

Effect of Radiation Processing on The Antioxidant Activity of Sage and Cinnamon

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تأثير المعالجة الإشعاعية على النشاط المضاد للأكسدة للمريمية والقرفة

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Abstract

In the present study, the effect of radiation processing on dried sage leaves and cinnamon barks samples were carried out at dose level of 25 kGy. Total phenolic content was determined in the extracts of these herbs alongside the antioxidant properties of their methanolic extracts were assessed using reducing power assay and by DPPH radical test within an extract concentration range 2.5 to 40 mg/ml of methanol. The

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result showed the total phenolic compounds were increased by 11.18% and 10.19% for irradiated sage and cinnamon, respectively. The values of EC₅₀ estimated from the results of reducing power assay and DPPH radical test showed that sage extracts had higher antioxidant activity than cinnamon extracts

In summary, gamma-irradiation of dried sage leaves and cinnamon barks was found to be significantly increase the total phenolic content, and on top of that the process not only maintained the antioxidant properties of dried sage leaves and cinnamon barks but also enhanced the antioxidant properties, to some extent.

خلاصة

تهدف الدراسة الى تقييم تأثير المعالجة بأشعة جاما على نشاط بعض مضادات الأكسدة الطبيعية الموجودة بالمرمية والقرفة والتي لها تأثيرات علاجية شائعة حيث تم تشيع المرمية والقرفة بالجرعة ٢٥ كيلو جرای . وقد تم تقدير المحتوى من الفينولات الكلية في المستخلص الميثانولي لهذه الأعشاب إلى جانب دراسة الخصائص المضادة للأكسدة لتلك المستخلصات الميثانولية وتم تقييم المقدرة الاختزالية reducing power والنشاط الكاسح للشقوق الحرة DPPH radical وذلك عند نطاق تركيزات من ٢,٥ الى ٤٠ ملجرام عينة لكل ١ مللي من الميثانول. وأظهرت النتائج زيادة المركبات الفينولية الكلية بحوالي ١١,١٨% و ١٠,١٩ لكل من المرمية والقرفة المعالجة اشعاعيا، على الترتيب. وأظهرت قيم EC₅₀ من نتائج المقدرة الاختزالية والنشاط الكاسح للشقوق الحرة أن مستخلصات المرمية كان أعلى في نشاطها كمضادات للأكسدة عن مستخلصات القرفة.

وعموما، وجد أن التشيع الجامي لأوراق المرمية المجففة ولحاء القرفة بالجرعة المستخدمة يؤدي الى زيادة المحتوى من الفينولات الكلية، وعلاوة على ذلك فإن المعالجة الاشعاعية لا تسبب فقط الحفاظ على الخصائص المضادة للأكسدة لأوراق المرمية المجففة ولحاء القرفة ولكن تؤدي إلى تعزيز الخصائص المضادة للأكسدة ، إلى حد ما.

Introduction

Cinnamon (*Cinnamomum verum*), and sage (*Salvia officinalis*) are important commercial spices valued for their flavouring properties. Phenolic compounds present in these spices are known to be responsible for their astringency and bitterness besides contributing to their medicinal properties [1].

The polar extracts of sage (*Salvia officinalis*) have strong radical-scavenging ability and superoxide anion radical-inhibiting ability [2]. The antioxidative activity of sage oil compounds, due primarily to the presence of compounds with vicinal -OH groups, is correlated with the oxygenated diterpene and sesquiterpene concentrations [3, 4].

Cinnamon contains a number of antioxidative components including vanillic, caffeic, gallic, protochatechuic, p-hydroxybenzoic, p-coumaric, and ferulic acids and p-hydroxybenzaldehyde [4]. Cinnamon has been reported to have the highest polyphenolic compound concentration (13.7mgGAE/g) [5]. Cinnamon bark, has been reported to have the strongest free radical-scavenging abilities [6]. At 5 mg/mL, cinnamon has a radical-scavenging activity of 92% [5]. Using a β -carotene-linoleate model system, the volatile oil of cinnamon inhibited 55.9% and 66.9% of the oxidation at 100 and 200 ppm, respectively, compared to the control [4]. Therefore, Sage and cinnamon tea, are of the popular plants since they have antioxidant activity and therapeutic advantage. Sage and cinnamon are used in traditional herbal medicine in the form of herbal infusion or essential oil [7].

