

Article

## Effect of $\gamma$ -irradiation on the Antioxidant Activity of *Salacia reticulata*

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Article history: Received 21 February 2015, Received in revised form 7 May 2015, Accepted 2 June 2015, Published 9 June 2015.

**Abstract:** The feasibility of using gamma irradiation to reduce microbial load and enhance shelf life of *salacia reticulata* was investigated. The irradiated and non-irradiated products were stored at room temperature (25-32 °C and 50-85% R.H) for 2 years. Acceptability of the irradiated product was assessed based on sensory, microbial and antioxidant status (estimating reducing power, lipid peroxidation inhibitory activity and scavenging activities of DPPH, NO and H<sub>2</sub>O<sub>2</sub> radicals). A dose of 10 kGy was sufficient to maintain microbial quality within acceptable limit up to 2 years of storage. No significant differences in sensory properties and antioxidant activities were observed between the non-irradiated and irradiated samples.

**Keywords:** salacia reticulata,  $\gamma$ -irradiation, antioxidant activity

### 1. Introduction

*Salacia reticulata* (SR) is an indigenous plant of the genus *Salacia* grown in Sri Lanka and India. It is usually used for the treatment of diabetes in ayurvedic medicine. The roots and stems of SR have also been used for prevention of rheumatism, gonorrhoea and skin disease. The root of *S. reticulata* has been reported to contain bioactive compounds such as salacinol, kotalanol, mangiferin, (-)-epicatechin, (-)-epigallocatechin, (-)-4'-O-methylepigallocatechin, (-)-epiafzelechin-(4 $\beta$ 8)-(-)-4'-O-methylepigallocatechin, (-)-epicatechin-(4 $\beta$ 8)-(-)-4'-O-methylepigallocatechin, hydroxyferruginol,

lambertic acid, kotalagenin 16-acetate, 26-hydroxy-1,3-friedelanedione, maytenfolic acid, 3 $\beta$ ,22 $\beta$ -dihydroxyolean-12-en-29-oic acid (Yoshikawa et al., 1998; Sekiguchi et al., 2010)..

SR is prone to microbial contamination and insect infestation during storage and transportation resulting in quality deterioration and economic loss. With a ban on chemical fumigation world over due to its adverse effects on human health and environment, processing of food by gamma radiation/electron beam has gained increased importance. Extensive studies have established the efficacy of this process as a safe method for preservation of herbs without producing any organoleptic changes, toxicological hazards, microbial and nutritional problems at the recommended decontamination doses (10 kGy) (Raso & Barbosa-Canovas, 2003; Diehl, 1995). The effects of radiation processing on the antioxidant properties of many spices and herbs have been reported earlier. Application of the  $\gamma$ -irradiation technology for the preservation of SR warrants proper post irradiation quality control of the plant and hence post-irradiation assessment of the medicinal values of SR is an essential requirement for the application of the technique. To the best of our knowledge, information on the effect of  $\gamma$ -irradiation on the antioxidant status of SR has not been investigated yet.

The present study was therefore carried out to check the feasibility of radiation processing to reduce microbial load, enhance shelf life and effect of  $\gamma$ -irradiation on the antioxidant activity of *Salacia reticulata*.

## 2. Materials and Methods

### 2.1. Materials

Three separate lots of *Salacia reticulata* (1 Kg each) were procured from local market. Each lot of all the powdered samples (mesh size 40) was divided into two sets. One set was kept as non-irradiated sample. The other set of samples, packed in high density polyethylene bags of 0.12 mm thickness were irradiated in a gamma chamber with cobalt-60 source (GC 5000, BRIT, DAE, Mumbai) to a dose of 10 kGy at the dose rate of 10 Gy/min. Dosimetry was performed using Fricke dosimeter. Irradiated and corresponding non-irradiated samples were stored under ambient conditions (25-32° C, 50-85% R.H.) for 2 years. Samples were taken at predetermined storage intervals (every three months) were analyzed in triplicate for the analysis of antioxidant activities. Three replicates of each sample were evaluated. All chemicals were purchased from Sigma-Aldrich Chemicals, USA. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). xylenol orange, Ethylenediaminetetraacetic acid (EDTA), trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and Folin-Ciocalteu reagent were purchased from Merck, India. Ferric chloride (FeCl<sub>3</sub>), sodium nitroprusside (SNP), sulfanilic acid and naphthylethylenediamine

