Journal of Medicinal Plants and By-products (2015) 2: 161-169

Original Article

The Effect of Non-thermal Processing of *Hyssopus officinalis* on its Antioxidant and Antimicrobial Activities

Mahboobeh Jamshidi, Mohsen Barzegar* and Mohammad Ali Sahari

Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

Article History: Received: Received: 31 December 2014/Accepted in revised form: 22 August 2015

Abstract

Hyssopus officinalis L. is one of the most important medicinal plants. Medicinal herbs are contaminated by microorganisms. Conventional methods for reducing of microbial loads such as ethylene oxide, propylene oxide and also use of steam are hazardous and instead, non-thermal process such as microwave and gamma radiation are being used widely in order to eliminate the microbial contaminations with no or a little side effect. In the present study the effect of gamma and microwave irradiation on antioxidant and antimicrobial activities of *Hyssopus officinalis* L. was investigated. Hyssopus samples were exposed to gamma irradiation at doses 10, 15, 20 and 25 kGy and microwave irradiation at power of 300, 450 and 600 W for 5 min. In order to undergo the sequence experiments, the hydroalcoholic (EtOH 50%) extracts of plant were prepared. The antioxidant activities 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. Results showed that gamma irradiation had no significant effect on antioxidant parameters, phenolic content and antimicrobial activities of sampels. Microwave treatment of Hyssopus at 300, 450 and 600 W for 5 min increased its antioxidant and antimicrobial activities. Results indicated that gamma and microwave irradiation do not have any negative effect on antioxidant and antimicrobial activities of Hyssopus.

Key words: *Hyssopus officinalis* L., Gamma irradiation, Microwave, Antioxidant activity, Antimicrobial activity

Introduction

Hyssopus officinalis L. is one of the most important medicinal plants. This plant is widely cultivated in central and south European countries such as Russia, Spain, France and Italy. Hyssop oil has antifungal and anti-bacterial activities [1]. It has shown that it could inhibit the growth of different microorganisms such as *Escherichia coli, Listeria monocytogenes* and *Staphylococcus aureus* [2]. Also, it had inhibiting effects on growth of *Pyrenophor aavenae, Pyricularia oryzae* and other fungi [3]. *Hyssopus officinalis* L. has antioxidant activities. It was able to reduce the oxidation rate of soybean oil in the oven test at 70 °C and it could act as an alternative of synthetic antioxidants [4]. Hyssop oil is used as a food and drink additives [5]. In spite of its bitter taste, it's used as a flavoring agent and also in sauce formulations [1].

Medicinal herbs like other agricultural products are contaminated by microorganisms [6]. Conventional methods for reducing the microbial loads such as ethylene oxide, propylene oxide and also use of

* Corresponding author: Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, P. O. Box 14115-336, Tehran, Iran
Email Address: mbb@tmu.ac.ir

steam are hazardous and banned in most countries [7,8]. Instead, today microwave, gamma radiation and ozone are being used widely in order to eliminate the microbial contaminations [9-11].

Non-thermal methods such as gamma irradiation are an effective method for decontamination of foodstuffs [12]. Many countries use gamma irradiation to increase the hygienic quality of spices and herbs. Commercial irradiation of spices in 2002 was approximately 100,000 tons world-wide [13]. The internationally safe dose is up to 10 kGy, while in some countries this level has increased to 30 kGy [12].

Microwave radiation is a region of electromagnetic waves with frequencies between 300 MHz and 300 GHz (wavelengths ranging from λ=1 mm to 1 m) and heat generated is called microwave heating [14]. Because of interaction between electromagnetic field and food ingredients for example water and NaCl lead to molecular friction and excitation and these causes to generate heat rapidly. Microwave heating is faster in comparison with conventional-heating, so, it can improve quality of food product by reducing heating time [15].

The aim of the present study is to examine the effect of two non-thermal processing namely, gamma and microwave irradiation, on antioxidant and antimicrobial activities of *Hyssopus officinalis* L.

Material and Methods

Materials

Dry sample of *Hyssopus officinalis* L. was 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 were obtained from Fluka (Germany) and linoleic acid from Sigma (USA). Muller Hinton Broth (MHB) was purchased from Scharlau microbiology (Spain). All other reagents used had the highest analytical grade.