Herbs and spices are prone to insect infestation and microbial contamination during sun-drying, storage, transportation and marketing, which can result in quality deterioration and economic loss. The use of herbs and spices to improve health has a long history including traditional and cultural aspects [8, 9]. Therefore the importance for adequate preservation methods for herbs has become more important. Use of ethylene oxide to decontaminate herbs and spices have recently been banned in many countries including the European Union and Japan on

account of possible toxic residues and the occupational health hazard for workers in the fumigation plant.

Treatment with ionizing radiation (i.e. gamma rays or electron beam irradiation) is an accredited preservation method. Irradiation treatment has been used for microbial decontamination of food, herbs, cosmetic raw materials and cosmetic products, and according to one study, the technology was applied to botanical raw materials at doses between 4 to 30 kGy for general contamination and 10 to 40 kGy for uncommon high contamination [10, 11]. Such treatment was found to have no adverse effect on the quality of herbs and spices. No significant changes in any of the major aromatic constituents have been reported so far. Although changes in aroma constituents of spices upon gamma-irradiation have been exhaustively studied. Little is known about the effects of irradiation on the antioxidant activity of phytochemicals. In addition, the observed effects appear contradictory: no significant effects of gamma-irradiation on the antioxidant capacity were found for the ethanolic extracts of oregano and black pepper [13, 14], whereas a significant decrease of the reducing power of the methanolic (80%) pepper extracts was observed after irradiation [15]. Decreased total ascorbate and carotenoid contents were found in some of nine irradiated herbs, among them oregano and sage [16]. Antioxidative activities in seven spices, among them nutmeg, were not affected by irradiation in different analyses [17].

The purpose of the present work was to examine the impact of gamma-irradiation treatment at the dose level of 25 kGy on the phenol compound contents and the antioxidant properties of sage and cinnamon.

Materials And Methods

Samples and chemicals

Cinnamon (*Cinnamomum verum*) barks and sage (*Salvia officinalis*) leaves were purchased from the local market (Cairo, Egypt). All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA.

Irradiation Treatment

For studying the effect of the gamma-irradiation, the samples of cinnamon and sage were packed in well-sealed polyethylene bags (1000 gauge). Each bag contained about 250 g. They were subjected at the ambient temperature to gamma irradiation from a ^{60}Co source at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The irradiation facility used was Indian Gamma Cell, delivered a dose rate of $2.95246 \text{ kGy h}^{-1}$ at the time of experimentation. The applied dose was 25 kGy, as monitored by FWT-60-00TM radiochromic film. Non- and irradiated sample were stored at 5 °C until being used to analyze.

Preparation of samples and extracts

The raw and irradiated samples were powdered. The extracts were obtained using methanol with 25 ml of 80% aqueous methanol and placed in an ultrasound bath for 2 h. the extracts were filtered through Whatman No. 4 filter paper and centrifuged at 2000 rpm for 10min and the supernatant used immediately for the experiments.

Determination of total phenolics content (TPC)

TPC values from 80% methanol extracts were determined according to the method of Shahidi and Naczki [19]. A 0.25 ml aliquot of the respective extract was mixed with 0.25 ml of Folin Ciocalteu reagent (previously diluted with water 1:1 v/v) and 0.5 ml saturated sodium carbonate (Na_2CO_3) solution and 4 ml water. The mixture was allowed to stand at room temperature for 25 min and then centrifuged at 5000 rpm for 10 min. Supernatant absorbance was measured at 725 nm, using a spectrophotometer. The results were expressed as mg ferulic acid eqivs./g dry matter of sample. All samples were analysed in triplicate.

