Annals of Agricultural Science 61 (2016) 175–179

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Annals of Agricultural Science

journal homepage:

Comparison of total phenolic and antioxidant activity of different *Mentha spicata* and *M. longifolia* accessions

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ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form 14 September 2016
Accepted 17 October 2016
Available online 24 December 2016

Keywords:
M. spicata
M. longifolia
Antioxidant activity
Phenolic compound

ABSTRACT

This study investigated total phenolic and antioxidant activity of five Iranian mint accessions from two different species, *Mentha spicata* L. (Mzin1, Mzin3 and Mzin8) and *M. longifolia* L. (Mzin5 and Mzin6). The content of total phenolics (mg tannic acid equivalent per g dry weight of the sample) differed from 50.1 in Mzin3 to 67.2 in Mzin6. The highest percent radical scavenging activity was observed with Mzin6 at all concentrations studied (50, 100, 250, and 500 ppm). Peroxide value of sunflower oil containing Mzin5 and Mzin6 was the lowest among the mint accessions and almost equivalent to that of butylated hydroxytoluene at 200 ppm concentration. In overall, *M. longifolia* was superior to *M. spicata*, as determined by two model systems, indicating its potential use as a natural source of dietary antioxidant.

Introduction

Lipid oxidation is a highly deteriorative process playing an important role in lowering the nutritional values of lipid-containing foods through rancidity reactions (Chanwitheesuk et al., 2005). These caustic changes affect the major quality-control parameters (*i.e.* aroma, color, and flavor), and thus negatively influence their suitability for consumption (Nogala-Kalucka et al., 2005).

Antioxidant is defined as a compound which exerts its effect at a low concentration in relation to the oxidant (Halliwell and Gutteridge, 1990). When antioxidant is added to lipid-containing foods, especially those rich in poly-unsaturated fatty acids, lipid oxidation is delayed or inhibited, owing to its free-radical scavenging capacity, thus increasing the shelf-life of foods during processing and storage (Wong et al., 2006; Liu et al., 2011).

In food industry, lipid oxidation is largely controlled by synthetic antioxidants (Wong et al., 2006), because they are inexpensive and available with consistent quality (Pokorný, 1991). However, synthetic antioxidants are suspected to be responsible for liver damage and carcinogenesis in laboratory animals (Hwang et al., 2001). Therefore, an increasing scientific and commercial interest from food and pharmaceutical industries is occurring for the use of plant-derived non-toxic antioxidants in food systems (Kanatt et al., 2007; Abdelli et al., 2016).

Mentha is a genus of an aromatic perennial herb belonging to *Lamiaceae* family (Arzani et al., 2007), which is well-known as a folk remedy for treatment of several disorders (Moreno et al., 2002). In addition, because *Mentha* spp. are a rich source of polyphenols, they are a potential source of natural antioxidant (Kanatt et al., 2007; Rita et al., 2016). *Mentha* spp. have a broad geographical range in Iran (Heydarizadeh et al., 2013), which necessitates extensive investigation on the antioxidant properties of their different accessions. Therefore, this study aimed to investigate the comparative antioxidant activities as well as oxidative stability of sunflower oil when supplemented with the methanolic extract of five Iranian mint accessions, three of which belonging to *M. spicata* (Mzin1, Mzin3 and Mzin8) and the remaining to *M. longifolia* (Mzin5 and Mzin6).

Materials and methods

Chemicals and reagents

Linoleic acid, β-carotene, α-tocopherol (TOC), and 1,1′-Diphenyl-2-picrylhydrazyl radical (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu phenol reagent, 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT), ascorbic acid, tannic acid, sodium carbonate and all other solvents/chemicals (analytical or HPLC grade) were obtained from Merck (Darmstadt, Germany).

Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.
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http://dx.doi.org/10.1016/j.aoas.2016.10.002
0570-1783/

Sample preparation

Five different fresh mint accessions from two different species of *M. spicata* and *M. longifolia* (Table 1) were kindly provided by Isfahan Agricultural Research Center (Isfahan, Iran). The fresh samples were air-dried at 25 °C in a dark place and then stored at −18 °C.

