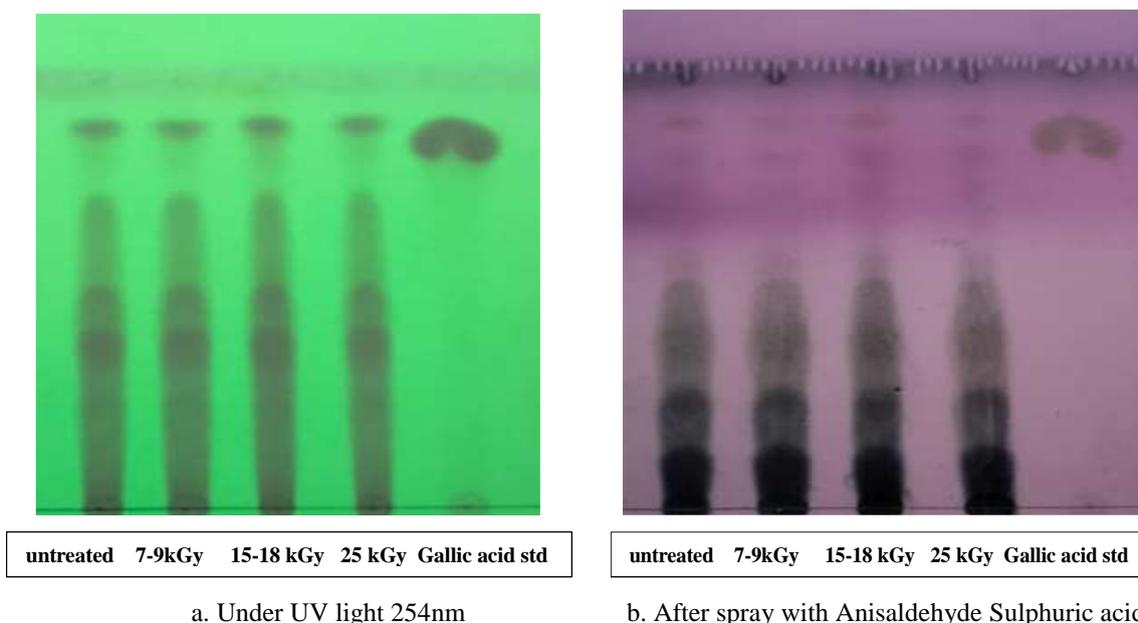


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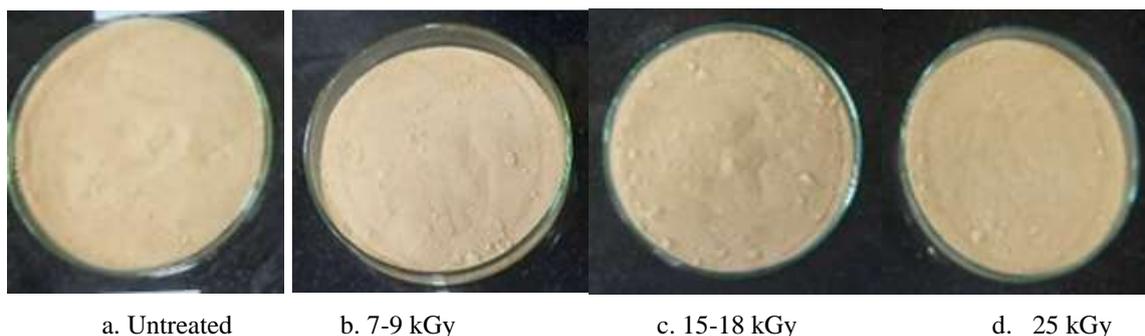


