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Research Article

EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF JUSTICIA ADHATODA: A GAMMA RADIATION STUDY

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ABSTRACT

The present paper deals with investigations on the effect of gamma irradiation on the natural antioxidants of *Justicia adhatoda*. For this purpose sample was irradiated in Co-60 irradiator (gamma chamber 900) at dose of 1, 2, 3, 5 and 10 kGy at ambient temperature. The effect of radiation on the methanol extract was investigated by different assays. In case of DPPH assay, at the dose of 5 kGy, the samples showed good scavenging activity (31.41%). Similarly in case of reducing power and FRAP assays values increased up to 38.68 mg equi of Fe^{+3}/g of dw and 25.90 mg equi of Fe^{+3} / g of dw respectively.

Keywords: *Justicia Adhatoda*, Antioxidant activity, Gamma irradiation, DPPH.

INTRODUCTION

Radiation processing technology has been used to improve food security and safety[1]. Radiation technology can produce desirable effects on food ranging from physiological impacts like sprout inhibition, delay of ripening and increase of juice yield. It can also destroy some harmful micro-organisms, bacteria and other pests that might be present in the food, so could be used to reduce or replace chemical preservatives used in foods[1]. There are a few reports in the literature on the effect of irradiation on antioxidant activity of herbs and medicinal plants. Plants have played a critical role in maintaining human health and civilizing the quality of human life for thousands of years. Hence, the present work was under taken to study the effect of gamma irradiation on antioxidant activity of *justicia adhatoda* (Family**:** Acanthaceae). The plant has been recommended by physicians for the management of various types of respiratory disorders. It possesses potent bronchodilatory, expectorant and anti-spasmodic properties[2]. The juice from the leaves and the decoction of the leaves and roots are helpful in diarrhea, dysentery and glandular tumor[3]. It is commonly used for bleeding disorders, it relieves muscular pain, cramps or convulsions due to its antispasmodic property. It is used for stimulating contraction of the uterine muscle, facilitating or speeding up childbirth. It is also used for lowering of blood pressure in mildly hypertensive patients.

The objective of this study was to assess the impact of gamma irradiation on antioxidant activity of *justicia adhatoda* leaves extract.

MATERIALS AND METHODS

Chemicals

All the chemicals used for experiments were of analytical grade. DPPH (2, 2-diphenyl, 1picrulhydrazyl) was purchased from Sigma chemicals. While methanol AR grade, Ascorbic acid, F.C reagent(folin-ciocalteau reagent), Ferulic acid, TCA (Trichloroacetic acid), TPTZ (tripyridyl triazine), Potassium ferricyanide, Ferric chloride and Sodium acetate were purchased from Sisco Research Laboratories, Mumbai, India.

Sample collection and preparation

The leaves of J*usticia adhatoda* were purchased from local retailer in Pune and identified. For preparation of extract 1 gm of sample was soaked in 100 ml methanol and stirred it for 3 hrs. The extract was then filtered and centrifuged (2000 rpm) for 15 min.

Sample irradiation

Samples tightly capped in glass containers were irradiated in ^{60}Co irradiator (Gamma chamber -900) at a dose rate of 3.72 Gy/min. The total dose absorbed was varied as 1, 2, 3, 5 and 10 kGy. Irradiation was carried out at room temperature. Dosimetry was performed using Fricke dosimeters. The non irradiated sample was kept in the irradiation room to attend the same experimental conditions.

FT-IR spectroscopy

The Fourier-transform infrared (FT-IR) spectra were acquired by using a Spectrometer Shimadzu (FTIR 8400) at the wavelength region between 4000 and 400 cm^{-1}.

Total phenolic

Total phenolic content was measured by using the Folin-Ciocalteau method[4]. 1000 µl of sample extracts irradiated at various doses were mixed with 1 ml distilled water and 500 µl of 1N Folin-Ciocalteau reagent, incubated for 1 min and then 3 ml of 5% Na_2CO_3 was added. After incubation of two and half hrs at room temperature, absorbance was measured at 765 nm on UV-Visible spectrophotometer (PC-1600, Shimadzu Co) by using methanol as blank.

