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***In vitro* antioxidant potentials and chemistry of essential oils and oleoresins from fresh and sun-dried *Mentha longifolia* L.**

Sunita Singh^a, S.S. Das^a, G. Singh^{a*}, Marina Perotti^b, Carola Schuff^b and César A.N. Catalán^b

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The present study was designed to assess differences in chemical composition and antioxidant potential of essential oils and oleoresins from fresh and sun-dried *Mentha longifolia* L. Essential oils and oleoresins were obtained by hydrodistillation and solvent extraction (*n*-hexane and ethanol), respectively. The chemical profile was evaluated by using gas chromatography (GC) and GC coupled to mass spectrometry (GC–MS). Antioxidant effectiveness was examined by five different methods, namely the ferric thiocyanate (FTC) method, the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, determination of the metal chelating power, and determination of the peroxide and thio-barbituric acid values in mustard oil at 0.02% concentration. The chemical composition was dominated by the presence of piperitenone oxide, an oxygenated monoterpene whose composition varied from 23.2% to 88.5% in both essential oils and oleoresins. Based on the antioxidant potential of essential oils, oleoresins and synthetic antioxidants can be sorted in the following descending order: butylatedhydroxytoluene > dried mint essential oil > fresh mint essential oil > propyl gallate > fresh mint ethanol oleoresin > dried mint ethanol oleoresin > dried mint hexane oleoresin > fresh mint hexane oleoresin. The drying process has a significant influence on the qualitative and quantitative content of the extracted substances.

Keywords: *Mentha longifolia* L.; essential oils; oleoresins; piperitenone oxide; oxygenated monoterpene

Introduction

The genus *Mentha* L. (Lamiaceae) includes more than twenty-five species, growing wild in damp or wet places throughout the temperate regions of Asia, Eurasia, Australia and South Africa (1). They are commonly cultivated in the world for essential oil production that is used extensively in the liquor and confectionary industries, flavoring, perfume production and for medicinal purposes (2). The leaves, flowers and stems of *Mentha* spp. are frequently used in herbal teas or as additives in commercial spice mixtures for many foods, to offer aroma and flavor (3). In addition, many species of *Mentha* have been used as folk remedies for the treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints due to its anti-inflammatory, carminative, antiemetic, antispasmodic, analgesic and stimulant activities (4). Furthermore, it is well-documented that the essential oils or/and extracts from some *Mentha* species possess antimicrobial and antioxidant properties (5, 6). Food spoilage caused by oxidation processes or by microorganisms during the production, storage and marketing is an important issue in the food industry. Antioxidants have been widely used as food additives to provide protection from the oxidative degradation of foods and oils. Synthetic

antioxidants, despite their effectiveness, are restricted in several countries because of their possible toxicity, absorption and accumulation in the body and also their carcinogenic effects (7). Consequently, there is much interest in substituting synthetic food preservatives and synthetic antioxidants for substances that can be marketed as natural.

The data on the antioxidant activities of *Mentha* species has been scattered in the literature and it is difficult to compare the available data because of differences in methodologies. Thus, comparison of the antioxidant activities of *Mentha* species using a similar approach is necessary. The main objective of the present study is to determine the chemical composition of essential oils and oleoresins obtained from sun-dried and fresh mint leaves, and then to compare their antioxidant potentials with those of synthetic additives using different antioxidant assays.

Materials and methods

The planting material has been collected from the botanical garden of the Botany Department, DDU Gorakhpur University, Gorakhpur, in June; voucher specimens (BG/ML2/6/13/DDU) were deposited at the

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Herbarium of the Science Faculty of DDU Gorakhpur University, Gorakhpur, India.

All solvents used were of analytical grade. Thiobarbituric acid (TBA), 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) and linoleic acid are from Acros (New Jersey, USA); butylatedhydroxytoluene (BHT), propyl gallate (PG) are from SD Fine Chemicals Ltd., Mumbai, India. Tween 20 and ferrozine (Merck Pvt. Ltd. Mumbai, India) were used as such. Crude mustard oil was obtained from a local oil mill in Gorakhpur.