Preparation of methanolic extract

Prior to extraction process, the air-dried samples were pulverized by a coffee grinder (Kenwood, CG 100, China) and then passed through a 40-mesh sieve. Methanolic extraction was performed according to the procedure outlined by Kähkönen et al. (1999). Briefly, after adding 10 ml of 80% methanol to 500 mg sample powder, the mixture was vortexed slightly for 1 min and then sonicated for 5 min using an ultrasonic bath (Bandelin Sonorex, Bandelin electronic, Berlin, Germany). The sonicated mixture was centrifuged (Sigma 2–16 centrifuge, Sigma Laborzentrifugen GmbH, Osterode, Germany) for 10 min at 1500*g*. The remaining solid residue was re-extracted twice according to the same procedure. Next, the collected filtrates were pooled, concentrated under reduced pressure at 40 °C in a rotary vacuum evaporator (R110, Buchi, Schweiz, Switzerland), and then freeze-dried (Edwards, Crawley, West Sussex, UK).

Total phenolic content

The total content of phenolic compounds in mint extracts was determined colorimetrically using the Folin–Ciocalteu reagent according to the procedure outlined by Pinelo et al. (2004). In brief, a sample of 0.5 mL plant extract was added to a test tube and mixed with 2.5 mL Folin-Ciocalteu reagent (0.2 N); after 5 min of reaction, 2.0 mL of sodium carbonate (7.5%, w/v) was added and reacted for 2 h at room temperature. Absorbance was measured at 765 nm using an M350 Double Beam UV–Visible Comspec spectrophotometer. A standard curve was established for the assay using tannic acid in 80% aqueous methanol. The phenolic content was determined using the standard tannic acid calibration curve and expressed as mg of tannic acid equivalents.

DPPH radical scavenging assay

The antioxidant activity was determined based on the radical scavenging capability of plant extracts (50, 100, 250 and 500 ppm) and synthetic antioxidants (as reference standard) on DPPH· free radical (Braca et al., 2002). DPPH solution (0.1 mM, 5 mL) and methanol (80%) were served as control and blank, respectively. Absorbance was determined at 517 nm.

β-carotene-linoleic acid assay

The lipid peroxidation inhibitory of methanolic extracts was determined in β-carotene bleaching model system according to the procedure proposed by Tepe et al. (2005). In brief, 1 mL of β-carotene solution (0.5 mg/1 mL chloroform; HPLC grade) was added into linoleic acid (25 μL) and 200 mg tween-40 emulsifier and vortexed for 1 min. Chloroform was evaporated from the mixture using a vacuum evaporator. The resulting mixture was diluted with 100 mL distilled water and vigorously agitated. The aliquots of the reagent (250 μL) were then dispensed into a series of test tubes containing mint extract (50, 100, 250, and 500 ppm), BHT or TOC (50 and 100 ppm). Immediately after reagent addition, the zero-time absorbance was measured against a blank (490 nm), which contained an emulsion without β-carotene. Next, the tubes were placed in a water bath (50 °C) to induce autoxidation, and the absorbance was recorded at 15 min intervals until the color of β-carotene disappeared (t = 180 min). Antioxidant activity (%) was calculated using the following equation:

$$\text{Antioxidant activity}(\%) = [1 - (A_0 - A_t)/(A^{\circ}_0 - A^{\circ}_t)] \times 100$$

where A_0 and A°_0 are the zero-time absorbance (t = 0) for test and control sample, respectively, and A_t and A°_t are the absorbance read after incubation (t = 180 min) of test and control sample, respectively.

Accelerated stability of sunflower oil

Methanolic mint extract (400 and 600 ppm) or BHT (100 and 200 ppm) was individually added into a 100 mL glass flask containing free-antioxidant sunflower oil (45 g), and then placed in an oven (60 ± 3 °C) to monitor the time course of oxidative stability. The degree of oil oxidation was determined periodically (at d 0, 4th, 8th, and 12th) in terms of peroxide value (mEq/kg oil) (AOCS, 2001).

Statistical analysis

Data were expressed as the means ± standard errors of at least three independent experiments, and analyzed using one-way analysis of variance with SPSS software (Ver. 16.0.0, 2007, SPSS Inc., Chicago, IL, USA). The level of significance was set at $P < 0.05$. Means comparison was performed by the Tukey's test.

Result and discussion

Amount of total phenolics

The total amounts of phenolic compounds in methanol extracts ranged from 50.1 to 67.2 mg tannic acid equivalent per 1 g dry weight of the sample. Mzin5 and Mzin6 had a significantly higher content of total phenolics ($P < 0.05$) compared to other accessions.