Microorganisms

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

Gamma Irradiation

The dry samples powder (500 g) was placed in polyethylen bags and irradiation was carried out at various doses 10, 15, 20 and 25 kGy at room temperature using a Gamma cell-220 irradiator (Nordion, Canada). The source strength was approximately 18 kCi with a dose rate of 4.18 Gy/s 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 Hyssopus. Dry samples powder (20 g) put into a glass plate and irradiated at power of 300, 450 and 600 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% (1:25 g/ml) and the mixture was shaken with laboratory shaker for 2 h at room temperature. All extracts filtered using Whattman filter paper No.1. Then, extracts was concentrated by using a rotary evaporator (Heidolph, Germany) at 40 ^{o}C and kept in a dark place at 4 ^{o}C.

Determination of Radical Scavenging Activity by DPPH Method

This assay was carried out as previously published method [16]. Two ml of various concentrations of 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 radical scavenging activity (RSA) of each extract was calculated by the following equation:

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

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

In this test, the EC_{50} index was calculated, which is the concentration of antioxidant required to reduce preliminary concentration of DPPH to 50%.

β-carotene bleaching assay (BCB)

The antioxidant activities of the Hyssopus extracts were determined using the following method [17]. 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 µl of linoleic acid, 400 µl 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 initial (t= 0) absorbance was measured at 470 nm, using a spectrophotometer. Then, the mixture was incubated in a water bath at 50 ^{o}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; $A_{(0)}$ and $A_{(2h)}$ are the absorbance of reaction mixture at zero and after 2 h.

Reducing Power

The reducing powers of Hyssopus extracts were determined according to the method of Oyaizu [18]. 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 ^{o}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} (RP) value was obtained by linear regression analysis. This parameter is defined as the sample concentration at which the absorbance was 0.5 for reducing power.

Determination of total Phenolic Content (TPC)

The total phenolic content of each extract was determined using the Folin–Ciocalteu reagent [19]. One hundered microliter of each extracts was mixed with 0.75 ml of Folin–Ciocalteu reagent which was diluted 10-fold with distilled water. After standing at room temperature for 5 min, 0.75 ml of 6% (w/v) sodium carbonate were added and mixed. The absorbance of the mixture was recorded after 90 min at 725 nm, using a spectrophotometer. 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 (mg gallic acid/gdw) of extract.

Determination of Antimicrobial Activity

Antimicrobial activities of irradiated and control extracts of samples were determined according to the method of Demirci *et al.* [20]. Two fold serial dilutions of extracts were made with MHB in 96-well microtiter plate (adding 100 microliter of herb extracts with 100 microliter of MHB). 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 ^{o}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 this test, the lower EC_{50} means, higher antioxidant activity. Fig. 1A and B show the effect of gamma and microwave irradiation on EC_{50} of Hyssopus, respectively. As seen, there was no significant difference between EC_{50} values of Hyssopus samples and control at various levels of gamma doses. Therefore, gamma irradiation treatment of Hyssopus up to 25 kGy had no influence on its antioxidant activity. Microwave treatment of Hyssopus leads to decrease the EC_{50} values. Therefore, antioxidant activity of treated sample was increased. Also, the result indicated, antioxidant activity of sample decreased at power of 600 W in comparison with 450 W.