dihydrochloride (NED) were purchased from SRL, India. All reagents used in this work were of analytical grade and solvents were redistilled before use.

## 2.2. Methods

### 2.2.1. Sensory analysis

Testing was carried out by an experienced sensory panel (15 members) using a 9 point scale with 1, dislike extremely or not characteristic of the product and 9, like extremely or very characteristic of the product (López-Rubira et al., 2005). Parameters evaluated were color, aroma, texture and overall acceptability. The sensory evaluation was done at every three months of storage intervals.

### 2.2.2. Microbial analysis

Standard methods were used to enumerate microorganisms present at each sampling time and treatment for 2 years of storage. The mesophilic bacteria, yeast and mold counts were carried out using Plate Count Agar and the pour plate method (Saroj et al., 2006). The sample (5 g) was homogenized in 50 ml of sterile physiological saline. After appropriate serial dilutions, the samples were pour plated on plate count agar. The colonies were counted after 24 h of incubation at 37°C. Total yeast and mold count was performed by pour plate method using potato dextrose agar supplemented with 10% tartaric acid to maintain pH of media to 3.5. Plates were incubated at 37°C for 48 h. Microbial counts were expressed as CFU g<sup>-1</sup> of herbal products. Each analysis was performed in triplicate.

### 2.2.3. Extraction

All samples (50 g each) were defatted with hexane and subsequently extracted with methanol (4 x 250 ml), 80% aq. methanol (4 x 250 ml) and finally with distilled water (4 x 200 ml) in an omnimixer. Three replicates were prepared. The respective extracts were dried in vacuum and made to 10% (w/v) solution of the respective solvents.

### 2.2.4. Total phenolics estimation

Total phenolic content was determined using Folin-Ciocalteu reagent according to the method of Tupe et al. (1999). Sample (40 µl, 1 mg/ml) was mixed with 200 µl Folin Ciocalteus reagent and 1160 µl of distilled water. After 3 min incubation 600 µl 20% sodium carbonate solution was added to the mixture and kept in the dark for 2 h at room temperature and absorbance was measured at 756 nm. Gallic acid was used as a standard and the total phenolic content was expressed as µg of gallic acid equivalents (GAE) per mg of extract.

### 2.2.5. DPPH radical scavenging Assay

The test extracts as well as the blank were incubated with 1,1-diphenyl-2-picrylhydrazyl solution in methanol (100  $\mu$ M) and the absorbance at 517 nm was monitored spectrophotometrically. Percentage inhibition of the radical was calculated according to the procedure reported earlier (Jao & Ko, 2002).

#### 2.2.6. Nitric oxide radical scavenging assay

The assay was carried out according to a previously described method, with modifications (Isfahlan et al. 2010). The reaction mixture contained 10 mM SNP, phosphate-buffered saline (pH 7.4), and the various concentrations of the samples. After incubation for 150 min at 25 °C, 1 mL of sulfanilic acid (0.33% in 20% glacial acetic acid) was added to 0.3 mL of the incubated solution, and the mixture allowed to stand for 5 min. NED (0.5 mL, 0.1% w/v) was added, and the mixture was incubated for 30 min at 25 °C. The pink chromophore, generated during diazotization of nitrite ions with sulfanilic acid and subsequent coupling with NED, was measured from the absorbance at 540 nm, using an appropriate blank.