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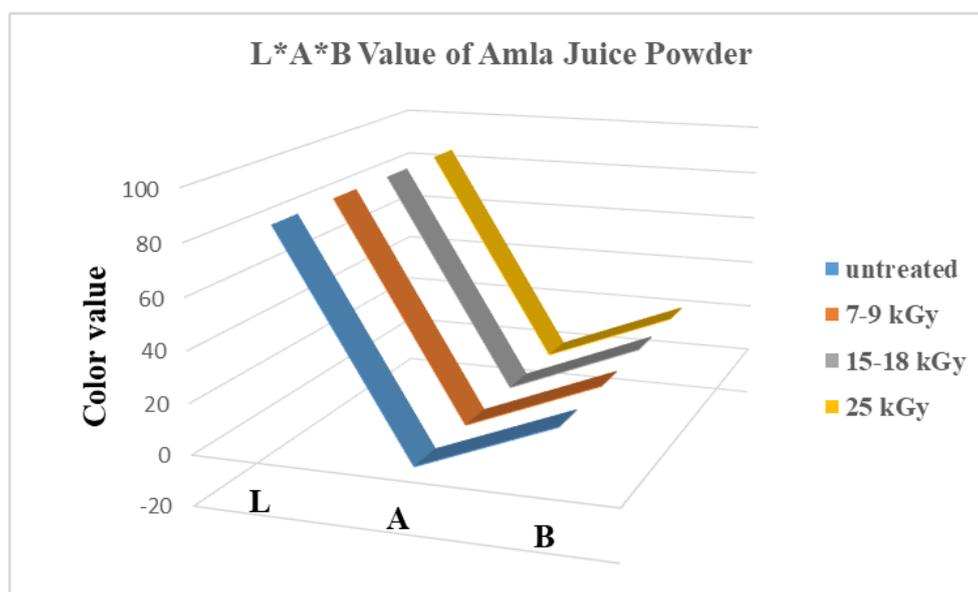


Figure 3. Graphical representation of LAB value of gamma treated and untreated amla juice powder sample.