Reducing power

The reducing power was determined according to the method of Oyaizu [19] (1986). Each extract ($0.5\text{--}10 \text{ mg ml}^{-1}$) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mmol L^{-1} sodium phosphate buffer (pH 6.6) and 2.5 ml of 10 mg ml^{-1} potassium ferricyanide and the mixture was incubated at 50 °C for 20 min. After 2.5 ml of 100 mg ml^{-1} trichloroacetic

acid were added, the mixture was centrifuged at 200g for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 1 mg ml⁻¹ ferric chloride, and the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power.

Scavenging ability against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Each extract (0.5–10 mg ml⁻¹) in methanol (4 ml) was mixed with 1 ml of methanolic solution containing DPPH (Sigma) radicals, resulting in a final concentration of 0.2 mmol L⁻¹ DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank [20]. The scavenging ability was calculated as follows: Scavenging ability (%) =

$$[(\Delta A_{517} \text{ of control} - \Delta A_{517} \text{ of sample}) / \Delta A_{517} \text{ of control}] \times 100.$$

Statistical analyses

Statistical analyses were performed using SAS for Windows version 9.1 [21]. Student's t-test analyzed differences between samples and controls. Regression analysis was also used to calculate EC₅₀. All data are presented as mean ± SD. The significance level was set at p < 0.05.

Result and Discussion

Phenolic compounds are hydroxylated derivatives of benzoic and cinnamic acids and contribute to overall antioxidant activities in the plants. The total phenolic content of irradiated and non-irradiated samples of dry sage and cinnamon barks was determined using the Folin-Ciocalteu's phenol reagent. The results are expressed as mg equivalents of gallic acid/100 g dry weight of extract and given Table 1. The phenolic contents of the control (non-irradiated) samples were found to be 21.29 and 15.02 mg per 100 g for extract from non-irradiated dry sage and cinnamon, respectively. Gamma-irradiation at 25 kGy significantly increased the total phenolic contents of sage and cinnamon, respectively, by 11.18% and 10.19%, as compared to their respective control. It seems that 25 kGy of irradiation might induce some chemical reactions in

components of sage and cinnamon, which possibly degrade or decompose large molecules into small phenolic molecules, and subsequently increased their ability to be soluble in methanolic extract.

Table 1. Total phenolic content (mg/100g) in extract from non-irradiated and irradiated dry sage and cinnamon. Values represents the average \pm standard error of three determinations.

Constituents	Irradiation Dose (kGy)			
	Sage		Cinnamon	
	0	25	0	25
Total phenols	21.29 \pm 0.109	23.67 \pm 0.1562	15.02 \pm 0.1026	16.55 \pm 0.1644
Difference	-2.38		-1.53	
Changes (%)	----	11.18	----	10.19
P value	0.0002		0.0014	

The available information in the literature on the effect of ionizing radiation on the phenolic content of sage and cinnamon is a limited. However, for other plant materials, diverse effects of radiation on the phenolic content have been reported. Khattak et al. [22] observed that irradiated *Nigella sativa* seed samples at dose levels of 2, 4, 8, 10, 12 and 16 kGy significantly increased the phenolic content. Variyar *et al.*[1] found increased amounts of phenolic acids in irradiated cloves and nutmeg. Similarly, Harrison and Were [23] also reported increases in total phenolic content of gamma-irradiated almond skin extract, as compared to the control samples. These increases in phenolic contents were associated with the degradation of tannins [1] and changes in the conformation of the molecules [24], as a result of the irradiation treatment. In contrast, Koseki *et al.*[25] reported a decrease in the amount of total phenolic compounds in dehydrated rosemary after irradiation doses of between 10 and 30 kGy, with respect to controls. The difference in the effect of radiation on total phenolic content may be due to plant type, geographical and environmental conditions, state of the sample

(solid or dry), phenolic content composition, extraction solvent, extraction procedures, temperature, dose of gamma irradiation.