#### 2.2.7. H<sub>2</sub>O<sub>2</sub> scavenging assay

Following a reported method, with some modifications, the H<sub>2</sub>O<sub>2</sub> scavenging activity was determined (Banerjee et al. 2003). H<sub>2</sub>O<sub>2</sub> (50  $\mu$ L, 1 mM) and various concentrations of the samples (each 100  $\mu$ L) were incubated for 30 min at room temperature. FOX reagent (0.85 mL, prepared from 100  $\mu$ M xylenol orange, 250  $\mu$ M ammonium ferrous sulfate, and 25 mM H<sub>2</sub>SO<sub>4</sub> in water) was added into the mixtures, which were allowed to stand for 30 min at room temperature. The absorbance of the ferric–xylenol orange complex at 560 nm was measured against an appropriate blank.

#### 2.2.8. Reducing power assay

The Fe<sup>3+</sup> reducing power of the extract was assayed following a reported method, with minor modifications (Oyaizu et al., 1986). Different concentrations of the sample were mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide (0.5 mL) and incubated at 50 °C on a water bath for 20 min. TCA (0.5 mL, 10%) was added to the mixture, which was centrifuged at 3000 rpm for 10 min. Finally, 0.5 mL of the upper layer was mixed with an equal volume of distilled water and 0.1 mL of 0.1% FeCl<sub>3</sub> solution. The reaction mixture was left for 10 min at room temperature, and the absorbance at 700 nm was measured against an appropriate blank.

#### 2.2.9. Isolation of mitochondrial fraction

Three month old female Wistar rats were used for the preparation of mitochondria. Rat liver was excised, homogenized in 0.25 M sucrose containing 1 mM EDTA. The homogenate was centrifuged at 3000 x g for 10 min to remove cell debris and the nuclear fraction. The resulting

supernatant was centrifuged at 10,000 x g for 10 min to sediment mitochondria. This pellet was washed with 50 mM Phosphate buffer, pH 7.4, to remove sucrose. The pellet was finally suspended in 50 mM phosphate buffer and protein content was estimated (Lowry et al., 1951).

#### 2.2.10. Exposure of mitochondria to agents inducing oxidative stress

The incubation mixture (0.5 ml) contained 0.35 ml of buffering medium (1 mM  $\text{KH}_2\text{PO}_4$  in 0.15 M Tris-HCl buffer, pH 7.4), 50  $\mu\text{l}$  of  $\text{FeSO}_4$  (final concentration 50  $\mu\text{M}$ ) in 0.1 N HCl, 50  $\mu\text{l}$  of membrane fraction containing 0.25 mg protein and 50  $\mu\text{l}$  of ascorbic acid to give a final concentration of 0.4 mM. The reaction was started by the addition of ascorbic acid. Incubation was carried out at 37°C in a shaker water bath (Devasagayam et al., 1983).

#### 2.2.11. Thiobarbituric Acid Reactive Substances (TBARS) assay

The method involved heating of biological samples with thiobarbituric acid (TBA) reagent (TBA-TCA-HCl-EDTA) for 20 min in a boiling water bath. TBA reagent contains 20% TCA, 0.5% TBA, 2.5 N HCl and 6 mM EDTA. After cooling, the solution was centrifuged at 2,000 rpm for 10 min and the precipitate obtained was removed. The absorbance of the supernatant was determined at 532 nm against a blank that contained all the reagents except the biological sample. Concentration of TBARS was then calculated with the help of standard graph using 1,1,3,3-tetraethoxypropane, as malonaldehyde equivalents (Devasagayam et al., 1983).

#### 2.2.11. Statistical analysis

Data presented for antioxidant activity assays are means of values obtained from three independent samples each analyzed in triplicate. Thus a total of nine replications was carried out for each complete set of analysis and the values reported as means  $\pm$  SD, n=9. One-way analysis of variance (ANOVA) followed by Tuckey's post-test was used to assess significant differences ( $p < 0.05$ ) between the samples. All the statistical analyses were accomplished using the computer software Graph Pad Prism 3.02 for Windows (Graph Pad Software, USA).

