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Effect of gamma and microwave irradiation on antioxidant and antimicrobial activities of *Cinnamomum zeylanicum* and *Echinacea purpurea*

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Abstract

The effect of gamma and microwave irradiation on antioxidant and antimicrobial activities of *Cinnamomum zeylanicum* and *Echinacea purpurea* were investigated. In this study, all samples were exposed to gamma irradiation at doses 10, 15, 20 and 25 kGy and microwave irradiation at power of 100, 180 and 300 W for 5 min. In order to undergo the sequence expriments, the hydroalcoholic (EtOH 50%) extracts of two medicinal plants were prepared. The antioxidant activity of irradiated and control samples were evaluated by DPPH radical scavenging (RS), ferric reducing power (FRP), β-carotene bleaching (BCB) and total phenolic content (TPC) of sampels. In order to study the antimicrobial activity, for determination of minimal inhibitory concentration (MIC) on *E. coli* and *S. aureus*, broth diluting method was used. Regarding gamma irradiation, no significant effect on antioxidant and antimicrobial activities of Cinnamon was observed. However, at doses higher than 10 kGy, higher radical scavenging activity (RSA) of was observed for Cinnamon extract (by DPPH method). By increasing the irradiation dose, FRP and TPC of samples increased and no adverse effect was observed on antimicrobial activity of Echinacea extract. Microwave treatments of Cinnamon, had no significant effect on antioxidant and antimicrobial activities of its extract. However, at 300 W an increase in antioxidative and antimicrobial activities of Echinacea extract was observed.

Introduction

Spices and aromatic herbs are well known for their antioxidant and antimicrobial activities as well as playing role as flavor agent. *Cinnamomum zeylanicum* is a popular pharmaceutical herbs belonging to the family *Lauraceae* comprises about 250 species which are dispersed in India, China, Srilanka and Australia. Cinnamon leaf and bark are widely used in food products as spices and production of essential oils (Jayaprakasha *et al*., 2003) as well as flavoring agent. Cinnamon has health beneficial properties, such as antimicrobial activity, controlling glucose intolerance and diabetes, inhibiting the proliferation of various cancer cell lines, and treating common cold (Anderson and Broadhurst, 2004). Cinnamon has cinnamaldehyde and eugenol which they have antimicrobial activity (Prasad *et al*., 2009). Also, Cinnamon has antioxidant activity, which is especially attributed to the presence of phenolic substances (Jayaprakasha *et al*., 2007).

Echinacea purpurea (*E. purpurea*) is another common pharmaceutical herbs also known as purple coneflower. *Echinacea purpurea* belonging to the family *Asteraceae* and it grows greatly in the North America and Europe (Speroni *et al.*, 2002). It is also popular because of its immunostimulatory, antiviral and antibacterial benefit to humans (Li, 1998; Percival, 2000). In Europe, *Echinacea purpurea* is the most common for treatment of cold in comparison of other species of *Echinacea*. Alkyl amides, polysaccharides, glycoproteins and chicoric acid are effective compounds of *Echinacea purpurea*. Chicoric acid is the main phenolic compound with antioxidant activity. Alkyl amide does not have antioxidant activity on its own, but increases the antioxidant activity of chicoric acid. Because of the presence of chicoric acid in leaf extract of *Echinacea purpurea*, it has most antioxidant effect in comparison to other species (Thygesen *et al*., 2007).

Medicinal herbs and spices like other agricultural products are contaminated by microorganisms; which can be occurred during the process, storage and transportation (Seo *et al*., 2007). Conventional methods for reducing of microbial loads are sterilization with gases such as ethylene oxide, propylene oxide and also use of steam (Dickman, 1991). Because of these are hazardous and banned in most countries (Uijl, 1992) instead, today microwave, gamma radiation and ozone are being used widely in order to eliminate the microbial contaminations (Emam *et al*., 1995; Farag *et al*., 1995; Zhao and Cranston, 1995).