Antioxidant activity assays

FRAP assay

The antioxidant activity of extract was determined by using a modified method of ferric reducing /antioxidant power (FRAP)[4] assay. The FRAP reagent contains 2.5 ml of 10 mM TPTZ solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3.6H_2O$ and 25 ml of 0.3 M Sodium acetate buffer at pH= 3.6. 100 µL extracts of the sample irradiated at various doses were mixed with 3.0 ml of freshly prepared FRAP reagent, and the mixture was incubated at 37 ^0C for 15 min. Absorbance was measured at 593 nm on UV-Visible spectrometer by using distilled water as blank.

DPPH assay

The DPPH assay was carried out similar to earlier report by Rajurkar and Gaikwad (2010)[4] with some minor modification. 100 µL extract of the sample irradiated at various doses was mixed with 2 ml of 0.1 mM DPPH solutions in methanol. The reaction mixture was kept in dark at room temperature. The absorbance was measured at 517 nm on UV-Visible spectrophotometer. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and % scavenging was calculated by using the following equation:

$$\% \; scavenging = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control reaction (containing all reagents except the test compound), and A_1 is the absorbance of the test compound.

Reducing power assay

The reducing power of extract was determined by the previously published method[4]. 1000 µl of sample extract irradiated at various doses were mixed with 500 µl of 0.2 M Phosphate buffer (pH=6.6), 500 µl of Potassium Ferri-cyanide solution, 500 µl of 10% Trichloroacetic acid solution and 100 µl of 0.1% Ferric Chloride. Absorbance was measured at 700 nm on UV-Visible spectrophotometer. An increase in absorbance was used as measure of the reducing power.

RESULTS

When FTIR radiation falls on a molecule it may be absorbed, reflected or transmitted. Absorption of radiation leads to the FTIR spectrum[5]. Figure 1 shows the FT-IR spectrum in the spectral range 4000–400 cm^{-1} for gamma irradiated *Justicia adhatoda* with 0, 1, 5 and 10 kGy doses. As can be seen from this figure at 0 kGy the main band indicates C=O stretching at 1730.21 cm^{-1}[ester group] and 1643.41 cm^{-1} corresponds to amides, C=O group at 1417.73[acid] and C–OH group at 1053.17 cm^{-1}. After irradiation it is observed that there are some differences in height and shape of certain absorption bands.

Total phenolic contents

Phenolic compounds are a unique category of phytochemicals especially in terms of their vast potential health benefiting properties. Hence it is important to evaluate the total phenolic content from medicinal plants. Total phenolic contents were obtained by preparing the calibration curve using different concentration of Ferulic acid (FA) as standard antioxidant (0.02-0.10 mg/ml). The effect of gamma irradiation on total phenolic of *Justicia adhatoda* leaves extract is shown in Fig.2. In the present study it is observed that there is non-significant change in the phenolic content at 1, 2, 3 and 10 kGy doses in the irradiated *Justicia adhatoda* as compared to non-irradiated one. At 5 kGy dose there is a significant increase in the phenolic content [22.311 mg equi of FA /g of dw] was observed.