Extraction of essential oil and oleoresins

Fresh *Mentha longifolia* L. (aerial parts, 250 g) was washed for extracting the essential oil (FMEO), whereas it was sun dried and powdered for extracting the dried mint essential oil (DMEO) using a Clevenger-type apparatus for 6 hours in accordance with *European Pharmacopoeia* (8) procedure. A light green-colored oil was obtained. The oil was dried over anhydrous sodium sulfate to remove traces of moisture and stored in a refrigerator at 4°C until use.

Oleoresins were obtained by extraction of fresh and dried aerial parts of *M. longifolia* L. with ethanol and *n*-hexane for 6 hours in a Soxhlet apparatus. The solvent was removed by distillation to yield viscous oleoresins, namely fresh mint ethanol (FMET), dry mint ethanol (DMET), fresh mint *n*-hexane (FMNH) and dry mint *n*-hexane (DMNH) oleoresins, which were stored in a refrigerator at 4°C until use.

Chemical composition analysis

Qualitative and quantitative analyses of the essential oils and oleoresins were carried out using a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and a capillary HP-5 column (30 m × 0.32 mm; 0.25 μm film thickness); the injector and detector temperature were maintained at 250° and 270°C, respectively. Injection size: 0.5 μL, split mode. Helium was used as a carrier gas at a flow rate of 1 mL/minute. For essential oils and oleoresins the oven was programmed as follows: 60°C (1 minute), 60–185°C at a rate of 1.5°C/minute), 185°C (1 minute), 185–275°C at a rate of 9°C/minute), 275°C (2 minutes) with a split ratio 80:1. Gas chromatography–mass spectrometry (GC–MS) analysis was carried out on an HP 6890 gas chromatograph (Agilent Technologies, Buenos Aires, Argentina) coupled to an HP 5973 quadrupole mass spectrometer (Agilent Technologies) equipped with a PerkinElmer Elite-5MS capillary columns (5% phenyl methyl siloxane, length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm). Helium was used as the carrier gas at a flow rate of

1 mL/minute. GC–MS interphase, ion source and selective mass detector temperatures were maintained at 280°, 230° and 150°C, respectively; ionization energy: 70 eV. The oven temperature program was the same as for the GC–FID analysis.

The components percentage was taken from capillary GC traces with the FID. The identification of the individual components was based on (i) computer matching with the Wiley 275 and National Institute of Standards and Technology (NIST) libraries provided with computer controlling GC–MS system; (ii) comparison with spectra available in our files and literature data (9–11); and (iii) comparison of their GC arithmetic indices (AI) on a HP-5 column. The AIs for the essential oil components were calculated using a homologous series of *n*-alkanes C₈–C₁₈. For oleoresins, a homologous series of *n*-alkanes C₈–C₂₂ was used.

Complementary antioxidant assays

DPPH free radical scavenging activity

The radical scavenging capacity of essential oils and oleoresins was monitored by measuring their ability to scavenge the DPPH radical by a method reported earlier (12). For this, 1 mL of freshly prepared DPPH radical solution (0.1 mM in methanol) was mixed thoroughly with 3 mL of methanolic solution of essential oil, oleoresins and synthetic antioxidants (5–20 μg/mL). The reaction mixture was left for 30 minutes in the dark at room temperature after which the resultant absorbance was recorded at 517 nm. Control (without any additive) and standards (containing BHT and PG; in place of oil and oleoresins) were also tested. The capability to scavenge the DPPH radical (% inhibition) was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \left\{ 1 - \left(\frac{A_t - A_b}{A_c} \right) \right\} \times 100$$

where A_t is the absorbance of the test sample, A_b is the absorbance of the blank and A_c is the absorbance of the control sample.

Ferrous ion chelating activity

In order to determine the ferrous ion chelating activity of essential oils and oleoresins, the method reported in literature (13) was used. A 200-μL amount of each essential oil/oleoresin was mixed with 0.1 mL of 2 mM FeCl₂ and 0.2 mL of 5 mM ferrozine solutions. After a 10-minute equilibrium period, the absorbance at 562 nm was recorded. A complex of Fe²⁺/ferrozine showed a strong absorbance at 562 nm.