Table 1
Accession number, landrace name, species, origin, source, and the correlation coefficient between concentration of phenolic compounds in mint extracts (50, 100, 250, and 500 ppm) and their free-radical scavenging activity.

Accession No.[a]	Landrace name	Species	Origin (city)	Source	R-squared value, Equation
Mzin1	American mint	*M. spicata*	Kashan, Iran	IARC[b]	0.997, y = 3.6x + 26.50
Mzin3	Isfahan poneh	*M. spicata*	Kashan, Iran	IARC	0.947, y = 5.4x + 20.50
Mzin8	Mahalaty mint	*M. spicata*	Kashan, Iran	IARC	0.971, y = 4.6x + 21.0
Mzin5	Piperita-2	*M. longifolia*	Kashan, Iran	IARC	0.998, y = 4.4x + 31.50
Mzin6	Poneh	*M. longifolia*	Kashan, Iran	IARC	0.997, y = 4.7x + 27.50

R-squared, correlation coefficient.
[a] Arzani et al. (2007).
[b] IARC: Isfahan Agricultural Research Center.

The total phenolic content of *M. spicata* extract, reported by Kanatt et al. (2007), was 25.6 mg in terms of catechin equivalent per g of wet weight of extract. In another study, Scherer et al. (2013) reported that the methanolic extract of *M. spicata* was 76.3 mg gallic acid equivalents per gram of dry extract. More recently, Benabdallah et al. (2016) reported that the total phenolic content of five *Mentha* spp. ranged from 14.7 to 43.2 mg of gallic acid equivalent per gram of dry weight, and from highest to lowest was as follows: *M. aquatica* > *M. arvensis* > *M. piperita* > *M. pulegium* > *M. rotundifolia* > *M. villosa*.

The scavenging effects of methanolic extracts on DPPH· radical

The DPPH radical scavenging assay is a convenient and fast technique to evaluate antioxidative activity (Li et al., 2012). DPPH is a deep-purple colored stable free radical, color of which changes from violet to yellow in the hydrogen- or electron donation process and becomes a stable diamagnetic molecule (Brand-Williams et al., 1995). Because antioxidant has the electron and hydrogen-donating ability, the discoloration degree of mixture indicates the free radical scavenging power of the antioxidant (Braca et al., 2001). The DPPH radical scavenging capacity of both natural and synthetic antioxidants, was evaluated in terms of percent reduction of the initial DPPH absorption, and the results were expressed as relative activities against control (Fig. 2). It was shown that 74.8 and 67.7% of DPPH radical was scavenged at 100 and 50 ppm ascorbic acid, respectively. Butylated hydroxytoluene exhibited a poor scavenging potency of 4.8% and 18.1% at 50 and 500 ppm concentrations, respectively. Among mint accessions, Mzin5 and Mzin6 exhibited the highest DPPH scavenging at 500 ppm concentration (49.3%; $P < 0.05$). This observation agrees with the extraction yield data presented in Fig. 1, which showed that Mzin5 and Mzin6 had the highest methanolic extract (58.2 and 67.2 mg tannic acid equivalent per g dry weight of the sample, respectively).

Fig. 2 illustrates the efficient capability of mint extracts, especially Mzin5 and Mzin6, in DPPH radical scavenging as compared with BHT, possibly because of their strong hydrogen donating ability. The poor DPPH scavenging capability of BHT originates perhaps from the fact that it possesses only one hydroxyl group (Bamdad et al., 2006).

Nickavar et al. (2010) reported that IC_{50}, defined as the concentration of sample extract necessary to obtain an activity of 50%, from lowest to highest was as follows: *M. piperita* < *M. pulegium* < *M. rotundifolia* ⩽ *M. longifolia* < *M. spicata*. Similarly, Gharib and Silva (2013) studied the antioxidative activity of *Lamiaceae* herbs where the IC_{50} value of *M. piperita* and *M. spicata* was determined to be 59.2 and 63.8 (μg/mL), respectively.