In recent year several researches have studied the effect of gamma and microwave irradiation on antioxidant and antimicrobial activities of some plant

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materials (Lim and Murtijaya, 2007; Perez *et al.*, 2007; Hayat *et al*., 2010; Perez *et al*., 2011). Studies showed that gamma and microwave irradiation have different affects on antioxidant and antimicrobial properties of studied plants.

The aim of the present study is to examine the effect of gamma and microwave irradiation on antioxidant and antimicrobial activities occurring in *Cinnamomum zeylanicum* and *Echinacea purpurea.*

Materials and Methods

Materials

Dry samples of Cinnamon and *E. purpurea* were obtained from the Institute of Medicinal Plants Research in Karaj, Iran. Folin-Ciocalteu phenol reagent, ethanol, potassium ferricyanide, trichloroacetic acid, ferric chloride and sodium carbonate were purchased from Merck (Darmstadt, Germany); 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene from Fluka (Germany); linoleic acid from Sigma (USA) and Muller Hinton Broth (MHB) from Scharlau microbiology (Spain). All other reagents used had the highest analytical grade.

Microorganisms

The test microorganisms used for the study were *Escherichia coli* (RITTC 1177) and *Staphylococcus aureus* (PTCC 1112). *E. coli* were purchased from Razi Vaccine and Serum Research Institute (Karaj, Iran) and *Staphylococcus aureus* from Iranian Research Organization for Science and Technology (Tehran, Iran).

Gamma irradiation

The dry samples powder (500 g) was placed in polyethelen bags and irradiation was carried out at various doses 10, 15, 20 and 25 kGy at room tempreture using a Gamma cell-220 irradiator (Nordion, Canada). The source strength was approximately 18 kCi with a dose rate of 4.18 Gy/s as determined with a Fricke dosimeter. Untreated sample was used as a control.

Microwave irradiation

Boutan microwave oven (model CE300 S-TDU, Tehran, Iran) was used for irradiating of Cinnamon and Echinacea. Dry samples powder (20 g) put into a glass plate and irradiated at power of 100, 180 and 300 w for 5 min. Untreated sample was used as a control.

Preparation of plant extracts

The dry irradiated and control samples were mixed with ethanol 50% (with ratio 1: 25 g/ml) and the mixture was shaken with laboratory shaker for 2 h at room temprature. All extracts filtered using Whattman filter paper No.1. Then, extracts was concentrated by using a rotary evaporator (Heidolph, Germany) and kept in a dark place at 4°C.

Determination of RSA by DPPH method

This assay was carried out as described by (Brand-Williams *et al*., 1995). 2 ml of various concentrations of sample extracts were mixed with 1 ml of a 0.2 M ethanolic DPPH solution and shaken vigorously. After incubation in a dark place at room temperature for 30 min, absorbance at 517 nm was determined using a spectrophotometer (Scinco, Seol, South Korea). The corresponding absorbance of blank (containing 2 ml ethanol and 1 ml DPPH solution) was also recorded. The RSA of each extract was calculated by the following equation:

$$\% \text{ RSA} = [1 - ((A_{Control} - A_{sample}) / A_{Control}) \times 100]$$

where RSA is the radical scavenging activity; $A_{Control}$ is the absorbance of the control at t = 0 min, A_{Sample} is the absorbance of the sample at t = 30 min.

β-carotene bleaching assay

The antioxidant activities of the Cinnamon and Echinacea extracts were determined using the following method. In brief, 10 mg of β -carotene was dissolved in 10 ml of chloroform and one ml of this solution were pipetted in to a 100 ml round-bottom flask. Then, chloroform was removed by nitrogen gas, 40 ml of linoleic acid, 400 ml of Tween 20 and 100 ml of distilled water were added. After vigorous shaking, 9.6 ml aliquots of this emulsion was added to 0.4 ml of the extract. The zero time absorbance was measured at 470 nm, using a spectrophotometer. Then, the mixture was incubated in a water bath at 50°C for 2 h and then its absorbance was recorded. Antioxidant activity was calculated according to the following formula:

$$AI \% = [A_{(2h)} / A_{(0)}] \times 100$$

where AI is a antioxidant index; $A_{(0)}$, $A_{(2h)}$ is the absorbance of reaction mixture at zero and after 2 h.