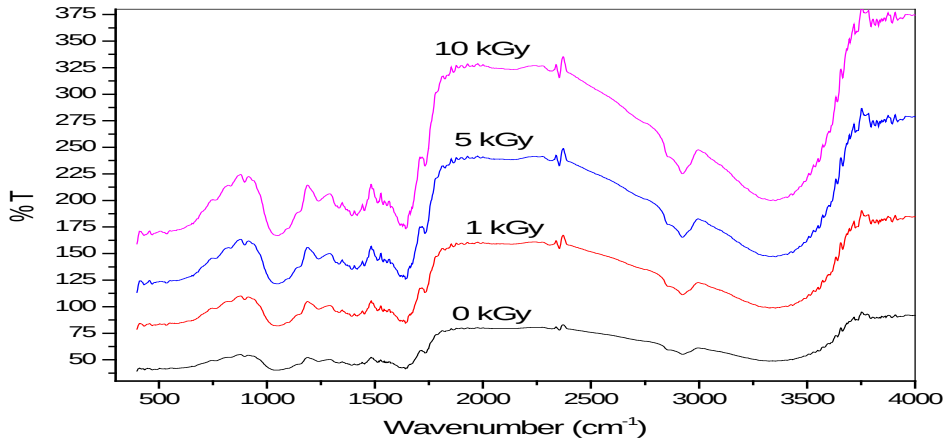

Fig. 1: FT-TR spectra of non-irradiated and irradiated *Justicia adhatoda* sample

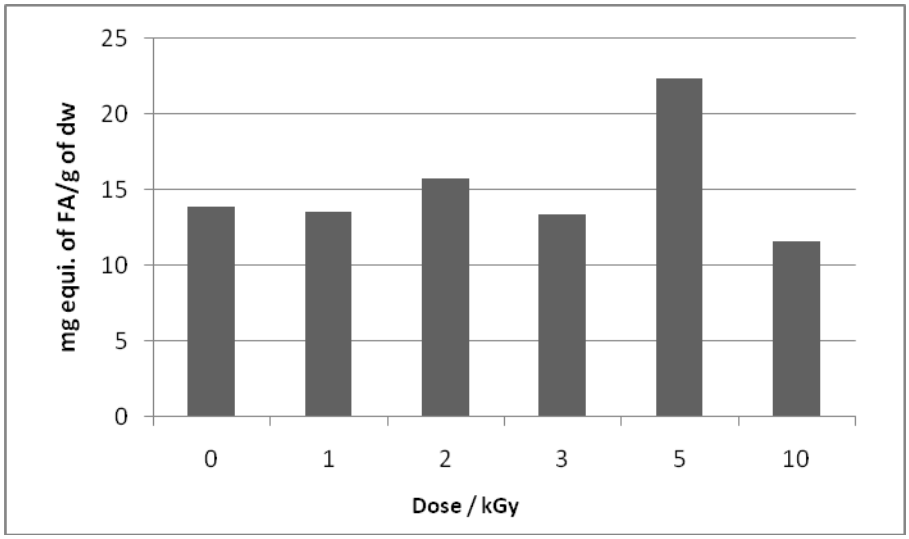

Fig. 2: Effect of gamma irradiation on total phenolic content of *Justicia adhatoda.* All results were expressed as mean ± standard deviation (N=2).

FRAP assay

The Effect of gamma irradiation on FRAP assay of *Justicia adhatoda* leaves extract is shown in Fig. 3. The results are expressed as mg equivalent concentration of Fe^{+3} reductions per gram dry weight of sample. As can be seen from the figure there is non-significant change in the Ferric reducing /antioxidant power (FRAP) assay was observed. At 5 kGy dose, the antioxidant activity was increases from 20.85 mg equi of Fe^{+3} /g dw to 25.909 mg equi of Fe^{+3} /g dw.

This increase in activity may be due to formation of Maillard reaction products (MRPs) which increases the reducing power and ultimately the antioxidant activity[6].