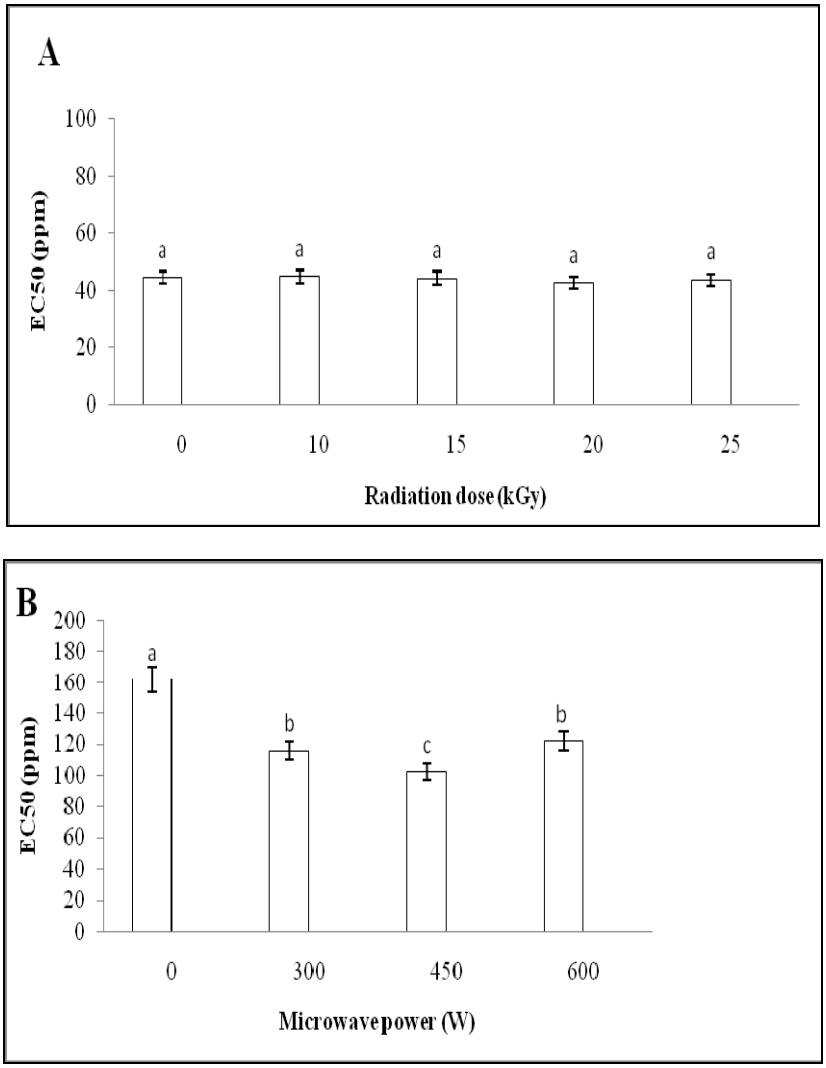


Fig. 1 EC_{50} values of hydroalcoholic extracts of Hyssopus (by DPPH method) treated by different doses of gamma ray (A) and various power of microwave (B). Values with same letters are not significantly different ($p< 0.01$).

β-carotene Beleaching Assay

Fig. 2A shows the antioxidant indices of Hyssopus treated with gamma ray. There was no significant difference between antioxidant activities of treated samples and control. Microwave treatment of Hyssopus increased its antioxidant activity. In addition, antioxidant activity of sample at 600 W was lower than 450 W (Fig. 2B).

Reducing Power

In this test the lower EC_{50} (RP) means the higher antioxidant activity. Fig. 3A shows the effect of gamma irradiation on EC_{50} (RP) values of Hyssopus extracts. Gamma irradiation had no significant effect on EC_{50} (RP) and reducing power of Hyssopus. Microwave treatment of sample resulted in reduced EC_{50} (RP) or increased of reducing power. But, reducing power of sample treated at 600 W was lower than sample treated at 450 W. (Fig. 3B).

Total Phenolic Content

The Folin–Ciocalteu method is used to measure the TPC. This method is based on the reduction of metal oxides by polyphenols. Gamma irradiation treatment did not have significant effect on TPC of Hyssopus. Microwave treatment at all levels increased the total phenolic content of samples (Fig. 4A and 4B).

Effect of Gamma and Microwave Irradiation on Antimicrobial Activity of Extracts

In the present study, broth dillution method was employed to determin MIC of Hyssopus extracts against *E. coli* and *S. aureus*. MIC of gamma treated Hyssopus and control, at all levels, were 1.5 and 6mg/ml, against *S. aureus* and *E. coli*, respectively.