Total antioxidant activity (FTC method)

The antioxidant activity was also determined by the ferric thiocyanate (FTC) method in linoleic acid emulsion system (14) with some modifications. Reaction media contained a 2.5-mL solution of essential oils and oleoresins (1 mg/100 mL in absolute alcohol), 2.5 mL of 2.51% linoleic acid emulsion and 5 mL of 0.05 M phosphate buffer (pH 7.0). The mixed solution (10 mL) was incubated at 40°C in the dark. The same solution without any test substance was used as a control. The peroxide level of each sample was determined by reading the absorbance at 500 nm after reaction with 0.1 mL of 20 mM FeCl₂ and 0.2 mL of 30% ammonium thiocyanate every 24 hours. BHT and PG were used as positive standards.

Lipid peroxidation assays

Sample preparation

The essential oils and oleoresins extracted from fresh and dried *M. longifolia* L. were added individually to unrefined crude mustard oil samples (30 g each) at the concentration of 200 ppm (w/v). Synthetic antioxidants such as BHT and PG were also added to mustard oil at the same concentration. Mustard oil without any additive was taken as a control sample. All the samples were exposed to accelerated oxidation by incubating at 70°C in the dark. The extent of oxidation of all samples was assessed periodically by various lipid peroxidation assays.

Peroxide value

This parameter measures the total peroxide and hydroperoxide oxygen content of the mustard oil systems. The peroxide value (PV) was measured at regular intervals according to the procedure described by Horwitz (15). A 5-g sample of mustard oil was dissolved in 30 mL of glacial acetic acid–chloroform (3:2) solution and mixed with 0.5 mL of saturated KI solution. After 1 minute, 30 mL of distilled water was added and the mixture was titrated with 0.01 N Na₂S₂O₃ using a starch indicator. Titration was continued, shaking the flask vigorously until the blue color just disappeared. The PV (meq of peroxide/kg of oil) was calculated as:

$$\text{meq of peroxide/kg of oil} = S \times N \times 1000 / \text{weight of oil}$$

where *S* is volume of Na₂S₂O₃ consumed and *N* is the normality of Na₂S₂O₃.

TBA value

The test was performed according to the method (16) previously reported. About 100 mg of the oil sample

was dissolved in 25 mL of 1-butanol, mixed thoroughly with 5.0 mL of TBA reagent (200 mg TBA in 100 mL 1-BuOH) and incubated at 95°C for 2 hours. After that, the reaction mixture was cooled to room temperature under running water and absorbance was measured at 530 nm. At the same time, a reagent blank test (without TBA reagent) was also done. The TBA value (meq of malondialdehyde/g) was calculated as:

$$\text{TBA value} = \frac{50 \times (A - B)}{M}$$

where *A* is the absorbance of the test sample, *B* is the absorbance of the reagent blank and *M* is the mass of the sample (mg).

Statistical analyses

For the essential oil or oleoresin, three samples were prepared for assays of every antioxidant activity. The data are presented as mean (standard deviation of three determinations; data are not shown). Statistical analyses were performed using a one-way analysis of variance (17). A probability value of *p* ≤ 0.05 was considered significant.

Results and discussions

Chemical composition

A detailed and careful interpretation of the experimental data (EM fragmentation and retention indices) obtained from GC–FID and GC–MS analysis resulted in the identification of a large number of components in the essential oils and oleoresins of fresh and dried leaves of *M. longifolia* (Tables 1 and 2). Table 1 shows the identification of twenty-three components in FMEO and DMEO, representing about 93.3% and 97.4%, respectively, of the total amount. Table 2 shows the chemical compositions of ethanol and *n*-hexane oleoresins extracted from fresh and dried *M. longifolia*. A total of twenty-one and twenty-three components were identified in FMET and FMNH, representing about 85.9% and 83.5%, respectively, of the total amount, whereas in DMET and DMNH, a total of twenty-four and twenty components were identified, representing about 72.6% and 83.1%, respectively, of the total amount.

