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Original Article

EFFECTS OF GAMMA IRRADIATION ON ACTIVE COMPONENTS, FREE RADICALS AND TOXICITY OF CASSUMUNAR GINGER RHIZOMES

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ABSTRACT

Objective: To study the effects of gamma irradiation (10 and 25 kGy) on volatile oil constituents, total phenolic content, and free radical scavenging activity of cassumunar ginger rhizomes. Moreover, the effects on toxicity were investigated on both non-irradiated and irradiated samples, and accompanied by measurements of free radical content.

Methods: Electron paramagnetic resonance spectroscopy (EPR) and GC-MS were used to determine free radicals and active compounds in essential oils, respectively. Toxicity was estimated using Toxi-Chromo Test**.** Total phenolic content and Antioxidant properties were determined using the Folin–Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, respectively.

Results: Irradiation at the doses of 10 and 25 kGy significantly ($P < 0.05$) increased free radicals in cassumunar ginger rhizomes. However, the volatile oils, total phenolic content, antioxidant activity and toxicity were not significantly ($P > 0.05$) affected by the irradiation doses.

Conclusion: The present results suggest that gamma irradiation at the doses up to 25 kGy can safely be used to sanitize dried cassumunar ginger rhizomes.

Keywords: Gamma irradiation, Cassumunar ginger, Essential oils, Antioxidant activity, Total phenolic content, EPR, Free radicals.

INTRODUCTION

Cassumunar ginger (*Zingiber montanum* (Koenig.) Link ex Dietr., *Zingiber cassumunar* Roxb.), commonly known as Plai in the Thai language, is an important medicinal plant in Thai traditional medicine, exhibiting many medicinal effects. Its rhizomes have long been used to relieve muscle and joint pain [1]. Further, several pharmacological studies have reported that the rhizomes of cassumunar ginger possess anti-allergic activity [2], anti-inflammatory activity [3-4] and antioxidant activity [5-6], which can be attributed to the essential oils and phenolic compounds.

Like other medicinal plants, cassumunar ginger rhizomes are prone to microbial contamination and insect infestation. Irradiation has been used to ensure that medicinal plants remain free of pathogenic microorganisms. The treatment by gamma irradiation reduces economic losses resulting from quality deterioration.

However, many studies have demonstrated that gamma irradiation affects the chemical constituents of the plants, such as total phenolic content [7-12], volatile oils [12,13-16] and biological activity such as antioxidant activity [9,12,17-20]. Moreover, irradiation resulted in the increase of free radicals in several herbs and spices [21].

The present work is designed to study the effects of gamma irradiation (10 and 25 kGy) on volatile oil constituents, total phenolic content, and free radical scavenging activity of cassumunar ginger rhizomes. Furthermore, the determinations of toxicity and free radical content will be investigated on both non-irradiated and irradiated samples.

MATERIALS AND METHODS

Sample irradiation

Spray-dried ethanolic extracts of cassumunar ginger rhizomes were purchased from a local company in Bangkok. The samples were irradiated by gamma rays from cobalt-60 at the doses of 10 and 25 kGy (Gammacell 220; dose rate12 kGy/h).

EPR Measurements

The amount of free radicals was determined by electron paramagnetic resonance (EPR) at room temperature using JEOL JES-RE2X (X band) provided with a TE_{011} cylindrical resonant cavity. The samples were introduced into quartz tubes (4 mm inner tube diameter). Measurements were made at room temperature on the same day as the irradiation treatment. Mn^{2+}/MgO was used for calibration with the g value of the fourth Mn^{2+} signal from the lowest magnetic field as g = 1.981, and that of the third Mn^{2+} signal as g = 2.034.

Analysis of volatile compounds by GC/MS

Volatile oils of cassumunar ginger rhizomes were obtained by steam distillation. The oils were diluted 1:100 in methanol before being injected into a gas chromatography-mass spectrometer (GC-MS). Volatile oil analyses were performed using an Agilent Technologies (model 6890 N) mass spectrometer interfaced to a Quadrupole mass selective detector (model 5973 inert). The ionization voltage was 70eV. HP-Innowax capillary column (30m x 0.25 mm i.d., 0.25mm film thickness) was used for the separation. The oven temperature was programmed to increase in a controlled and constant manner, from 50 °C to 240 °C, increasing at 4 °C/min.