Reducing power

The reducing power of Cinnamon and Echinacea extracts were determined according to the method of Oyaizu (1986). One ml of various concentrations of sample extracts was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (1%, w/v). The mixture was incubated

at 50°C for 20 min. Subsequently, after rapid cooling, 2.5 ml of trichloroacetic acid were added (10%, w/v) and the mixture was centrifuged at 2000 rpm for 10 min. Then, 5 ml of upper layer was mixed with 5 ml of distilled water and 1 ml of ferric chloride (0.1%). After vigorous shaking, the absorbance of the solution was measured at 700 nm by spectrophotometer. In this test, the EC_{50} value was obtained by linear regression analysis.

Determination of total phenolic content

The total phenolic content of each extract was determined using the Folin–Ciocalteu reagent (Mottaleb *et al*., 2005). The TPC was calculated on the basis of a calibration curve of gallic acid (y = 0.004x + 0.0082) and results were expressed as mg gallic acid per gram dry weight.

Determination of antimicrobial activity

Antimicrobial activities of irradiated and control extracts of samples were determined according to the method of Demirci *et al*., 2008. Two fold serial dilutions of extracts were made with MHB broth in 96-well microtiter plate (adding 100 microliter of herb extracts with 100 microliter of MH broth). Then, 10 microliter of bacterial suspensions standardized to 0.5 Mac Farland was added. A positive control (containing 10 microliter inoculum and 100 microliter MHB) and negative control (containing 10 microliter herb extracts and 100 microliter MHB) were prepared. The contents of the wells were mixed and the microplates were incubated at 37°C for 18-24 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract that inhibited microorganism growth.

Statistical analysis

All statistical analyses were carried out by SAS ver 9.1.3 software and differences among the means were determined using least significant differences (LSD) test at $\alpha = 0.01$. All tests were performed in triplicate and results are presented as mean ± standard deviation of three independent determinations.

Results

Radical scavenging activity

In DPPH radical scavenging test, in order to report the power of antioxidant, the EC_{50} index was calculated, which is the concentration of antioxidant required to reduce preliminary concentration of $DPPH^{\cdot}$ to 50%; therefore, the lower EC_{50} means, higher antioxidant activity. Figure 1A and B show the effect of gamma and microwave irradiation on

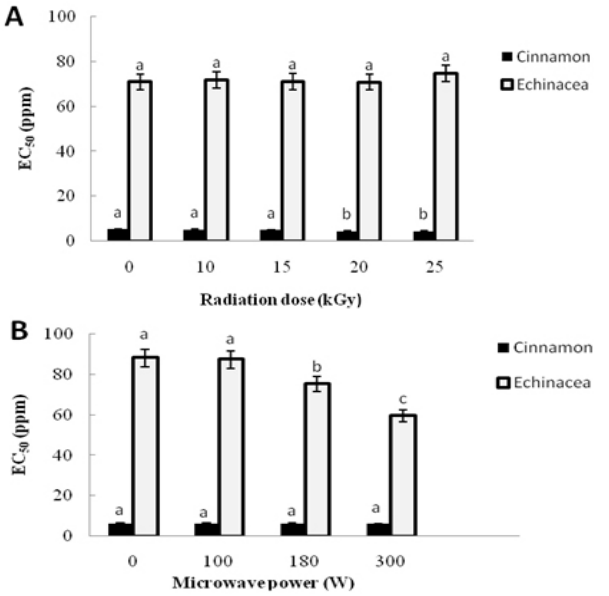

Figure 1. Changes of EC_{50} values (ppm) of gamma irradiated Cinnamon and *Echinacea* extracts (A) and microwave treated (B). Values expressed as mean ± s.d. of three replicates (DPPH method).