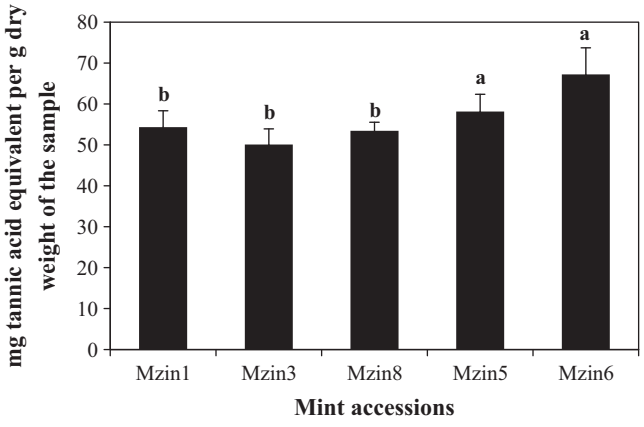

Fig. 1. Methanolic yield of total phenolic compounds in five mint accessions. Each data represents the mean of three replicates, and error bars indicate SE.

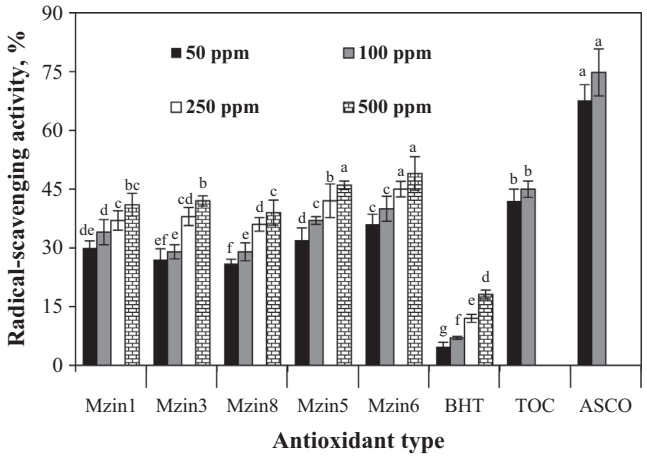

Fig. 2. Radical-scavenging activity of five mint accessions, BHT, ascorbic acid (ASCO), and α-tocopherol (TOC) by DPPH assay at different concentrations (ppm). Each data represents the mean of three replicates, and error bars indicate SE.

In a study conducted in Iran in 2006, the DPPH scavenging power of Iranian *M. piperita* oil was determined to be 23.5%. However, it was not in agreement with the results of Mimica-Dukić et al. (2003) who reported a DPPH scavenging power of 50% for *M. piperita* oil. A dose dependence of radical scavenging capacity of the oils was known the reason for this discrepancy, because Yadegarinia et al. (2006) used a fivefold more diluted oil in their experiments, compared with the study of Mimica-Dukić et al. (2003). The inconsistency among in the data of the current experiment and other reports might be explained by the differences in cultivars, growing conditions (Häkkinen et al., 1999), as well as the methodology used to determine the antioxidant activity.

Relationship between total phenolics and antioxidant activity

Table 1 presents the correlation coefficient between concentration of phenolic compounds in methanolic extract and its antioxidant activity (in terms of free-radical scavenging activity). The data showed a strong correlation (higher than 90%) between phenolic concentration and antioxidant activity in *M. species*. Earlier studies found no agreement in correlation between total phenolics and antioxidant activity. Although some reports showed a strong positive correlation (Djeridane et al., 2006; Katalinic et al., 2006; Katsube et al., 2004), other studies (Capecka et al., 2005; Wong et al., 2006) reported a poor correlation.

β-carotene–linoleate model system

Oxidation of linoleic acid promotes formation of free radicals, which subsequently causes discoloration of the highly unsaturated β-carotene molecules (Sarikurkcu et al., 2008). Therefore, adding an antioxidant to β-carotene-linoleic emulsion would neutralize the linoleate free radicals and inhibit β-carotene bleaching (Singh et al., 2002). Antioxidant activity of phenolic compounds in different concentrations of mint extract accessions, as well as BHT and TOC, as measured by β-carotene–linoleate model system, is presented in Fig. 3(A). Fig. 3(B) also illustrates the time course of a decreasing change in absorbance of β-carotene emulsion supplemented with various concentrations of Mzin6, BHT and TOC for a total test period of 180 min (15 min interval, in total 13 data points). The antioxidant activity of various mint accessions evaluated in β-carotene–linoleate model system, was 46.6 and 53.2% at 100 ppm concentration for Mzin8 and Mzin5, respectively. However at higher concentrations, the difference became more