Determination of total phenolic content

The total phenolic content was investigated by using the Folin–Ciocalteu method. First, ten microliters of the extract (10 mg/ml) were added to 100 µl of 7% aqueous sodium carbonate solution and mixed well. Then 10 µl of Folin–Ciocalteu reagent was added to the mixture and the total volume normalized to 250 µl using distilled water. After shaking, the extract was incubated for 90 min and the blue complex absorbance was measured at 750 nm against a blank control. All spectrophotometric work was performed using a Benchmark plus microplate spectrophotometer (Bio-Rad Laboratories (UK) Ltd). The total phenolic content was calculated on the basis of a calibration curve of gallic acid. The results were expressed as gallic acid equivalents (mg) per 10 mg of dry weight of the extract.

Free radical-scavenging assay with DPPH

The free radical scavenging activity was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. One hundred microliters of sample (10 mg/ml) were mixed with 100 µl of 0.022% 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution in MeOH, and left standing for 30 min. Absorbance of the mixture was then determined at 517 nm and the percentage activity was calculated.

Toxicity test using *E. coli* (K12 OR85)

Toxi-Chromo Test was determined according to the method as reported in the literature [22]. The Toxi-Chromo Test (Environmental Bio-Detection Products Inc., Ontario, Canada) is a bacterial assay based on the ability of toxic substances to inhibit the de novo synthesis of an inducible enzyme, beta-galactosidase, in a strain of the bacteria, *E. coli* (K12 OR85). The absorbances of the tests, blanks and controls were measured at 615 nm with a microplate reader. Mercuric chloride in water was used as a positive control.

Statistical analysis

All measurements were performed in triplicate. Excel 2007 (Microsoft, Inc.) was used for correlation and t-test analysis. $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Effects of gamma irradiation on free radical generation

The EPR spectra and the height of the samples are shown in Figure 1 and Table 1, respectively. Our results showed that on the day of the irradiation, non-irradiated cassumunar ginger showed a small singlet peak. EPR singlet signals at g = 2.006 might be due to semiquinone radicals [21,23-27]. Gamma irradiation at the doses of 10 and 25 kGy increased free radicals 14.6 and 17.8 times, respectively. Similarly to our results, Gamma irradiation treatments resulted in the increase of the free radicals in basil, bird pepper, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage [21]. The free radicals might be produced by the oxidation of polyphenolic compounds which are ubiquitously present in plants [23].

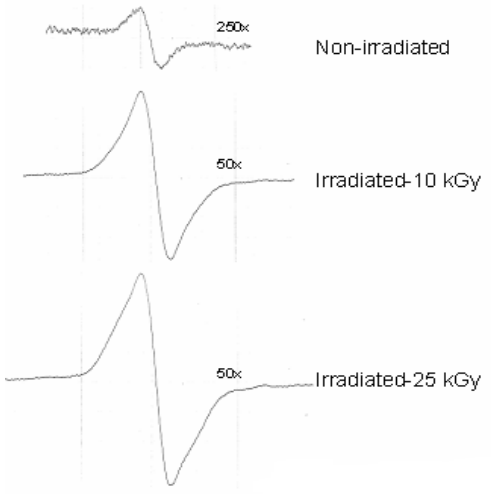

Fig. 1: EPR spectra of non-irradiated, 10 and 25 kGy irradiated cassumunar ginger samples with amplitude 250x, 50x, and 50x, respectively

Table 1: Free radical induction of control and irradiated cassumunar ginger

Radiation doses (kGy)	Free radical induction (height;amplitude 50x; mean ± SD)
0	164.5 ± 7.8
10	2397.0 ± 94.8
25	2927.5 ± 4.9