The total monoterpenoids fractions in FMEO and DMEO were 91.9% and 95.1%, respectively, consisting almost entirely of oxygenated monoterpenes (89.1% and 94.7%, respectively). The dominant component in both essential oils was piperitenone oxide, with amounts of 79.9% in FMEO and 88.5% in DMEO, followed by small amounts of piperitenone, terpinen-4-ol, lippiphphenol and caryophyllene oxide. The content of piperitenone in essential oils and oleoresins from our

Table 1. Gas chromatography–mass spectrometry (GC–MS) analysis of fresh mint and dried mint (*Mentha longifolia* L.) leaves essential oils.

Compounds	FME0, %, FID	DME0, %, FID	AI ^a	AI (lit.) ^b	Identification ^c
α -Pinene	0.2	tr	928	932	MS, RI, co-GC
β -Pinene	0.3	tr	973	974	MS, RI, co-GC
3-Octanol	1.1	0.3	985	988	MS, RI, co-GC
<i>p</i> -Cymene	0.1	tr	1019	1020	MS, RI, co-GC
Limonene	2.2	0.4	1024	1024	MS, RI, co-GC
1,8-Cineole	0.3	tr	1025	1026	MS, RI, co-GC
Linalool	0.3	0.2	1096	1095	MS, RI, co-GC
Borneol	1.3	0.5	1166	1165	MS, RI, co-GC
Terpinen-4-ol	0.4	0.1	1172	1174	MS, RI, co-GC
<i>p</i> -Cymen-8-ol	0.2	0.6	1179	1179	MS, RI
α -Terpineol	0.5	0.3	1188	1186	MS, RI, co-GC
Shisofuran	0.1	0.6	1199	1198	MS, RI
Carvone	0.2	tr	1241	1239	MS, RI, co-GC
Piperitone	0.3	tr	1248	1249	MS, RI, co-GC
<i>cis</i> -Piperitone epoxide	0.6	tr	1251	1250	MS, RI
Isopiperitenone	1.1	0.6	1272	–	MS, RI
Piperitenone	2.9	1.5	1338	1340	MS, RI
Piperitenone oxide	79.9	88.5	1369	1366	MS, RI
Lippiaphenol (diosphenolene)	1.0	1.8	1401	–	MS, RI
β -Caryophyllene	tr	1.0	1415	1417	MS, RI, co-GC
Germacrene-D	0.3	0.5	1487	1484	MS, RI
Caryophyllene oxide	0.7	0.8	1579	1582	MS, RI
Dill apiole	tr	0.4	1615	1620	MS, RI
Total identified %	94.0	98.1			
Monoterpene hydrocarbons	2.8	0.4			
Oxygenated monoterpenes	89.1	94.7			
Total monoterpenoids %	91.9	95.1			
Sesquiterpene hydrocarbons	0.3	1.5			
Oxygenated sesquiterpenes	0.7	0.8			
Total sesquiterpenoids %	1.0	2.3			
Others	1.1	0.7			

Notes: Trace (tr), <0.05%. ^aThe arithmetic index (AI) was calculated on an HP-5 capillary column using a homologous series of *n*-alkanes C₈–C₁₈. ^bAI (lit.), values reported in Adams (37). ^cRI, retention index; Co-GC, co-injection with an authentic sample. Percentages were obtained from electronic integration measurements using flame ionization detection (FID). DME0, dried mint essential oil; FME0, fresh mint essential oil.

collection of *M. longifolia* was at very low levels ranging from 0.1% to 2.9%. Regarding sesquiterpenes, β -caryophyllene and germacrene-D were present in both essential oils. Previous studies on *M. longifolia* L. essential oil reported that germacrene-D is a typical sesquiterpene of different varieties of this species (18). Oleoresins were also dominated by oxygenated monoterpenes with piperitenone oxide as the major component in all cases, accompanied by phytosterols such as stigmast-5-en-3 β -ol (β -sitosterol), stigmast-5,22-dien-3 β -ol (stigmasterol), ergost-5-en-3 β -ol and fatty acids (palmitic, oleic and stearic) (Table 2). Interestingly, stigmastan-3,5-diene was found only in alcoholic oleoresins of fresh (FMET) and dried (DMET) mint leaves (13.5% and 1.9%, respectively), indicating that the polar solvent extracts selectively this steroidal compound. The significantly lower content of stigmastan-3,5-diene in sun-dried leaves may be due to the ease of oxidation of this steroidal diene in the presence of air and light. It is well known that sterols as well as

steroidal dienes are easily oxidized and degraded when exposed to light and air.