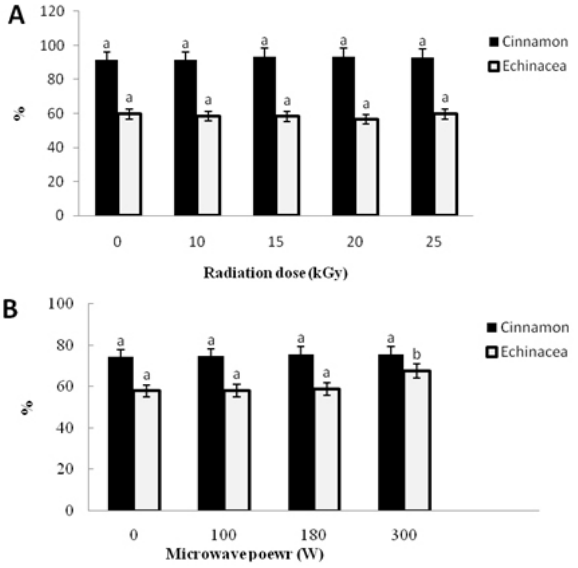

Figure 2. Changes of antioxidant indices of gamma irradiated Cinnamon and Echinacea extracts (A) and microwave treated (B). Values expressed as mean ± s.d. of three replicates (BCB method).

EC_{50} of Cinnamon and Echinacea, respectively. As seen, regarding antioxidant activity, similar results were obtained from irradiated Cinnamon at dose 10 and control, although EC_{50} showed reduction as the applied dose increased. EC_{50} values, as well, at 20 and 25 kGy, did not have significant difference ($p < 0.01$). There was no significant difference between EC_{50} values of Echinacea samples and control at various levels of gamma doses. In addition, the same results were observed for microwave treated samples. Also, no significant difference was observed between EC_{50} values of Echinacea samples treated with microwave

Table 1. Effect of gamma irradiation on TPC of Cinnamon and Echinacea extracts

Gamma dose (kGy)	TPC (mg GAE/g)	
	Cinnamon	Echinacea
0	55.69 ± 1.43 [a]	35.05± 2.57 [a]
10	55.62 ± 0.54 [a]	35.27± 0.38 [a]
15	55.57 ± 0.40 [a]	35.22±2.18 [a]
20	56.50 ± 0.22 [a]	39.11± 0.96 [b]
25	56.50 ± 0.32 [a]	39.54± 0.54 [b]

Means within each column with the same superscript letters are not significantly different ($p < 0.01$).

at 100 w and control. However, higher powers (180 and 300 w) decreased the EC_{50} of samples.

β-carotene beleaching assay (BCB)

The BCB method is based on the loss of the yellow color of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (Wettasinghe and Shahidi, 1999). Hence, there is a straigh relashionship between antioxidant potentioal of compounts available in this test and preventing the reduction of β-carotene color. Figure 2A shows the results of Cinnamon and Echinacea treated with gamma ray. There was no significant difference between antioxidant activities of treated samples and control. Microwave treatment of Cinnamon did not significantly affect the Cinnamon antioxidant activity. Also, microwave treatment of Echinacea up to 180 w had no influence on its antioxidant activity. However, at 300 w, its antioxidant activity has been increased (Figure 2B).