Reducing power

The antioxidant activity of dry sage leaves and cinnamon bark extracts has been also associated with its reducing power [26]. Reducing powers (expressed as absorbance at 700 nm). The reducing power of extracts from non- irradiated and irradiated sage and cinnamon, with concentrations between 2.5 and 40 mg dry herb/ml methanol, is presented Fig.1. All extracts were able to reduce Fe^{3+} depending on concentration and increasing linearly (Table 2) with concentration increase.

The data of reducing power test were annualized by linear regression analysis to estimate the EC_{50} values for the extracts of non-irradiated and irradiate sage and cinnamon (Table 2). For the, this parameter is defined as the sample concentration with an absorbance value of 0.5. According to EC_{50} estimated values, it can be seen that, similarly to the DPPH radical test, cinnamon extracts have a higher reducing power than sage extracts. It also observed that irradiation treatment increased the reducing power of cinnamon by 1.3%.

Using the ferric reducing/antioxidant power method, Ahn et al. [27] reported that the reducing power of phytic acid was significantly increased by gamma-irradiation. In addition, using the same method, Ahn et al. [28] found that 0.5 kGy of irradiation increased or maintained the antioxidant activity of the minimally processed Chinese cabbage. However, Byun et al. [29] applied gamma-irradiation to Korean red ginseng powder and found that its hydrogen donating activity was not significantly changed by up to 10 kGy of gamma-irradiation.

Depending up on the above-mentioned results, it could be concluded that dry sage and cinnamon barks might contain some reductones, which could react with free radicals to stabilize and terminate the radical chain reaction. Apparently, the irradiation energy at 25 kGy on dry cinnamon barks might produce and release more reductones than in dry sage leaves.