On the other hand, several long chain *n*-alkanes C₂₅–C₃₁ that amounted 32.7% of the *n*-hexane oleoresin of fresh mint (FMNH) were only present as minor components or absent in the remaining oleoresins (Table 2).

Many workers (19–24) reported that *M. longifolia* essential oil is a good source of piperitenone oxide, *cis*-piperitone oxide, menthone, pulegone and menthol. Sharopov et al. (20) reported piperitenone oxide, *cis*-piperitone oxide and menthone as major components in several collections from Tajikistan; Saedi et al. (21) found piperitenone oxide and pulegone as major components in collections from Iran but in our case menthone, menthol and pulegone were not detected despite being sought specifically. Qualitative and quantitative variations in the chemical composition of an essential oil may be due to the presence of different chemotypes (genotypes), to phenological factors or environmental conditions (25).

Table 2. Gas chromatography–mass spectrometry (GC–MS) analysis of fresh and dried mint (*Mentha longifolia* L.) leaves oleoresins in different solvents.

Compounds	FMET,%, FID	FMNH,%, FID	DMET,%, FID	DMNH,%, FID	AI ^a	AI (lit.) ^b	Identification ^c
<i>p</i> -Cymene	tr	0.1	tr	–	1019	1020	MS, RI, co-GC
Limonene	tr	0.1	tr	–	1024	1024	MS, RI, co-GC
1,8-Cineole	tr	tr	tr	–	1025	1026	MS, RI, co-GC
<i>p</i> -Cymenene	4.3	tr	3.0	tr	1091	1089	MS, RI, co-GC
Isopiperitenone	tr	0.5	tr	tr	1272	–	MS
Piperitenone	1.5	0.7	1.1	0.1	1338	1340	MS, RI
Piperitenone oxide	53.3	30.2	47.7	23.2	1369	1366	MS, RI
Germacrene-D	tr	tr	1.2	–	1490	1484	MS, RI
Neophytadiene	–	–	1.3	3.5	1830	–	MS, RI
Palmitic acid	3.0	1.6	5.1	4.8	1967	–	MS, RI, co-GC
Phytol	tr	tr	2.2	2.5	2012	1942	MS, RI
Stearic acid	tr	1.7	tr	1.9	2157	–	MS, RI, co-GC
Oleic acid	–	–	–	15.7	2128	–	MS, RI, co-GC
Monoolein	–	–	–	4.4	–	–	MS, RI, co-GC
Pentacosane	tr	0.6	tr	–	–	2500	MS
Hexacosane	tr	1.3	tr	–	–	2600	MS
Heptacosane	tr	2.5	tr	–	–	2700	MS
Octacosane	tr	3.9	tr	tr	–	2800	MS
Nonacosane	tr	6.0	0.1	tr	–	2900	MS
Triacontane	tr	6.3	0.1	tr	–	3000	MS
Hentriacontane	–	12.1	0.1	3.7	–	3100	MS–co-GC
Stigmastan-3,5-diene	13.5	–	1.9	–	–	–	MS
Vitamin E	–	–	0.5	1.1	–	–	MS
Tritriacontane	–	–	–	3.3	–	3300	MS
Pentatriacontane	–	5.9	–	0.9	–	3500	MS
Ergost-5-en-3 β -ol	2.7	0.4	1.3	0.1	–	–	MS, co-GG
Hexatriacontane	–	7.9	–	–	–	3600	MS
Stigmast-5-en-3 β -ol	7.5	1.6	6.9	15.0	–	–	MS, co-GC
Stigmast-5,22-dien-3 β -ol	0.1	0.1	0.1	2.9	–	–	MS, Co-GC
Total	85.9	83.5	72.6	83.1			