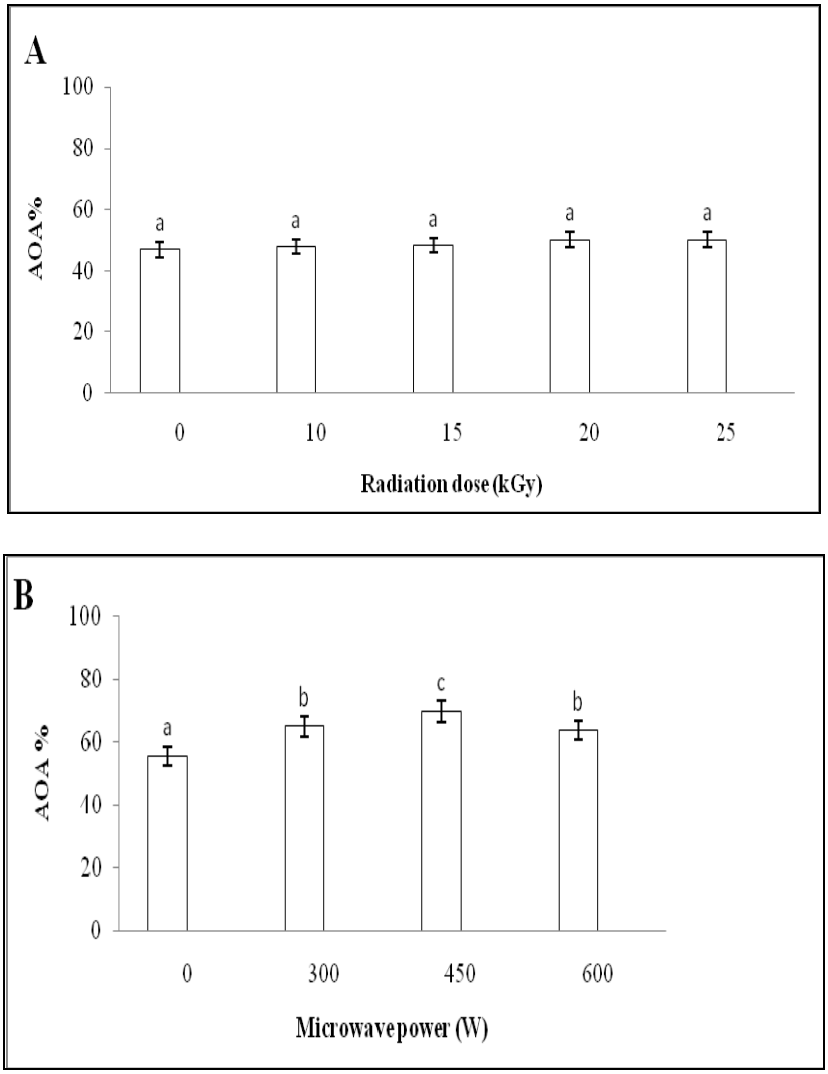
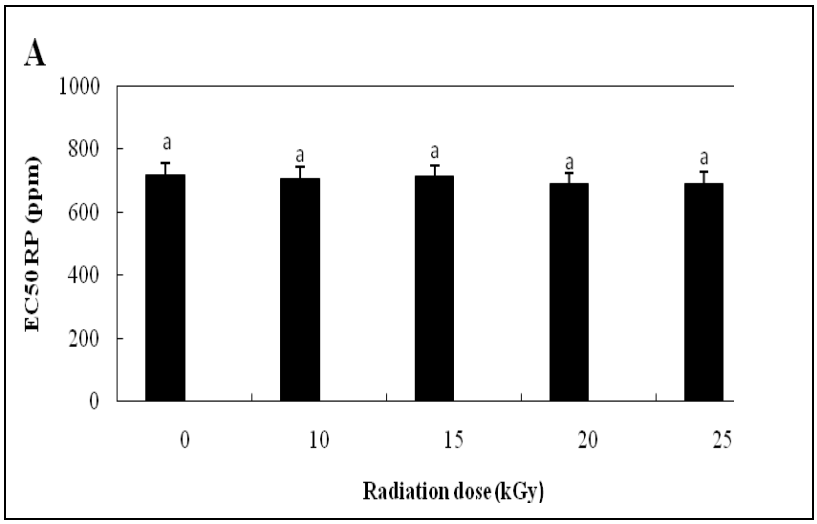


Fig. 2 Antioxidant index of hydroalcoholic extracts of Hyssopus (by BCB assay) treated by different doses of gamma ray (A) and various powers of microwave (B). Values with same letters are not significantly different ($p< 0.01$).