Effect of gamma irradiation on volatile oils

Monoterpenes (C_{10}), sesquiterpenes (C_{15}) and phenylbutanoids are typical constituents of cassumunar ginger volatile oils, with representative structures shown in Figure 2. The results of irradiation on volatile oils of cassumunar ginger rhizomes are shown in Table 2. In this present work, seventeen compounds were identified and the major volatile constituents of non-irradiated samples were (E)-1-(3,4-dimethoxylphenyl)butadiene (30.46%), terpinen-4-ol (14.33%), 4-(3',4'-dimethoxy phenyl)but-3-ene (9.18%), beta-sesquiphellandrene (7.04%), 4-(2',4',5'-trimethoxy phenyl) but-3-ene (6.16%), 4-(2',4',5'-trimethoxyphenyl)but-1,3-ene (3.88%) and gamma-terpinene (3.82%). No qualitative or major quantitative changes were observed in volatile oil constituents of irradiated cassumunar ginger rhizomes at both doses of 10 and 25 kGy. Phenylbutanoids were increased but this increase was not statistically significant ($P > 0.05$). Terpinen-4-ol has been shown to exhibit topical anti-inflammatory and antimicrobial activity. (E)-1-(3, 4-dimethoxyphenyl) butadiene (DMPBD) has shown anti-inflammatory effects [1]. Since our results showed that the active volatile compounds of cassumunar ginger rhizomes were not significantly changed ($P > 0.05$) after irradiation, it may be concluded that biological activities of the irradiated herbs might be maintained.

Effects of gamma irradiation on total phenolic content

The results of total phenolic content expressed as mg equivalents of gallic acid/10 mg dry weight of extract are given in Table 3. Gamma irradiation (10 kGy and 25 kGy) did not induce any detectable significant changes in total phenolic content ($P > 0.05$). In accordance with our results, other published accounts have indicated that irradiation treatment did not show any significant effects on the total phenolic content in turmeric [28], artichoke, sweet basil [29], cardamom and cinnamon [30]. Conversely, the ability of gamma irradiation to increase phenolic content in plant material has also been observed in many herbs and spices including velvet beans [7], fresh-cut vegetables (romaine, iceberg lettuce, endive) [8], Niger seeds [9], *Citrus unshiu* pomaces [10], rosemary [11], clove and nutmeg [30]. On the other hand, significant decreases of phenolic content were observed in dehydrated rosemary [29] and tomato [31]. The uncertain effects of irradiation on total phenolic content may be due to various factors such as plant type, dose of gamma irradiation, extraction solvent, extraction procedures, type of phenolic compounds, sample state, temperature, etc [9].

Effects of gamma irradiation on free radical-scavenging activity

The radical-scavenging activities of the samples were 62.92%, 61.13% and 61.79% for non-irradiated, 10 and 25 kGy irradiated cassumunar ginger samples, respectively (Table 3). No significant differences ($P > 0.05$) were observed in the scavenging activity of the control and irradiation-processed samples at 10 and 25 kGy. Consistent with our results, Murcia, et al (2004) report irradiated dessert spices (cinnamon, ginger, nutmeg, anise, vanilla, licorice, and mint) did not show significant differences in their scavenging activity as a result of irradiation at doses of 1, 3, 5, and 10 kGy [32]. Gamma-irradiation treatment (at 2.5 and 20 kGy) did not cause any significant effect on antioxidant capacity of methanol extracts of freeze-dried mushrooms [33]. However, some reports showed different results for the effects of gamma irradiation on antioxidant properties. Research conducted by Ahn et al (2005) and Suhaj et al (2006) indicated a decrease of antioxidant levels [17,19], whereas Khattak et al. (2008) and Variyar et al, (2004) reported increased antioxidant levels [9,20]. The inconsistent effects of irradiation on antioxidant content may be dependent on several factors including the dose delivered, exposure time, the technological criteria, raw material used and extraction solvents, etc [34].

Effects of gamma irradiation on toxicity

Our toxicity results as shown in Figure 3, demonstrated that toxic effects were in a dose-dependent manner but were not significantly ($P > 0.05$) affected by the irradiation doses. In addition, our data suggested that the irradiation-induced free radicals did not cause magnification of the toxicity.