Notes: Trace (tr), <0.05%. ^aThe arithmetic index (AI) was calculated on an HP-5 capillary column using a homologous series of *n*-alkanes C₈–C₂₂. ^bAI (lit.), values reported in Adams (37). ^cRI, retention index; Co-GC, co-injection with an authentic sample. Percentages were obtained from electronic integration measurements using a flame ionization detector (FID). FMET, fresh mint leaves ethanol oleoresin; FMNH, fresh mint leaves *n*-hexane oleoresin; DMNH, dried mint leaves *n*-hexane oleoresin; DMET, dried mint leaves ethanol oleoresin.

Antioxidant potentials

The DPPH radical scavenging activity was found to increase with increasing concentration (Figure 1). The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant. The essential oils (DMEO and FMEO), synthetic antioxidants (BHT and PG) and oleoresins (FMNH, FMET, DMET and DMNH) reduced the DPPH radical formation in a dose-dependent manner. FMEO and DMEO showed better scavenging power (40.5–73.4%) than those of PG but lower than BHT at all concentrations tested. Among the oleoresins, FMET showed a better scavenging effect than the remaining oleoresins. The results reported by Azizkhani and Ataei (26) are nicely correlated with our work, where the essential oil and methanol extract obtained from Iranian *M. longifolia* L. showed weaker antioxidative potentials in comparison with the synthetic antioxidant, BHT.

Iron is a transition metal is capable of generating free radicals from peroxides by Fenton reactions.

Because Fe²⁺ also has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe²⁺ concentration in Fenton reactions afforded protection against oxidative damage. Ferrozine can quantitatively form complexes with Fe²⁺. In the presence of other chelating agents, the complex formation is disrupted, with the result that the red color of the complexes decreases. The absorbance of Fe²⁺–ferrozine complex was decreased dose dependently, i.e. the activity was increased on increasing concentration. Among the tested substances, DMEO exhibited a higher chelating activity (up to 67%) in comparison with the oleoresins but was not as effective a chelator as EDTA (Figure 2). Maximum chelating of metal ions at 200 μ g/mL for DMEO and EDTA was found to be 67.0% and 96.2%, respectively, whereas the oleoresins were less effective in metal chelation and their metal chelating activity ranges from 13% to 36%.

The FTC method measures the amount of peroxide formed during the initial stages of oxidation.

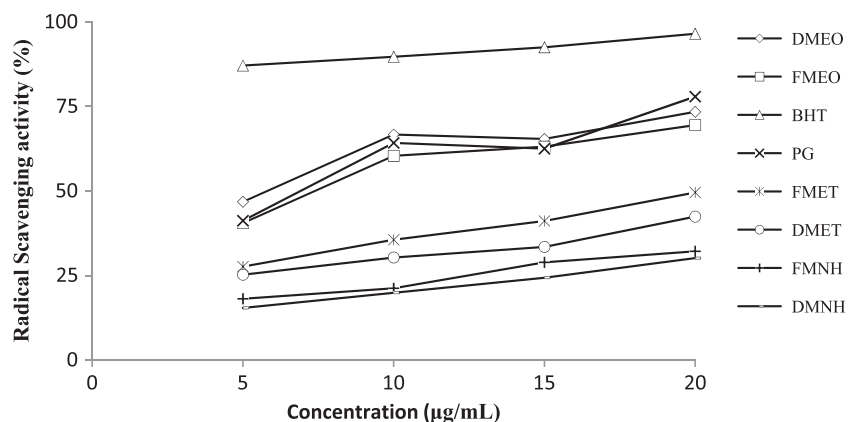


Figure 1. Scavenging effect (%) of *Mentha longifolia* essential oils and its oleoresins on 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical.