Reducing power

Antioxidant activity of samples was also tested by FRP method. This test is based on potential of antioxidants occuring in samples to reduce Fe^{3+} to Fe^{2+}. In this test, the blue color is observed and determined by measuring its absorbance at 700 nm. Therefore, the higher absorption means the higher ability of reduction power and therefore, the better antioxidant activity (Horvathova *et al.*, 2007). In this test, EC_{50} (RP) was calculated by linear regression. This parameter is defined as the sample concentration at which the absorbance was 0.5 for reducing power. Same as DPPH test, the lower EC_{50} (RP) means the higher antioxidant activity. Figure 3A and B show the effect of gamma and microwave irradiation on EC_{50} (RP) values of Cinnamon and Echinacea extracts, respectively. Gamma irradiation had no significant effect on EC_{50} (RP) and reducing power of Cinnamon. For Echinacea samples, below 15 kGy, there was no significant difference between irradiated sample and control. However, increasing the irradiation dose reduced EC_{50} (RP) or increased the antioxidant activity. In addition, EC_{50} (RP) values of samples treated at 20 and 25 kGy did not have significant difference ($p < 0.01$). Microwave treatment of Cinnamon did not have any unwanted effect on its reducing power, however, microwave treatment of Echinacea at 300 w resulted in an increased of reducing power.

Table 2. Effect of microwave irradiation on TPC of Cinnamon and Echinacea extracts

Microwave power (W)	TPC (mg GAE/g)	
	Cinnamon	Echinacea
0	58.88± 1.79 [a]	27.91± 2.49 [a]
100	58.92± 2.00 [a]	27.97± 2.43 [a]
180	58.40± 2.01 [a]	27.88± 3.21 [a]
300	56.04± 1.19 [a]	42.31± 1.40 [b]

Means within each column with the same superscript letters are not significantly different ($p < 0.01$).

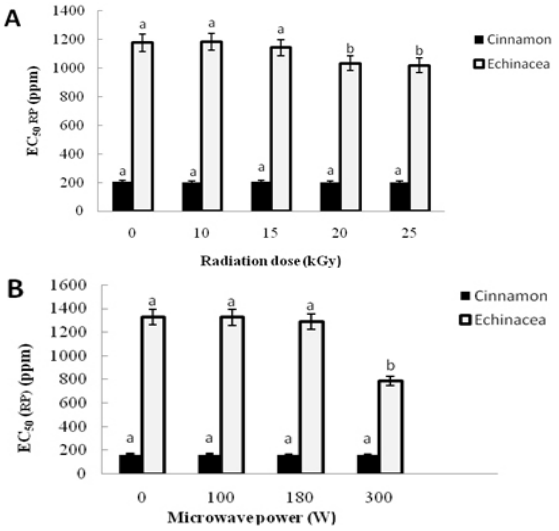

Figure 3. Changes of EC_{50} (RP) values (ppm) of gamma irradiated Cinnamon and Echinacea extracts (A) and microwave treated (B). Values expressed as mean ± s.d. of three replicates (reducing power method).

Total phenolic content

The Folin–Ciocalteu method is usaully used in various biological systems to measure the TPC. This method is based on the reduction of metal oxides by polyphenols (Horvathova *et al.*, 2007). Table 1 presents the effect of gamma irradiation on TPC of Cinnamon and Echinacea extracts. No significant difference was observed in TPC of treated and untreated samples of Cinnamon. Also, radiation up to 15 kGy did not have significant effect on total phenolic content of Echinacea, but increasing the irradiation dose up to 20 and 25 kGy showed a significant increase in total phenolic content. Microwave treatment at all levels did not have significant effect on TPC of Cinnamon. Also, microwave treatment of Echinacea up to 180 w did not have significant effect on its TPC. However, applying 300 w increased the total phenolic content of sample (Table 2).