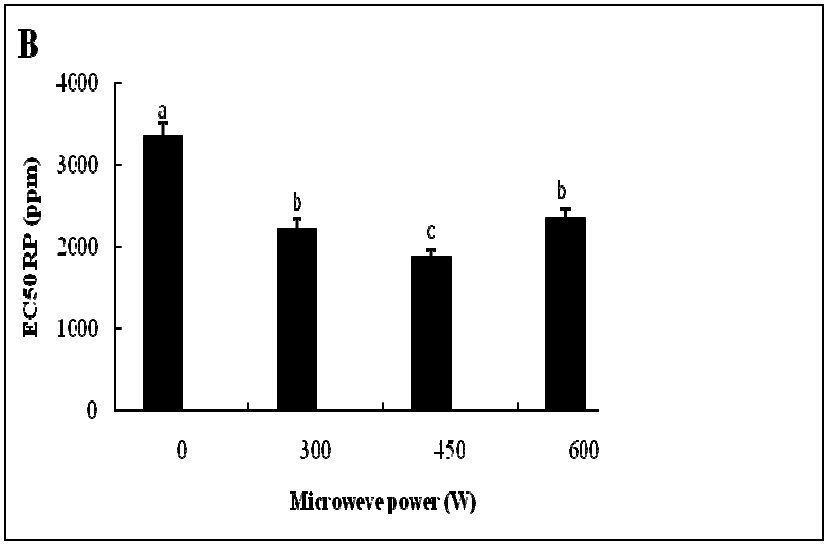


Fig. 3 EC_{50} (RP) values of hydroalcoholic extracts of Hyssopus (by reducing power test) treated by different doses of gamma ray (A) and various powers of microwave (B). Values with same letters are not significantly different ($p< 0.01$).