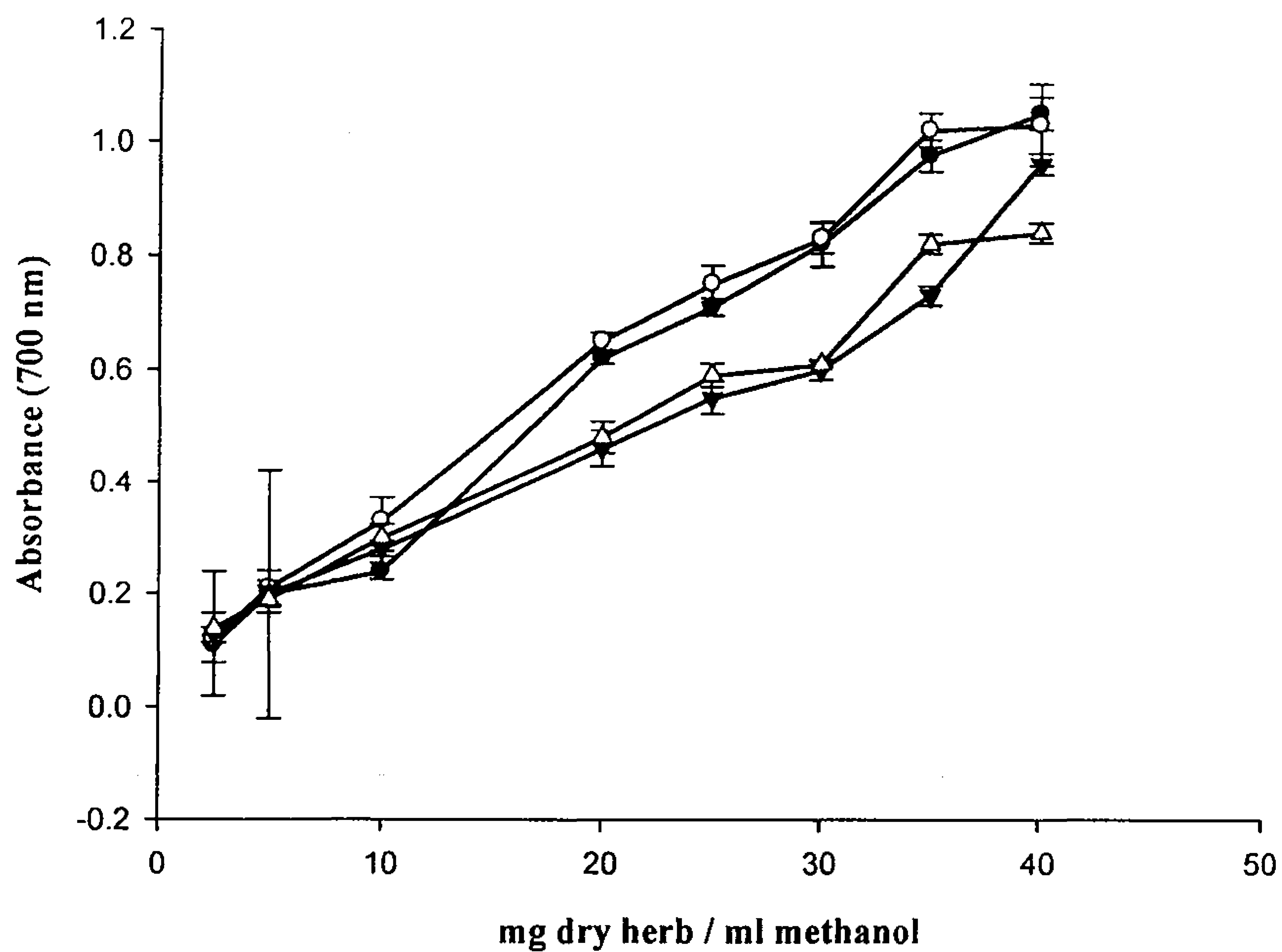


Fig 1. Reducing power of methanol extract of non-irradiated and irradiated dry sage and cinnamon.

Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals of raw and irradiated dry sage leaves and cinnamon barks

Antioxidants are substances that react and inactivate free radicals, thus preventing and reducing their adverse effects on biological targets. Accordingly, the synthetic DPPH radical, although not biologically relevant, is widely used as an indicator compound to test the radical scavenging ability and therefore the antioxidant activity, both of simple compounds and plant extracts [30]. The scavenging ability of dry sage leaves and cinnamon barks extracts on the DPPH radical, stated as inhibition percentage, was analyzed in a concentration range between 2.5 and 40 mg/ml of methanol (Fig. 2).

Scavenging abilities of methanolic extracts of raw dry sage leaves

and cinnamon barks at 25 kGy on DPPH radicals was 84.97 ± 0.260 and $54.63 \pm 1.828\%$ respectively at 20 mg/ml, indicating that raw dry sage leaves and cinnamon barks were good in this scavenging ability (Fig 2). Irradiating sage and cinnamon post the scavenging ability of their extracts. As the concentration of samples increased, scavenging ability increased linearly. At 40 mg/ml, methanolic extracts of non-irradiated exhibited excellent scavenging abilities of 88.3 ± 0.115 and $81.83 \pm 1.271\%$, respectively. Meanwhile, irradiated dry sage leaves and cinnamon barks at 25 kGy showed scavenging abilities of 89.77 ± 0.767 and $77.10 \pm 0.866\%$, on DPPH radicals, respectively. Obviously, these scavenging abilities were comparable with methanolic extracts from 35mg raw and irradiated dry sage leaves or cinnamon barks per ml methanol.

For a better comparison of the antioxidant activity, it is common practice to use the EC_{50} parameter. A low EC_{50} value states a strong antioxidant activity. For the DPPH radical test, EC_{50} (DPPH) is defined as the sample concentration that reduces the radical initial concentration by 50%. The values of EC_{50} (DPPH) were obtained by lineal regression analysis (Table 2). However Table 3 presents EC_{50} (DPPH) values for the metanolic extract of sage and cinnamon. Both non irradiated and irradiated cinnamon extracts had higher EC_{50} values than raw and irradiated sage, which means a lower scavenging ability (Table 3). It was observed the irradiation treatment increased the EC_{50} values of studied herbs.

Earlier research studies showed different results for the effect of gamma irradiation on the antioxidant properties of plant materials. A research study conducted by Jo [31] indicated that the scavenging ability on DPPH radicals was increased in green tea extracts following irradiation at 10 and 20 kGy.