Effect of gamma and microwave irradiation on antimicrobial activity of extracts

In the present study, broth dillution method was employed to determin MIC of Cinnamon and Echinacea extracts against *E. coli* and *S. aureus*. MICs of gamma treated Cinnamon and control, at all levels, were 0.15 and 1.19 mg/ml against *S. aureus* and *E. coli*, respectively. Also, MICs of gamma treated Echinacea and control, at all doses, were 0.86 and 6.87 mg/ml, respectively. Therefore, gamma irradiation did not have significant effect on antimicrobial activity of Cinnamon and Echinacea extracts. Also, microwave treated Cinnamon and, MIC were 0.15 and 1.19 mg/ml against *S. aureus* and *E. coli* for control and microwave treated Cinnamon at all levels, respectively, which indicated microwave treatment had no significant effect on antimicrobial activity of Cinnamon. MIC against *S. aureus* and *E. coli* for control and microwave treated Echinacea at 100 and 180 W were 0.86 and 6.87 mg/ml, respectively. However, for samples treated at 300 W MIC against *S. aureus* and *E. coli* were 0.43 and 3.43 mg/ml, respectively, which show that treatment at 300 W resulting better antimicrobial activity of Echinacea.

Discussion

The results of present study demonstrated that gamma radiation did not have negative effect on antioxidant activity, phenolic content and antimicrobial activity of Cinnamon. An increase just was observed at higher dose (>10 kGy, in DPPH radical scavenging test). An increase in DPPH scavenging without any alteration in total phenolic content (at higher dose) could be associated to the structural changes of phenolic acids that induced by irradiation (Khattak *et al*., 2008). This observation may be attributing to the presence of sugar and amino acids in Cinnamon which they cause formation of Millard reaction products, which they are capable to scavenge the radical (Chawla *et al*., 2007). In Echinacea, as well, an increase in reducing power and phenolic content, at higher dose was observed. However, the other tests did not show any significant difference. A gragual increase in TPC could be related to release of phenolic compounds from glycoside components and decomposition of bigger phenolic compounds to smaller ones as a result of gamma irradiation. Adamo *et al*. (2004) believed that destructive oxidation reaction and gamma irradiation are able to break the chemical bonds of poly phenols; therefore, soluble phenols with low molecular weight are released. Hence, irradiation showed no considerable effect on antioxidant and antimicrobial activities, which is explained through chemical principles. In low water medium (dry condition) formation of free radicals is restricted and in dry condition, produced radicals as a result of ionizing radiation are not able to move freely and/or have limited movement; thereby, they react with each other, which results in protecting other components. There are literatures available wherein the effect of gamma irradiation on antioxidant and antimicrobial activities of plant material has been investigated. Gamma irradiation of *Glycyrrhiza glabra* root (at 5-25 kGy), significantly increased the radical scavenging activity and also increased considerably the total phenolic at higher doses and irradiation up to 20 kGy did not have any effect on antimicrobial activities. Gamma irradiation of *Nigella sativa* seed at 2-16 kGy increased DPPH radical scavenging activity and total phenolic content (Khattak *et al*., 2008). Irradiation of rosemary at 30 kGy increased the antioxidant activity of ethanol and water extracts, although it did not have any effect on its methanol extract (Perez *et al.*, 2007). Gamma irradiation of green tea at 10-20 kGy increased DPPH radical scavenging activity of its methanol extract immediately after irradiation (Jo *et al*., 2003). Irradiation of *Nigella sativa* seed up to 10 kGy did not have detrimental effect on antimicrobial activities (Khattak *et al.*, 2004). Gamma irradiation of sage and oregano at 30 kGy did not have any significant effect on DPPH radical scavenging activity, reducing power and TPC (Perez *et al*., 2011). Also, gamma irradiation of sage, thyme and oregano at 10 kGy showed slight effect on their antioxidant activities (Brandstetter *et al*., 2009). Therefore, gamma irradiation can have various effects on tested samples. These reported variations could be related to differences in plant type, chemical components, geographical and environmental conditions, solvent type and extraction method, phenolic content, dose of gamma irradiation and etc.