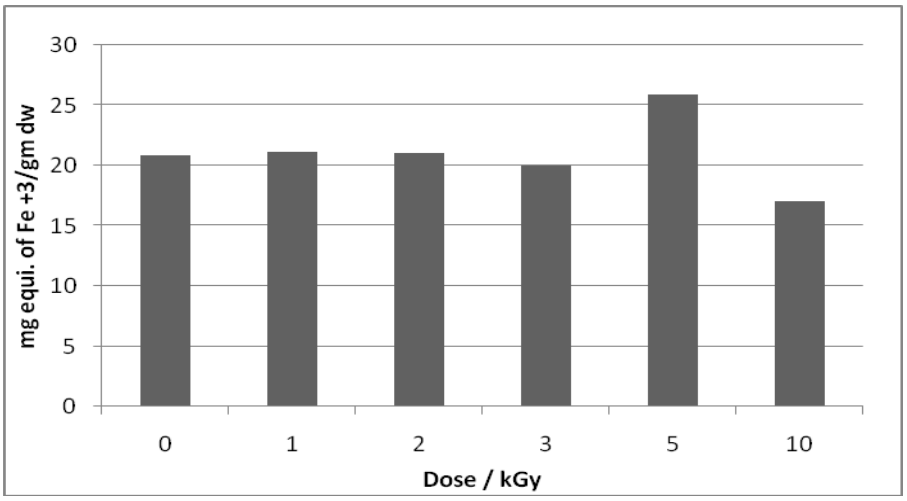

Fig. 3: Effect of gamma irradiation on FRAP assay of J*usticia adhatoda.* All results were expressed as mean ± standard deviation (N=2).

DPPH assay

A rapid, simple and inexpensive method to measure antioxidant capacity of foods involves the use of DPPH. It is widely used to test the ability of compounds to act as free radical scavenger or hydrogen donor and to evaluate antioxidant activity[7]. The reduction capability of DPPH was determined by decrease in its absorbance at 517 nm induced by antioxidants. The effect of gamma irradiation on DPPH radical scavenging activity of J*usticia adhatoda* is shown in Fig.4. As can be seen from figure 4 there is a significant change in DPPH activity after irradiation and maximum increase is found at 5 kGy (31.41 %). This increase in activity may be attributed to Maillard reaction products (MRPs) formed during irradiation of sample.

Presence of sugar and amino acids leads to formation of these Maillard reaction products (MRPs). These MRPs are able to scavenge hydroxyl radical and superoxide anion radical. Variyar et al (2004)[8] have also reported similar enhancement of antioxidant activity by gamma irradiation in soyabean.

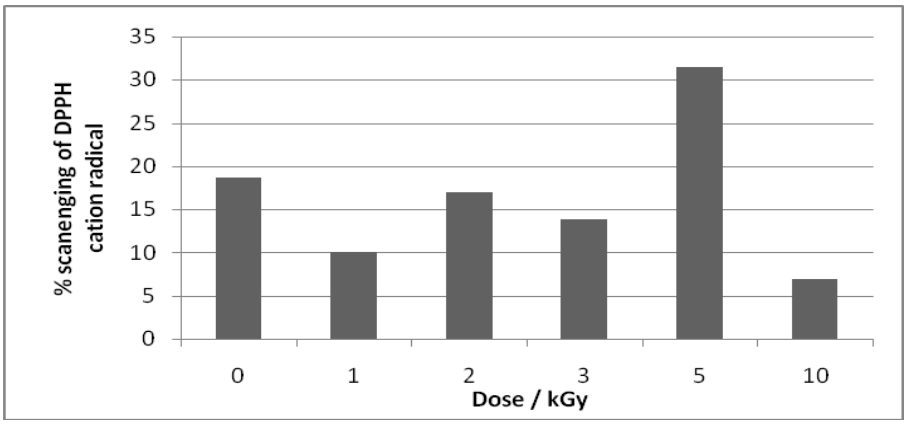

Fig. 4: Effect of gamma irradiation on DPPH scavenging ability of J*usticia adhatoda.* All results were expressed as mean ± standard deviation (N=2).