### 3. Results and Discussion

Sensory parameters and microbiological status are very crucial attributes that affect the consumer acceptance of a produce. Sensory and microbial analyses were therefore carried out for optimizing the radiation dose with a view to improve the shelf life of SR. 10 kGy was found to be the optimized dose from both analyses. No significant changes in the sensory attributes like color, aroma, texture, taste and overall acceptability was noted by the panelists during the entire storage period of 2 years (data not shown). Microbial status of irradiated and non-irradiated samples is shown in Table 1.

**Table 1.** Microbial load (cfu/g) of salacia reticulata samples during storage

Storage time	Microbial load (cfu/g)		yeast & mould count (cfu/g)	
	NI	I (10 kGy)	NI	I (10 kGy)
0 month	$2 \times 10^2$	0	35	0
3 months	$2.3 \times 10^2$	0	192	0
6 months	$2.9 \times 10^4$	0	350	0
9 months	$3.2 \times 10^6$	0	650	0
12 months	$3.2 \times 10^7$	0	$1.1 \times 10^3$	15
15 months	$3.2 \times 10^8$	50	$2.4 \times 10^3$	29
18 months	$>10^9$	$1.2 \times 10^3$	$3.6 \times 10^5$	43
21 months	$>10^9$	$2.4 \times 10^4$	$5.3 \times 10^5$	61
24 months	$>10^9$	$3.6 \times 10^5$	$7.9 \times 10^5$	82

The counts for non-irradiated samples increased significantly (more than acceptable limit,  $<5 \times 10^5$  CFU/g as prescribed for herbal medicines) after nine months. No bacterial load was detected in the radiation processed samples (10 kGy) either immediately or up to 2 years of storage. Yeast and mold counts also demonstrated a similar trend. Thus radiation treatment (10 kGy) was highly effective in reducing total aerobic, yeast and mold counts and extended shelf life up to 2 years. 80% aq. methanol extract was found to possess the maximum antioxidant activity among the several extracts. Hence all studies related to antioxidative capacities were performed using aq. methanol extracts only. Total phenolic contents, RP, LPO inhibition activity and DPPH, ABTS, NO and  $H_2O_2$  radical scavenging activities of non-irradiated and irradiated samples are presented in **Table 2**. Similar results for non-irradiated samples were also shown by Tupe et al. (2013). Radiation processing was not found to have any significant impact on them. Similar effect of radiation processing on the antioxidant properties of several medicinal plants have been reported earlier (Suhaj et al., 2006; Chatterjee et al., 1999). In sample matrices, besides phenolics several other constituents are irradiated together and hence mutual protection by the different constituents could result in a lower impact of radiation on the phenolics of the sample (Diehl, 1995). This could possibly explain the unaltered antioxidant status of the herb.

**Table 2.** Antioxidant status of non-irradiated and irradiated *salacia reticulata* samples during storage

Antioxidative Assays	Immediately after irradiation		After 2 yrs of storage	
	Non-irradiated	Irradiated	Non-irradiated	Irradiated
Total Phenolic content (mg Gallic Acid Equivalent /gm sample)	103.2 ± 1.28	105.9 ± 1.05	105.8 ± 1.25	104.6 ± 1.71
Reducing Potential	0.84 ± 0.09	0.85 ± 0.07	0.85 ± 0.09	0.84 ± 0.08
Lipid peroxidation (% inhibition)	85.29 ± 1.94	86.14 ± 1.85	84.83 ± 1.79	85.76 ± 1.67
DPPH (% scavenging activity)	80.59 ± 0.62	81.78 ± 0.59	82.65 ± 0.62	81.12 ± 0.37
Nitric oxide (% scavenging activity )	26.37 ± 0.85	25.57 ± 0.93	24.81 ± 0.76	25.21 ± 0.68
H <sub>2</sub> O <sub>2</sub> (% scavenging activity )	3.87 ± 0.19	3.64 ± 0.27	3.95 ± 0.13	3.88 ± 0.21

Values are expressed as mean ± S. D., n=9

#### 4. Conclusion

In the present study radiation processing was successfully applied for the preservation of *Salacia reticulata* up to two years while improving microbial quality. Radiation processing did not significantly affect its sensory quality and antioxidant activity checked by various *in vitro* assays.