In evaluation the effect of microwave treatment on antioxidant and antimicrobial activities of Cinnamon and Echinacea, the obtained results showed that microwave at 100, 180 and 300 w for 5 min did not have any significant effect on antioxidant and antimicrobial activity of Cinnamon and no significant differences were observed among different treatments. However, in the case of Echinacea, by increasing the power to 300 w, antioxidant activity and total phenolic content were significantly increased. Therefore, based on obtained results, it can be concluded that microwave treatment released the phenolic components, which is resulted from break of covalence bonds of phenolic components. Therefore, there is an increase in phenolic

content, which result in an increase in antioxidant capacity of extract. Free phenolic components and antioxidant activity of *Citrus mandarin* peels and pomace have increased with the applied microwave power (Hayat *et al*., 2010a; Hayat *et al*., 2010b). Microwave treatment of Thai red curry powder increased its antioxidant activity in comparison with control sample and an increase in microwave power increased the antioxidant activity (Inchun *et al.*, 2010). Also, results showed that antimicrobial activity of extract at 300 w have been increased, which could be resulted from the increase of phenolic content by increasing the microwave power. Some of the studies showed that there is direct relation between phenolic components available in herbs and spices with antimicrobial activities and sample with higher phenolic content present higher antimicrobial activity (Hara-Kudo *et al*., 2004; Shan *et al.*, 2007). Inhibition of microbial growth by phenolic components could be associated with formation of hydrogen bonds with vital proteins such as microbial enzymes and lake of iron (Scalbert, 1991). After treatment of samples with gamma and microwave, it has been observed that MIC against *S. aureus* was lower than MIC against *E. coli*. Thereby, *S. aureus* was more sensetive to the studied extracts. Gram negative bacteria, because of having hydrophile surface at outer layer of their membranes and presence of a unique preplasmic space, are resistant against antimicrobial materials (Nikaido, 1996; Duffy and Power, 2001).

An increase in phenolic content and antioxidant activity as a result of microwave treatment compared to gamma irradiation could be related to break of covalance bonds between phenolic components and an increase in free phenolic components with low molecular weight, which could be associated to heat generated by absorption of electromagnetic rays during microwave treatment; however, gamma irradiation is considered as a cold treatment. Results of some studies showed that heat is responsible for increasing the antioxidant activity and total phenolic content of herbal extracts. Applying heat treatment up to 80^{o}C for 1, 2 and 3 h increased antioxidant activity and phenolic content as well as terpenic components of thyme essential oil (Alavi *et al.*, 2010). Heat treatment at 100^{o}C for 15 min increased antioxidant activity of mint extract (Arabshahi *et al.*, 2007). In heat treatment of turmeric essential oil and powder at 100^{o}C for 1 hour increased antiradical activity (Tiwari *et al*., 2006). Heat treatment of tanic acid at 121^{o}C for 15 min incresed its antioxidant and antimicrobial activities (Kim *et al*., 2010). Also, antioxidant activity and phenolic content of heated peanut hull extracts at 150^{o}C had been increased (Lee *et al*., 2006). The obtained results showed that Cinnamon and Echinacea extracts have antioxidant and antimicrobial activities and Cinnamon extract had higher antioxidant and antimicrobial activities in comparison to Echinacea extract.

Conclusions

Gamma irradiation had no adverse effect on antioxidant and antimicrobial activities and phenolic content of Cinnamon, but at higher dose (>10 kGy) radical scavenging activity of Cinnamon extract increased (in DPPH test). Also, using of higer dose of gamma ray caused an increasing in reducing power and phenolic content of Echinacea extract and gamma irradiation had no significant effect on its antimicrobial activity. Microwave treatments of Cinnamon and Echinacea had no significant effect on antioxidant and antimicrobial activities. Just at higher power (300 w) an increase in antioxidant and antimicrobial activities of Echinacea extract was observed. Finally, the results indicated that gamma and microwave irradiation do not have any negative effect on antioxidant and antimicrobial activities of Cinnamon and Echinacea. In addition, by using an appropriate power of microwave, it is possible to increase the antioxidant and antimicrobial activities of samples.

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