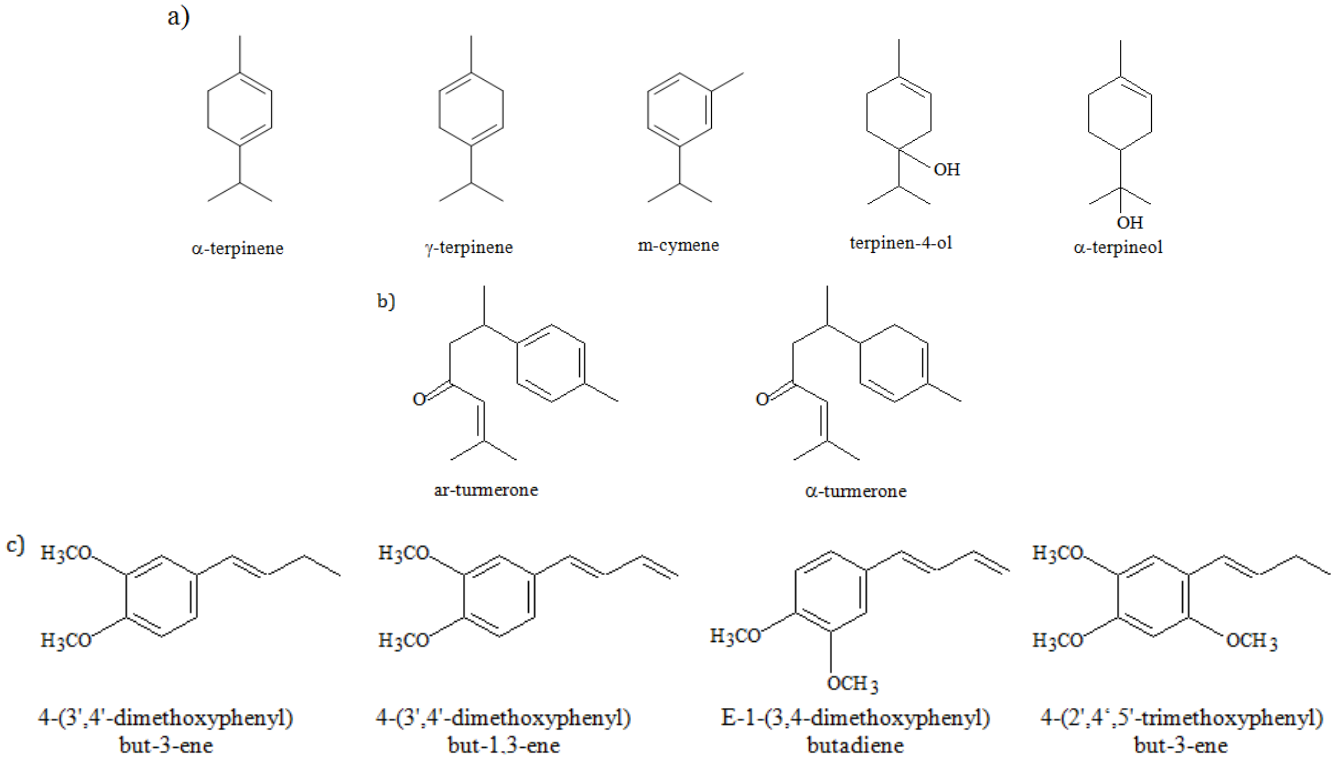

Fig. 2: Structure of some volatile oils of cassumunar ginger rhizomes a) monoterpenes (C_{10}), b) sesquiterpenes (C_{15}) and c) phenylbutanoids.