Variyar et al. [1] found that the scavenging ability of soybean, irradiated at 0.5 to 5 kGy on DPPH radicals, was increased with doses used. Huang and Mau [32] reported that, methanol extracts of irradiated freeze-dried mushrooms did not show significant modifications in their

scavenging activity as a result of irradiation doses between 2.5 and 20 kGy.

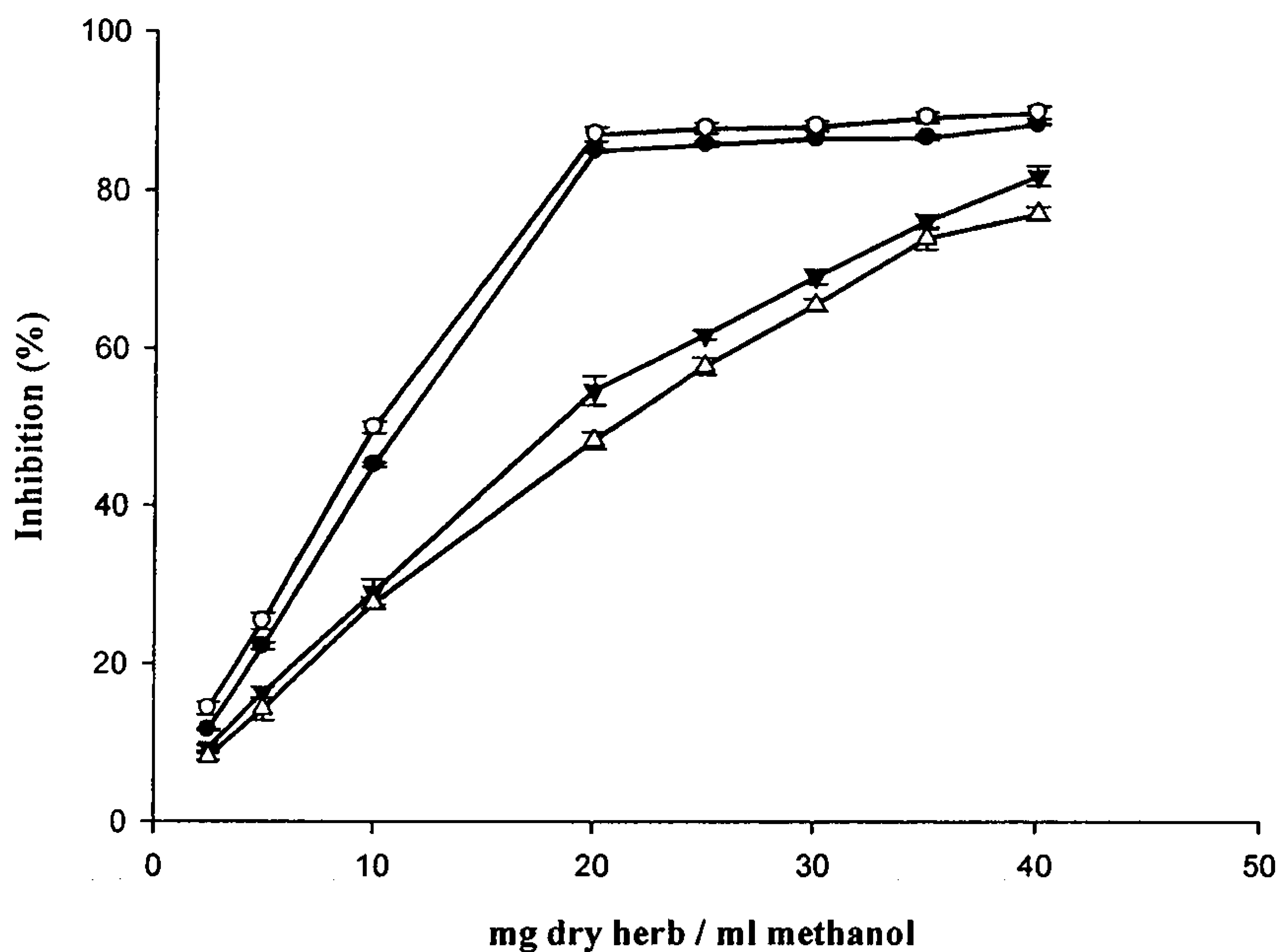


Fig 2. Scavenging ability of methanol extract of non-irradiated and irradiated dray sage and cinnamon on DPPH radicals.