#### References

- Banerjee, D., Madhusoodanan, U. K., Sharanabasappa, M., Ghosh, S., and Jacob, J. (2003). Measurement of plasma hydroperoxide concentration by FOX-1 assay in conjunction with triphenylphosphine. *Clin. Chim. Acta*, 337: 147-152.
- Chatterjee, S., Padwal Desai, S. R., and Thomas, P. (1999). Effect of  $\gamma$  -irradiation on the antioxidant activity of turmeric (*Curcuma longa* L.) extracts. *Food Res. Int.* 32: 487-490.
- Devasagayam, T. P. A., Pushpendran, C. K., and Eapen, J. (1983). Differences in lipid peroxidation in rat liver rough and smooth microsomes. *Biochim. Biophys. Acta*, 750: 91-97.
- Diehl, J.F. (1995). *Safety of Irradiated Foods*. New York: Marcel Dekker.
- Isfahlan, A. J., Mahmoodzadeh, J., Hassanzadeh, A., Heidari, R., and Jamei, R. (2010). Antioxidant and antiradical activities of phenolic extract from Iranian almond (*Prunus amygdalus* L.) hulls and shells. *Turk J Biol*, 34: 165-173.
- Jao, C. H., and Ko, W. C. (2002). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. *Fisheries Sci.*, 68: 430-435.

- Lopez-Rubira, V., Conesa, A., Allende, A., and Artes, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biol Technol.* 37: 174-185.
- Lowry, O. H., Rosenbrough, M. J., Parr, A. L., and Fandall, R. J., (1951). Protein measurement with folin phenol reagent. *J Biol. Chem.* 193: 265 - 275.
- Oyaizu, M. (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Jap. J. Nutr.*, 44: 307-315.
- Raso, J., and Barbosa-Canovas, G.V. (2003). Nonthermal preservation of foods using combined processing techniques. *Crit. Rev. Food Sci. Nutr.* 43: 265-285.
- Saroj, S. D., Shashidhar, R., Pandey, M., Dhokane, V., Hajare, S., Sharma, A., and Bandekar, J. R. (2006). Effectiveness of radiation processing in elimination of *Salmonella typhimurium* and *Listeria monocytogenes* from Sprouts. *J. Food Protect.* 69: 1858-1864.
- Sekiguchi, Y., Mano, H., Nakatani, S., Shimizu, J., and Wada, M. (2010). Effects of the Sri Lankan medicinal plant, *Salacia reticulata*, in rheumatoid arthritis. *Genes Nutr.* 5: 89-96.
- Suhaj, M., Racova, J., Polovka, M., and Brezova, V. (2006). Effect of  $\gamma$ -irradiation on antioxidant activity of black pepper (*Piper nigrum* L.). *Food Chem.* 97: 696-704.
- Tupe, R. S., Kemse, N. G., and Khaire, A. A. (2013). Evaluation of antioxidant potentials and total phenolic contents of selected Indian herbs powder extracts. *Int. Food Res J.* 20: 1053-1063.
- Yoshikawa, M., Murakami, T., Yashiro, K., and Matsuda, H. (1998). Kotalanol, a potent alpha-glucosidase inhibitor with thiosugar sulfonium sulfate structure, from antidiabetic ayurvedic medicine *Salacia reticulata*, *Chem. Pharm. Bull. (Tokyo)* 46: 1339-1340.