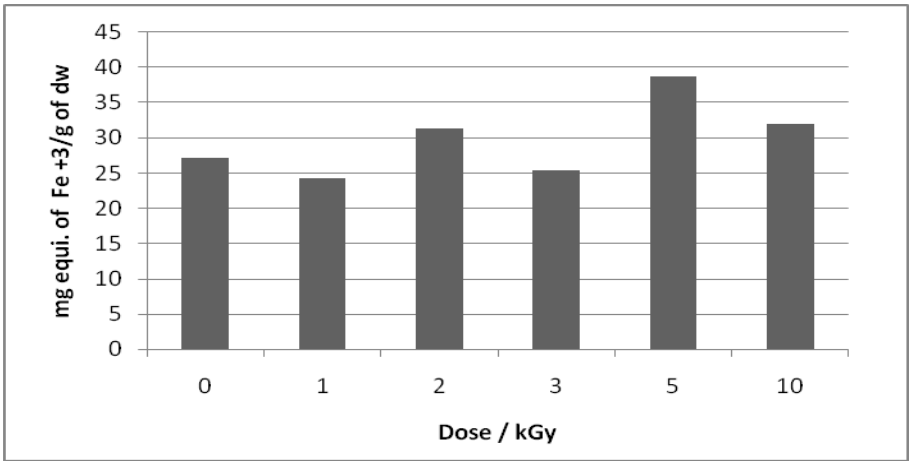

Fig. 5: Effect of gamma irradiation on reducing power of J*usticia adhatoda.* All results were expressed as mean ± standard deviation (N=2).

Reducing power assay

The antioxidant ability of certain compounds is associated with their reducing power. The reducing power was measured by reduction of Fe^{+3} to ferrous (Fe^{+2}) form. The effect of gamma irradiation on reducing power of J*usticia adhatoda* leaves extract is shown in Fig.5. It is observed that the sample irradiated at 5 kGy shows maximum reducing power [38.683 mg equi of Fe^{+3} /g of dw]. This increase in reducing power may be due to the formation of MRPs during irradiation, which help in reducing Fe^{+3} and hence increase in an antioxidant activity.

DISCUSSION

J*usticia adhatoda* is a well known plant drug in Ayurvedic and Unani medicines. It is used by Ayurvedic physicians and possesses some medicinal properties. It has been used for the treatment of various diseases and disorders particularly for the respiratory tract ailments. It is one of the primary herbs of the Ayurvedic system. In the present study we evaluate its antioxidant activity and effect of gamma radiation on antioxidant activity. Phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen atoms or electrons but also because of their stable radical intermediates which prevent the oxidation of various food ingredients particularly fatty acids and oils. Here the increase in phenolic acid after irradiation could be attributed to the release of phenolic compounds and degradation of larger phenolic compounds into smaller phenolic compounds by gamma irradiation[9]**.**

Maillard reaction is one of the major reactions taking place during thermal processing, cooking, and storage of foods and is responsible for change in colour and flavour of food. These MRP's were also form during the irradiation process. Further these MRPs were able to scavenge hydroxyl radical ($HO^{\cdot}$) and superoxide anion radical ($O_2^{\cdot-}$) and hence increase the antioxidant activity. Our current investigation shows significant increase in the DPPH scavenging activity at 5 kGy. This correlates with the earlier studies by Variyar et al. (2004)[8] in which the scavenging ability of soybean with 0.5–5 kGy of gamma irradiation on DPPH radicals increased with the doses used. It observed that as irradiation dose increases the antioxidant activity also increased for DPPH and reach to maximum. On further irradiation up to 10 kGy the activity start decreasing. In case of FRAP and reducing power assay also the FRAP value and absorbance was maximum at 5 kGy dose. This increase may be due to the formation of MRPs as a result of gamma irradiation. So we conclude that, at 5 kGy *justicia adhatoda* shows highest antioxidant activity. It also suggests that gamma irradiation can be efficiently increase antioxidant activity of *justicia adhatoda* by changing fixed antioxidant phenolic compounds into more free phenolic compounds.

CONCLUSION

From the results we can conclude that the low dose irradiation technology can be used to enhance the antioxidant activity of *justicia adhatoda* without any adverse change in physiological activity. Difference in the height and shape of certain absorption bands of FT-IR spectra shows that, there may be some structural changes in *justicia adhatoda.* Therefore, further study needs to evaluate structural changes after gamma irradiation of *justicia adhatoda.*

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