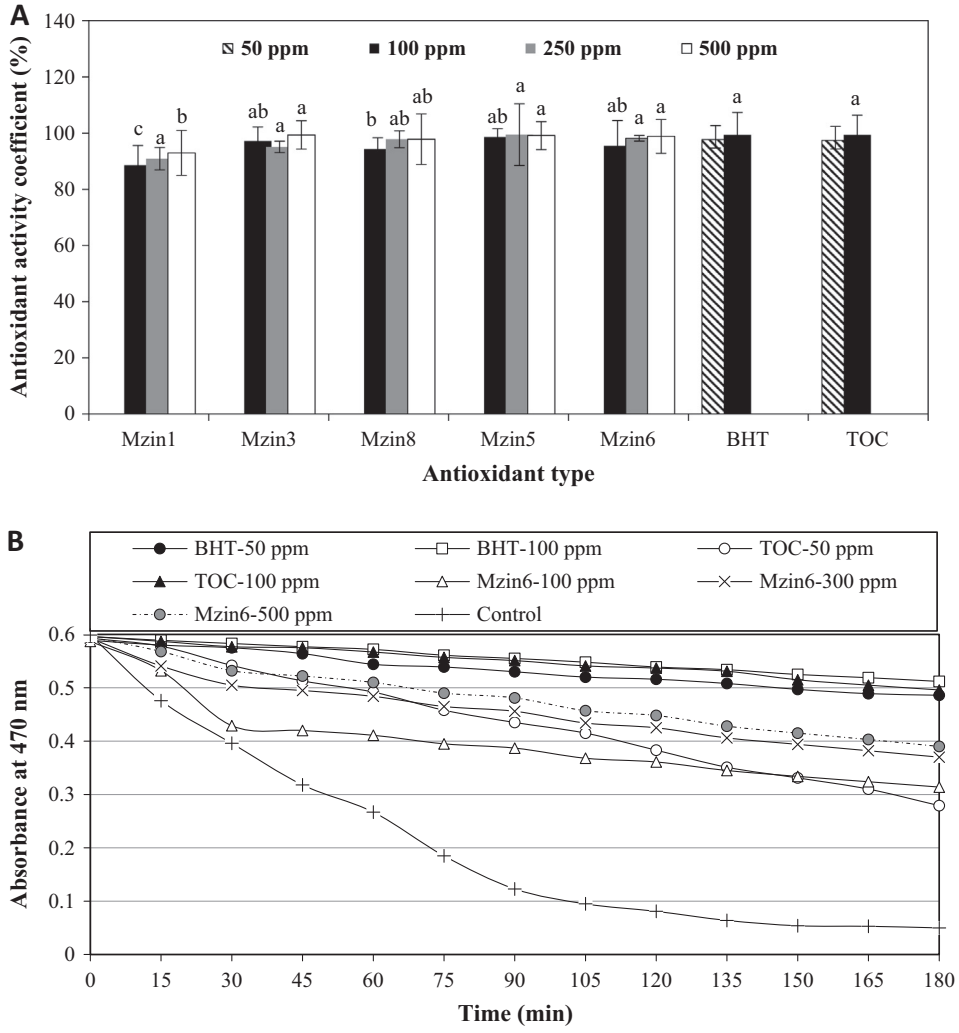

Fig. 3. (A) Antioxidant activity (%) of various concentrations of mint extract accessions by β-carotene–linoleate model system. (B) The time course of changes in absorbance intensity of various concentrations of Mzin6 by β-carotene–linoleate model system during a total test period of 180 min. BHT and α-tocopherol (TOC) were also included as standard reference for comparison. Each data represents the mean of three replicates, and error bars indicate SE.