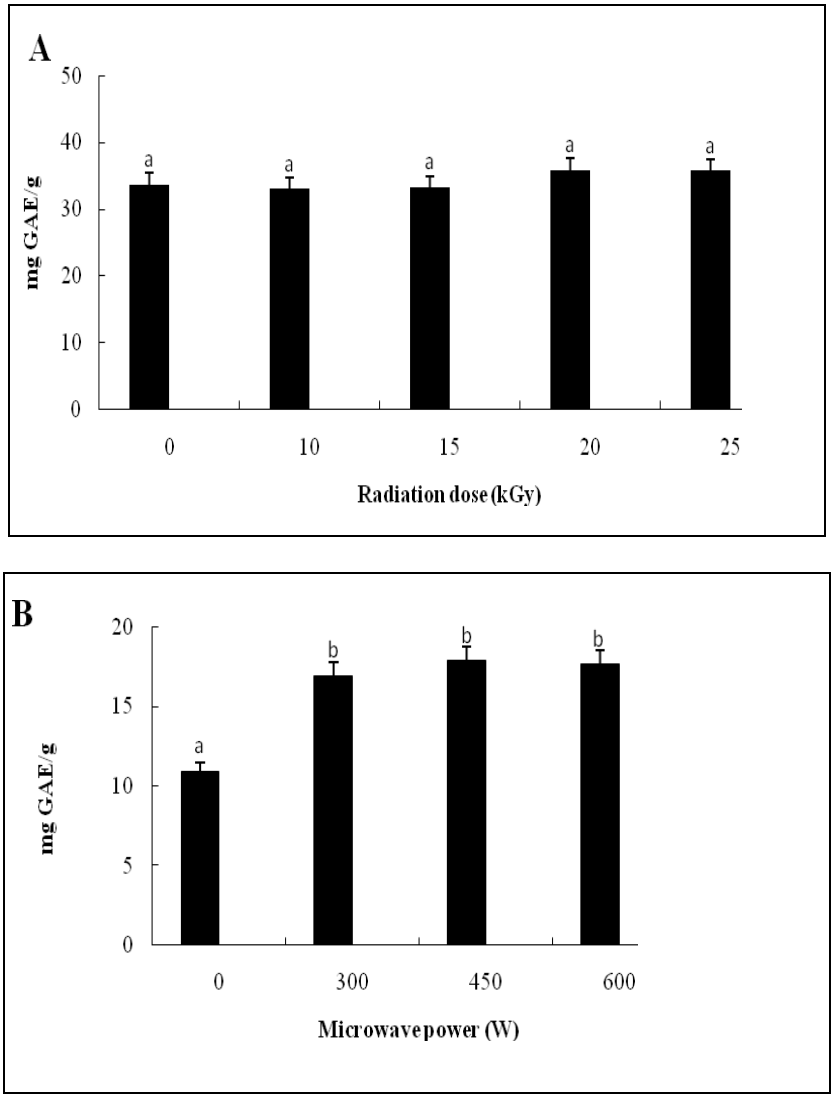


Fig. 4 Total phenolic content of hydroalcoholic extracts of Hyssopus at different doses of gamma ray (A) and various powers of microwave (B). Values with same letters are not significantly different ($p< 0.01$).

Therefore, gamma irradiation did not have significant effect on antimicrobial activity of Hyssopus extracts. Also, in microwave treatment, MIC against *S. aureus* and *E. coli* for control samples were 1.5 and 6mg/ml, respectively. However, for samples treated at 300, 450 and 600 W MIC against *S. aureus* and *E. coli* were 0.75 and 3mg/ml, respectively, which show that microwave treatment resulting decrease in MIC and enhancement in the antibacterial activity of Hyssopus extracts.

Discussion

Results of the present study demonstrated that gamma irradiation doses not have significant effect on antioxidant activity, phenolic content and antimicrobial activity of Hyssopus. It has been observed that formation of free radicals is difficult in samples with lower water content also, produced free radicals are not able to move freely; thereby, they react with each other. Therefore, other components presented in the sample are protected. There are some literatures available that show gamma irradiation had no effect on antioxidant and antimicrobial activities of plant materials. In studying of gamma irradiation of *Glycyrrhiza glabra* root at 5-25 kGy, result showed that radiation dose up to 20 kGy did not have any effect on antimicrobial activities. However, radiation at 25 kGy against some special bacteria declined the antibacterial activity of sample [21]. Irradiation of *Nigella sativa* seed up to 10 kGy did not have detrimental effect on antimicrobial activities [22]. Gamma irradiation of sage and oregano at 30 kGy did not have any significant effect on DPPH radical scavenging activity, reducing power and TPC [23]. Also, gamma irradiation of sage, thyme and oregano at 10 kGy showed slight effect on their antioxidant activities [24]. Kim *et al* (2009) reported gamma irradiation of cumin seed at 1- 10 kGy protected its antioxidant features [17]. Also, in another study, it was observed that radiation of licorice, mint, nutmeg, and vanilla at 1- 10 kGy did not have any significant effect on antioxidant activity of sample as tested by DPPH radical scavenging assays [25].

The result of the effect of microwave treatment on antioxidant and antimicrobial activities of Hyssopus at 300, 450 and 600 W for 5 min showed antioxidant activity, phenolic content and antimicrobial activity were increased. Therefore, it can be concluded that microwave treatment released the phenolic components; it might be attributed to break of covalence bonds of phenolic components. Hence, phenolic content increased, which lead to enhancement of antioxidant capacity of extract. Microwave treatment of citrus mandarin peels and pomace showed that antioxidant activities have been increased by increasing the applied microwave power [26,27]. 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 [28]. Also, our results showed that antioxidant activity decreased at 600 W in comparison with 450 W. Therefore, by applying the power higher than 450 W, some antioxidant compounds were degraded to other compounds with lower or no antioxidant activity. Hayat *et al.*, was observed a decreasing trend in antioxidant activity and phenolic acid content of citrus mandarin peel by increasing the processing time and microwave power [26].

Regarding to antimicrobial results, antimicrobial activity of treated samples at 300, 450, and 600 W have been increased, which could be resulted from the increase of phenolic content and direct relation between phenolic components with antimicrobial activities. Some of the studies showed sample with higher phenolic content show higher antimicrobial activity [29,30]. 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 [31]. Also, result showed gram negative bacteria (*E. Coli*.) are resistant against antimicrobial materials than gram possitive bacteria *(S. aureus)*, because they have a hydrophile surface at outer layer of their membranes and unique preplasmic space [32, 33].