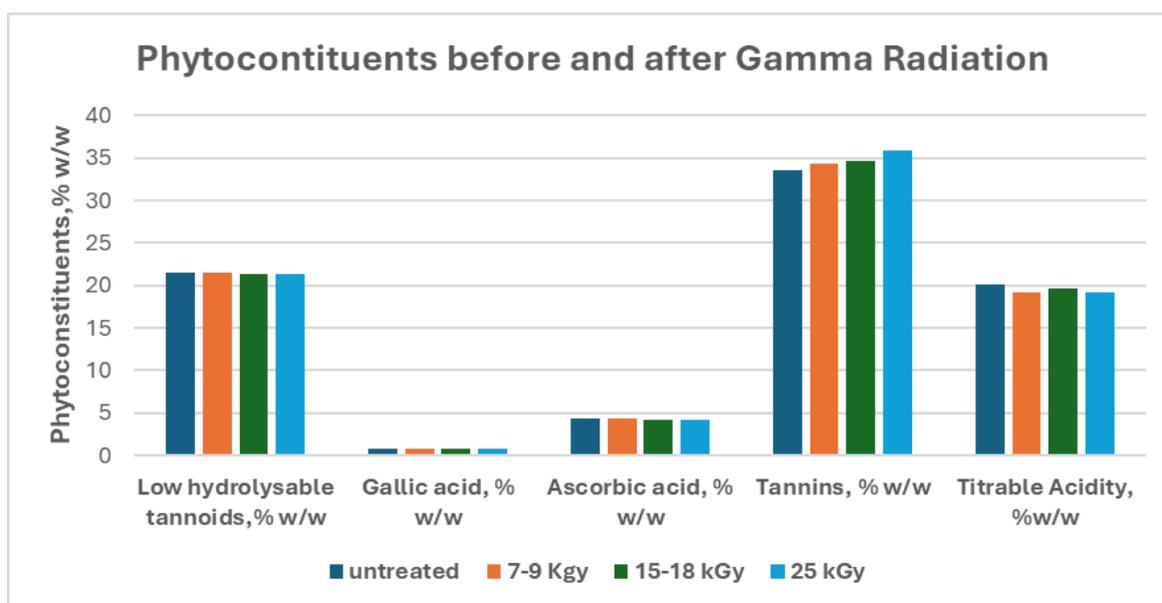


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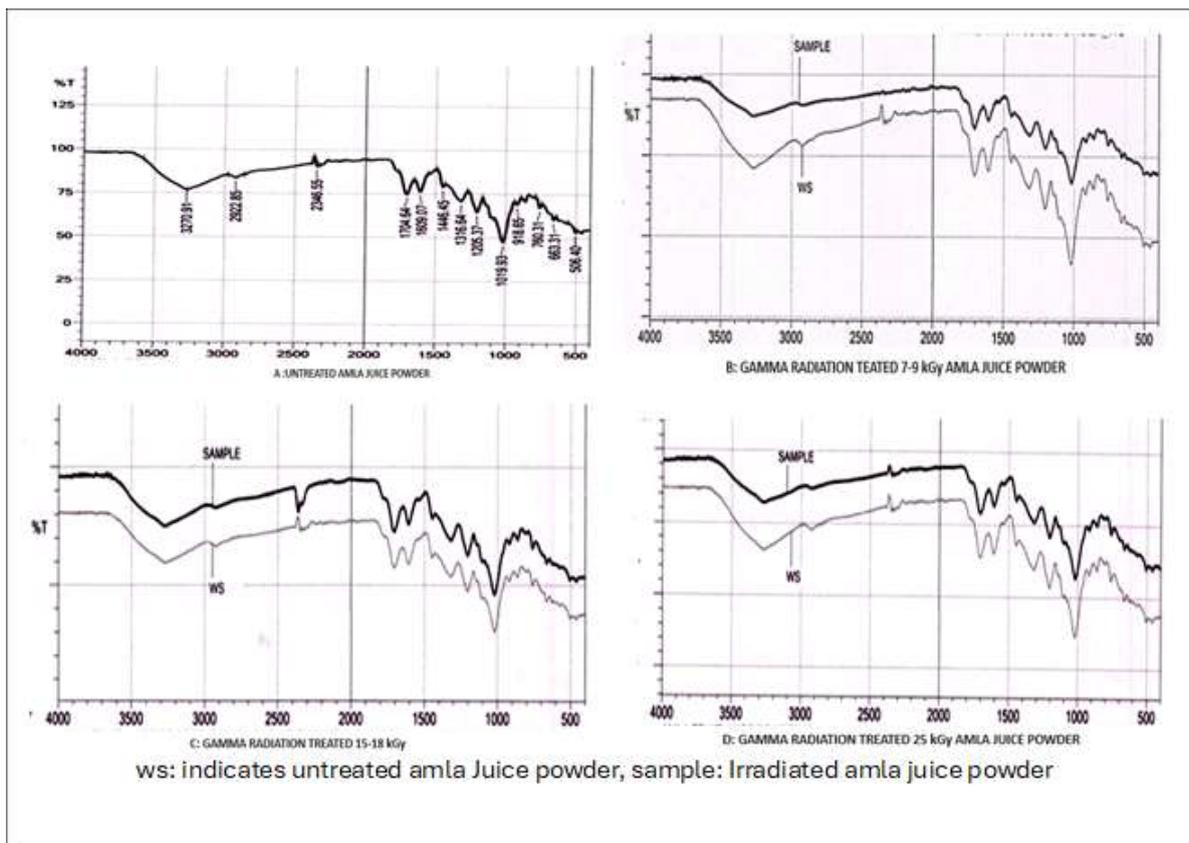


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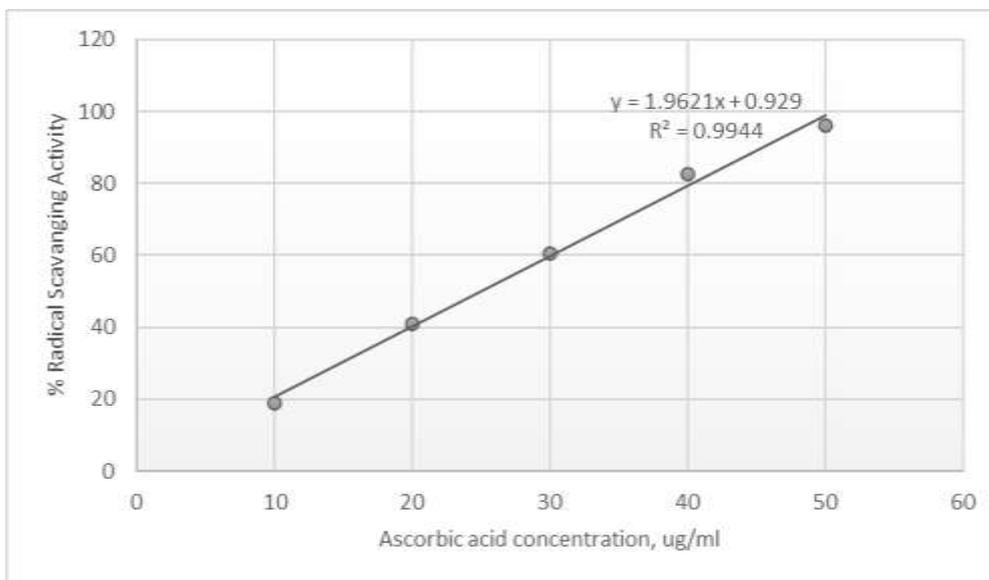