Table 2: Volatile oil contents (Mean ± SE) in untreated and gamma irradiated cassumunar samples

RT	Volatile oils	Relative percentage		
		Non-irradiated	irradiated 10 kGy	irradiated 25 kGy
3.92	sabinene	1.277±0.301	0.977±0.240	0.977±0.085
4.92	alpha-terpinene	2.677±0.808	2.460±1.032	2.297±0.140
6.28	gamma-terpinene	3.823±1.000	2.017±1.450	2.780±0.139
6.84	cymene	0.527±0.253	0.390±0.128	0.343±0.038
7.11	alpha-terpinolene	1.230±0.295	0.997±0.300	0.770±0.030
15.9	terpinen-4-ol	14.327±3.877	12.143±2.838	7.593±1.529
18.42	alpha-terpineol	1.070±0.205	1.030±0.108	0.857±0.144
20.45	beta-sesquiphellandrene	7.043±1.846	5.640±0.484	4.570±0.373
20.54	ar-curcumene	0.905±0.078	1.043±0.145	0.957±0.101
30.39	ar-turmerone	2.067±0.691	1.923±0.396	1.410±1.320
31.76	alpha-turmerone	0.960±0.384	0.940±0.205	1.423±0.576
32.09	beta-turmerone	1.147±0.439	1.377±0.291	2.010±0.624
32.58	4-(3',4'-dimethoxyphenyl)but-3-ene	9.183±1.348	9.777±1.410	10.307±0.878
32.83	4-(3',4'-dimethoxyphenyl)but-1,-3-ene	1.557±0.530	1.523±0.335	2.050±0.453
35.9	(E)-1-(3,4-dimethoxylphenyl)butadiene	30.457±1.848	33.167±1.550	33.800±1.572
38.07	4-(2',4',5'-trimethoxyphenyl)but-3-ene	6.160±0.893	7.147±0.268	8.290±0.953
41.33	4-(2',4',5'-trimethoxyphenyl)but-1,3-ene	3.880±0.816	4.973±1.090	5.600±1.434

Table 3: Total phenolic content and free radical scavenging activity of control and irradiated cassumunar ginger

Radiation doses (kGy)	Gallic acid equivalent (mg per 10 mg of dry weight; mean ± SD)	% Inhibition of DPPH (mean ± SD)
0	0.0108±0.0007	62.92±0.93
10	0.0116±0.0007	61.13±0.82
25	0.0111±0.0005	61.79±0.14

Irradiation is not the only process to produce free radicals. Common physical processes such as roasting, heating, pounding, and crushing can also generate free radicals similar to irradiation [7,35-36]. The carcinogenic radiolytic products such as 2-alkylcyclobutanones, benzene and furan are found from both irradiation and cooking processes. Therefore, irradiation and the resulting changes in free radical formation should not be considered alarming and may be proven to be safe.

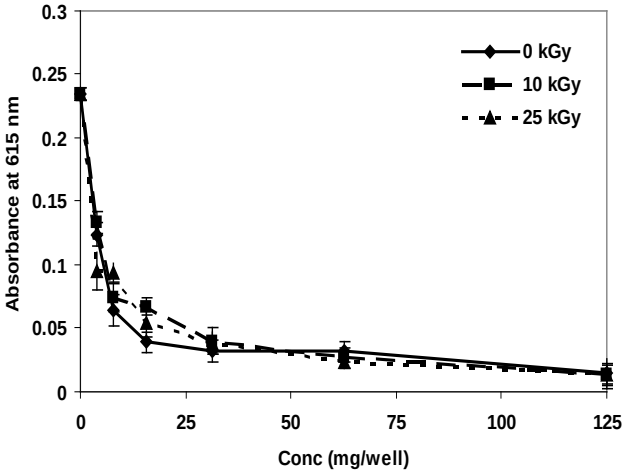

Fig. 3: Toxicity of non-irradiated, 10 and 25 kGy irradiated cassumunar ginger samples.

CONCLUSION

Our results showed that gamma irradiation at the doses of 10 and 25 kGy significantly increased free radical formation ($P < 0.05$). However, the volatile oil content, total phenolic content, free radical scavenging activity and toxicity were not significantly ($P > 0.05$) affected by the irradiation doses and the increased free radicals. In conclusion, the present results suggest that gamma irradiation doses up to 25 kGy could safely be used to sanitize dried cassumunar ginger rhizomes

CONFLICT OF INTERESTS

Declared None

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