Ahn *et al.* [27] reported that, after irradiation, scavenging ability of phytic acid on DPPH radicals was recorded. However, they pointed out that unirradiated phytic acid did not show scavenging ability. Green tea leaf extracts with 10 and 20 kGy of gamma-irradiation significantly increased the scavenging ability on DPPH radicals at 4°C [31]. Khattak *et al.* [22] studied the effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella staiva* seed. They reported that gamma-irradiation enhanced the scavenging activity in acetone and methanol extracts by 10.6% and 5.4%,

respectively, at 16 kGy. They also found that gamma irradiation increased the extraction yield and total phenolic content, as well as enhancing the free radical-scavenging activity. By contrast, Ahn *et al.*[28] found that, immediately after gamma-irradiation, the scavenging ability of Chinese cabbage was reduced by 2 kGy of gamma-irradiation. No significant changes of the scavenging abilities were observed in unirradiated and 5, 10 and 20 kGy-irradiated *Chungkookjang* and *Doenjang* (Korean fermented soybean) [33]. However, electron donating activities of some Korean medicinal herbs were not influenced by 10 kGy at the ambient temperatures [34]. Based on the results of these study, it seems that the irradiated sage and cinnamon had been scavenging ability on DPPH radical and were comparable with their raw state (non-irradiated) . Therefore, it could be concluded that gamma irradiation did not impair the scavenging ability on DPPH radical of irradiated these herbs at 25 kGy. Accordingly, the confirm the significant of irradiated sage and cinnamon at 25 kGy as dietary sources of natural antioxidants.

Table 2 Linear regression equations for calculation EC_{50} (DPPH) and EC_{50} (RP) of extracts extracted from non-irradiated and irradiated dry sage and cinnamon .

EC ₅₀ values		
	non- irradiated sage	irradiated sage at 25 kGy
DPPH Radical scavenging activity	y= 19.821+ 2.105X R ² = 0.825943	y= 23.406 + 2.054X R ² = 0.818108
Reducing Power	y=0.0439+0.0261X R ² = 0.978557	y=0.0879 + 0.0253X R ² = 0.963980
	Extract of non- irradiated cinnamon	Extract of irradiated cinnamon at 25 kGy
DPPH radical scavenging activity	y= 8.539 +1.969X R ² = 0.974851	y= 6.782 + 1.901X R ² = 0.981130
Reducing Power	y=0.0799 + 0.0202X R ² = 0.970160	y= 0.0975 + 0.0191 X R ² = 0.970461

RP, Reducing Power

Table 3. EC₅₀ values (mg dry herb / ml methanol) for sage and cinnamon extracts according to DPPH radical and reducing power test. Values were obtained by lineal regression analysis*.

Herb extract	0 kGy	30 kGY
EC ₅₀ according to DPPH radical test		
Sage	14.34	12.95
Cinnamon	21.06	22.73
EC ₅₀ according to reducing power test		
Sage	17.48	16.29
Cinnamon	20.80	21.07

*See Table 2.

Conclusion

The present study leads to following conclusions:

From the analysis of the present results, it can be inferred that gamma radiation at 25 kGy to the dry sage leaves and cinnamon barks reveal an important modifications on the their antioxidant activity, in this respect, it is a safe process. The total phenolic content was increased at the applied dose. Gamma-irradiation not only preserve the quality of dry sage leaves and cinnamon barks and maintained their reducing ability, and scavenging activity but also enhanced them. Therefore, from the standpoint of functional properties of irradiated sage and cinnamon, it could be useful for food industry to use The extracts of irradiated herbs as dietary sources of natural antioxidants .

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