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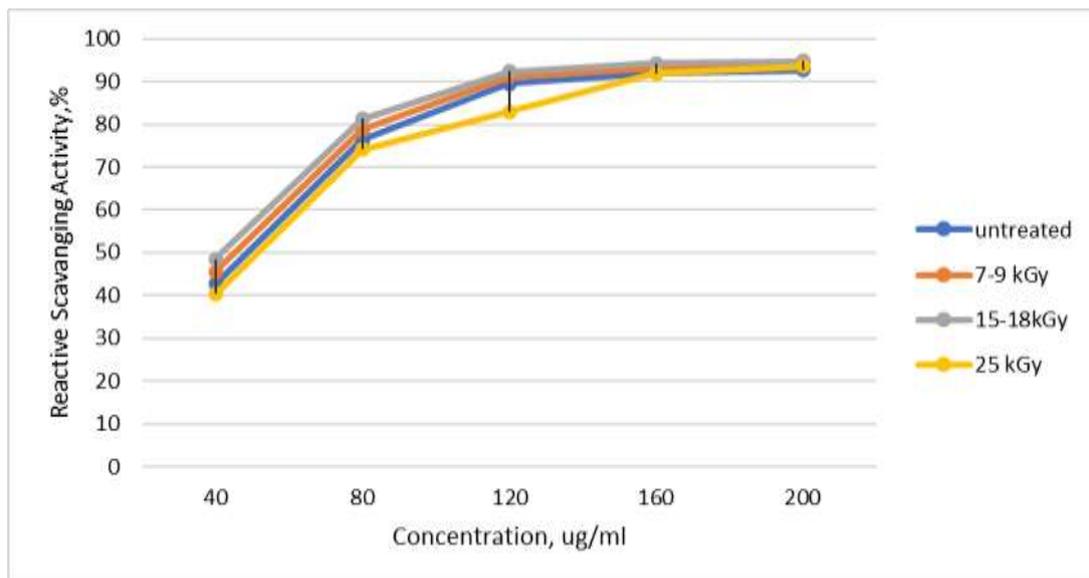


Figure 7. Antioxidant activity by DPPH assay

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Table 1: Organoleptic analysis of irradiated and non-irradiated fresh amla fruit juice powder.

Table 2: Phytochemical analysis.

Table 3: Microbial load analysis before and after gamma radiation treatment.

Table 1: Organoleptic Analysis of irradiated and non-irradiated fresh amla fruit juice powder

Sensory Parameter	Standards	Non irradiated	7-9 kGy radiated	15-18 kGy radiated	25 kGy radiated
Color	Off white	+++++	+++++	+++++	+++++
Aroma	Characteristic	+++++	+++++	+++++	+++++
Taste	Sour	+++++	+++++	+++++	+++++
Texture	Free flowing powder	+++++	+++++	++++	+++

Table 2: Phytochemical analysis

Phytoconstituents	Method	Non irradiated	7-9 kgy	15-18 kgy	25 kgy
Low hydrolysable tannoids (% w/w)	HPLC	21.55±0.22	21.42±0.16	21.27±0.11	21.3±0.24
Gallic acid, (% w/w)	HPLC	0.76±0.02	0.82±0.0081	0.83±0.0047	0.84±0.005
Ascorbic acid (% w/w)	HPLC	4.30±0.016	4.27±0.016	4.19±0.017	4.14±0.012
Tannins (% w/w)	Titration	33.50±0.80	34.30±0.36	34.70±0.56	35.90±0.330
Titrate Acidity (% w/w)	Titration	20.10±0.53	19.15±0.14	19.59±0.49	19.22±0.28

Table 3: Microbial load analysis before and after gamma radiation treatment

Parameter	Untreated	Gamma irradiated fresh amla juice powder		
		7-9kGy	15-18 kGy	25 kGy
Total bacterial count (cfu/g)	1.3 x 10 ³ ±3.8x10 ² cfu/g	ND	ND	ND
Total yeast and mould count (cfu/g)	8.7 x 10 ² ±2.0x10 ² cfu/g	ND	ND	ND