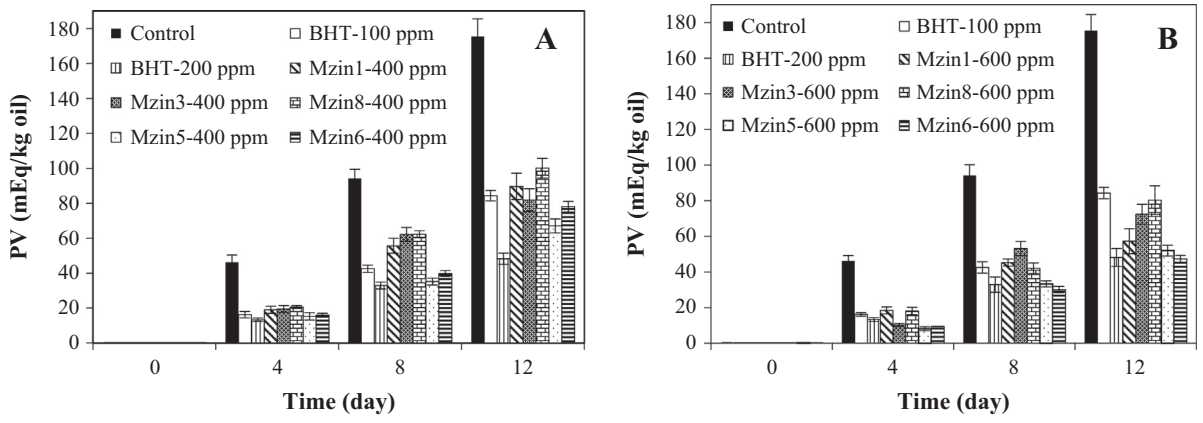

Fig. 4. Effect of various accessions of mint extract on sunflower oil oxidation expressed as peroxide value (PV) formation at 60 °C. (A) Different mint accessions at 400 ppm concentration; (B) Different mint accessions at 600 ppm concentration. BHT was included as a standard reference for comparison. Each data represents the mean of three replicates, and error bars indicate SE.

apparent. At 500 ppm concentration, the antioxidant activity ranged from 55.0% for Mzin8 to 82.9% for Mzin6. At 500 ppm concentration, the antioxidant activity of Mzin3, Mzin5, and Mzin6 was comparable to that of BHT or TOC at 100 ppm concentration ($P < 0.05$), highlighting their efficiency in hindering the oxidation of linoleic acid.

Antioxidant activity of the methanolic extract of *M. longifolia*, its essential oil, and BHT expressed as inhibition percentage in β-carotene–linoleic acid model system was reported to be 24, 36, and 96%, respectively (Gulluce et al., 2007). The antioxidant activity of Iranian *M. piperita* oil and BHT, as determined in β-carotene/linoleic acid assay was 50.2 and 86.8%, respectively.

Effect of adding mint extract on oxidation of sunflower oil

Antioxidants lower the rate of oxidation reactions by increasing the overall energy of activation, leading to an increase in the activation energy of lipid oxidation (Frankel, 1996). The effect of adding natural and synthetic antioxidants to sunflower oil in terms of peroxide value was monitored during a test period of 12 d at 60 °C. During the course of test period (0, 4, 8, and 12 days), BHT at 200 ppm concentration effectively improved sunflower oil stability and showed the lowest peroxide value. Among mint extracts, Mzin5 and Mzin8 (at 400 ppm concentration) exhibited the lowest and the highest peroxide value, respectively (Fig. 4A). In overall, at 600 ppm concentration, peroxide value of five mint accessions from highest to lowest was follows: Mzin8 > Mzin1 > Mzin3 > Mzin5 > Mzin6 (Fig. 4B). On day 12, peroxide value of Mzin6 was comparable to BHT at 200 ppm (47.4 *vs.* 48.2 mEq/kg oil).

Marinova and Yanishlieva (1997) sudied the kinetics of peroxide accumulation during oxidation of sunflower oil, and found that the ethanol extract of *M. piperita* effectively improved the oxidation stability of sunflower oil. In contrast, Anwar et al. (2003) investigated the antioxidant activity of seven plant extracts in corn oil and found that rosemary extract and *M. piperita* exhibited the highest and lowest inhibitory effects on corn oil oxidation, respectively.

Conclusion

DPPH and β-carotene–linoleate model systems confirmed that the phenolic antioxidant of Mzin5 and Mzin6, at 500 ppm concentration, was comparable to the synthetic antioxidants in terms of oxidation inhibition, indicating their potential use as safe, natural alternatives for synthetic antioxidants in food industry. The strong oil-stabilizing effect of Mzin5 and Mzin6 at 600 ppm concentration was comparable to BHT at 200 ppm concentration, highlighting their potential use as promising alternatives for synthetic antioxidants. Further research is required in order to isolate and identify the bioactive components of these mint accessions.

Acknowledgements

This research was financially supported by the grant from Isfahan University of Technology.

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