In addition, our results showed microwave treatment has a better effect compared to gamma irradiation. It could increase phenolic content and antioxidant and antimicrobial activities of samples. It could be related to generation of heat by absorption of electromagnetic rays during microwave treatment which result in break of covalance bonds between phenolic components and increase of free phenolic components, antioxidant and antimicrobial activities; however, gamma irradiation is considered as a cold treatment. Some studies showed that heat can increase the antioxidant and antimicrobial activities. Heat

treatment of tanic acid at 121 ^{o}C for 15 min increased its antioxidant and antimicrobial activities [34]. Heat treatment of thyme essential oil (up to 80 ^{o}C for 1, 2 and 3 h) increased its antioxidant activity and phenolic content as well as terpenic components [35]. In another research, by thermal processing of turmeric essential oil and powder (at 100 ^{o}C for 1 hour), an increased antiradical activity has been reported [36].

Conclusion

Gamma irradiation had no adverse effect on antioxidant and antimicrobial activities and phenolic content of Hyssopus. Microwave treatment of Hyssopus increased its antioxidant and antimicrobial activities. Finally, the results indicated that gamma and microwave irradiation do not have any negative effect on antioxidant and antimicrobial activities of Hyssopus. In addition, appropriate and reasonable power of microwave can increase the antioxidant and antimicrobial activities of samples.

Acknowledgements

The authors wish to thank Tarbiat Modares University Research Council for its financial support.

References

1. Omidbaigi R. Production and Processing of Medicinal Plants, Vol. 3, Astan Quds Razavi Publications, Mashad, 2000.
2. Nedorostova L, Kloucek P, Kokosha L, Stolkova M, Pulkrabek J. Antimicrobial properties of selected essential oils in vapor phase against foodborne bacteria. Food Control. 2009;20:157-160.
3. Letessier MP, Svoboda KP, Walters DR. Antifungal activity of the essential oil of hyssop (*Hyssopus officinalis*). J. of Phytopathol. 2001; 149:673- 8.
4. Soleimani H, Barzegar M, Sahari MA, Naghdi Badi H. An investigation on the antioxidant activities of *Hyssopus officinalis* L. and *Echinacea purpurea* L. plant extracts in oil model system. J. Med. Plants 2011;10:61-72.
5. Murakami Y, Omoto T, Asai I, Shimomura K, Yoshihira K, Ishimaru K. Rosmarinic acid and related phenolics in transformed root cultures of Hyssopus officinalis. Plant Cell Tiss. Org. 1998; 53:75-78.
6. Seo H, Kim J, Song H, Kim D, Byun M, Kwon J, Kim K. Effects of gamma irradiation on the yields of volatile extracts of Angelica gigas Nakai. Radiat. Phys. Chem. 2007;76:1869-1874.
7. Dickman S. Compromise eludes EC. Nature. 1991; 349:273.
8. Uijl CH den. Heat treatment of spices. Beating the bugs!. Int. Food Ingr. 1992;3:9-11.
9. Emam OA, Farag SA, Aziz NH. Comparative effects of gamma and microwave irradiation on the quality of black pepper. Z. Lebensm. Unters. For. 1995;201:57-561.
10. Farag SA, Aziz NH, Attia A. Effect of irradiation on the microbiological status an flavouring materials of selected spices. *Z. Lebensm.* Unters. For. 1995;201:283-298.
11. Zhao J, Cranston PM. Microbial decontamination of black pepper by ozone and effect of the treatment on volatile oil constituents of the spices. J. Sci. Food Agric. 1995;68:11-18.
12. IAEA Database on approvals for irradiated food. Food and environmental protection newsletter Suppl., 2006;pp.21-59.
13. Farkas J. Radiation decontamination of spices, herbs, condiment, and other dried food ingredients. In: Molins, R.A. (Ed.), Food Irradiation: Principles and Application. Wiley- Interscience, New York, 2001; pp.291-312.
14. Sanga E, Mujumdar AS, Raghavan GSV. Chapter 10: Principles and applications of microwave drying. In: Drying Technology in Agricultural and Food Science, A.S. Mujumdar (ed.). Science publishers, Enfield, USA, 2000; pp.283-289.
15. Sung T, Tang J, Powers JR. Antioxidant activity and quality of asparagus affected by microwave-circulated water combination and conventional sterilization. Food Chem. 2007;100:813-819.
16. Brand-Williams SW, Cuvelier ME, Breset C. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 1995;28:25-30.
17. Kim JH, Shin MH, Hwang YJ, Srinivasan P, Kim JK, Park HJ, Byun MW, Lee JW Role of gamma irradiation on natural antioxidant in cumin seeds. Rad. Phys. Chem. 2009;78:153-157.
18. Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. JPN. J. Nutr. 1986;44:307-315.
19. Mottaleb G, Hanachi P, Kua SH, Fauziah O, Asmah R. Evaluation of phenolic content and total antioxidant activity in Berberis vulgaris fruit extract. J. Biol. Sci. 2005;5:648-653.
20. Demirci F, Guven K, Demirci B, Dadandi MY, Baser KHC. Antibactrial activity of two Phlomis essential oils against food pathogens. Food Control 2008;19:1159-1164.
21. Khattak KF, Simpson TJ. Effect of gamma irradiation on the antimicrobial and free radical scavenging activity of *Glycyrrhiza glabra* root. Rad. Phys. Chem. 2010;79:507-512.
22. Khattak KF, Ihsanullah Ali L. Effect of gamma radiation on antimicrobial compounds of *Nigella sativa*.

In Ihsanullah, Khattak, S.U. (Eds.), Proceedings of the National Executive Symposium on Technologies Developed for Commercialisation. Challenges and Opportunities. NIFA Peshawar, Pakistan, 2004; pp.170-175.
23. Perez MB, Banek SA, Croci CB. Retention of antimicrobial activityin gamma irradiated argentinian sage and oregano. Food Chem. 2011;126:121-126.
24. Brandstetter S, Berthold C, Isnardy B, Elmadfa I. Impact of gamma-irradiation on the antioxidative properties of sage, thyme, and oregano. Food Chem. Toxicol. 2009;47:2230-2235.
25. Murcia MA, Eges I, Romojaro J. Antioxidant evaluation in dessert spices compared with common food additives. Influence of irradiation procedure. J. Agric. Food Chem. 2004;52:1872-1881.
26. Hayat K, Zhang X, Chen H, Xia S, Jia C, Zhong F. Liberation and separation of phenolic compounds from citrus mandarin peels by microwave heating and its effect on antioxidant activity. Sep. Purif. Technol. 2010a;73:371-376.
27. Hayat K, Zhang X, Farooq U, Abbas S, Xia S, Jia C, Zhong F, Zhang J. Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. Food Chem. 2010b;123:423-429.
28. Inchuen S, Narkrugsa W, Pornchaloempong P. Effect of drying methods on chemical composition, color and antioxidant activity of Thai red curry powder. Kasetsart J. Nat. Sci. 2010;44:142-151.
29. Hara-Kudo Y, Kobayashi A, Sugita-Konishi Y, Kondo K Antibacterial activity of plants used in cooking for aroma. J. Food Protect. 2001;67:2820 - 2824.
30. Shan B, Cai YZ, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int. J. Food Microbiol. 2007;117:112-119.
31. Scalbert A. Antimicrobial properties of tannins. *Phytochem.* 1991;30:3875-3883.
32. Nikaido H. Outer membrane. In: Neidhardt, F.C. (Ed.), *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology. American Society for Microbiology Press, Washington, D.C., 1996; pp.29-47.
33. Duffy CF, Power RF. Antioxidant and antimicrobial properties of some Chinese plant extracts. Int. J. Antimicrob. AG. 2001;17:527-529.
34. Kim TJ, Silva JL, Kim MK, Jung YS. Enhanced antioxidant capacity and antimicrobial activity of tannic acid by thermal processing. Food Chem. 2010;118:740-746.
35. Alavi L, Barzegar M, Jabbari A, Naghdi Badi H. Effect of heat treatment on chemical composition and antioxidant property of *Thymus daenensis* essential oil. J. Med. Plants. 2010;9:129-138.
36. Tiwari V, Shanker R, Srivastava J, Vankar PS. Chenges in antioxidant activity of spices- tumeric and ginger on heat treatment. Electron. J. Environ., Agric. Food Chem. 2006;5:1313-1317.