Total antioxidant activity of essential oils, oleoresins and synthetic antioxidants was determined in the linoleic acid system. Figure 3 clearly shows the antioxidative effects of various additives. Both DMEO and FMEO exhibited better antioxidant activity compared with oleoresins. FMET was found to be the most effective among the oleoresins in inhibiting the peroxides formation. The absorbance of linoleic acid emulsion without the addition of essential oils, oleoresins or synthetic antioxidant increased rapidly and there was significant ($p \geq 0.05$) difference between the blank and the tested additives. A higher absorbance indicates a higher concentration of peroxides formed. The amount of peroxides and hydroperoxides formed during the initial stages of lipid peroxidation are determined by measuring their PV. Figure 4 shows the PV for all the tested antioxidative substances. It was observed that all the tested additives showed significantly lower peroxide content than the control, for which the PV increased

from 21.2 to 213.2 Meq/kg. The TBA value assay measures the inhibitory effect of various additives on the formation of malondialdehyde, a secondary oxidation product. The result (Figure 5) clearly shows that both DMEO and FMEO have good inhibition at a concentration of 200 ppm in comparison with PG but lower than BHT. The effectiveness of the additives in stabilizing mustard oil from both PV and TBA values was found to be in the order:

BHT > DMEO > FMEO > PG > FMET > DMET > DMNH > FMNH > control.

Due to the chemical complexity of the organic extracts, determination of antioxidant activity using different techniques can give scattered results. Therefore, an approach with multiple assays is highly advisable. Previous works (27–29) on the antioxidant activity investigated by DPPH scavenging (30) and metal chelating power supported our investigations. By

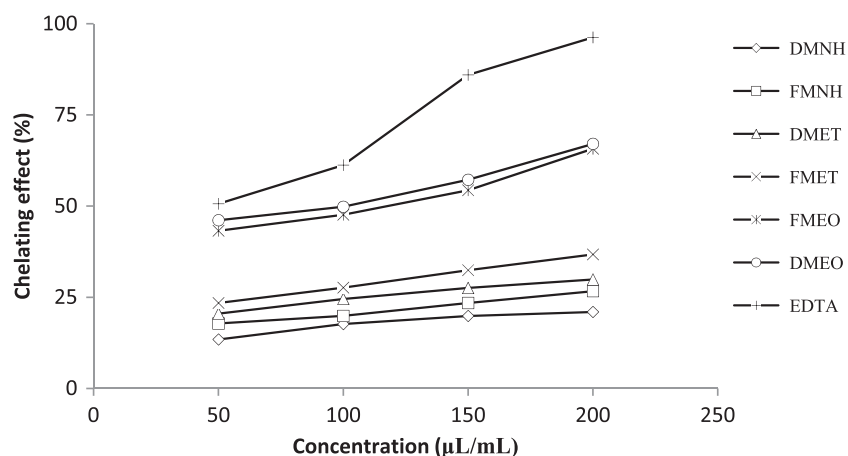


Figure 2. Chelating effect of *Mentha longifolia* essential oils and its different oleoresins.

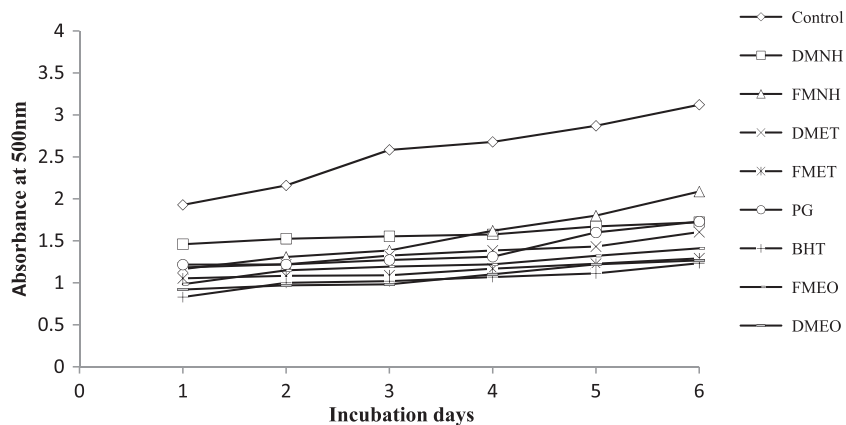


Figure 3. Inhibitory effect of *Mentha longifolia* essential oils and its oleoresins on the primary oxidation of linoleic acid system measured using the ferric thiocyanate method.

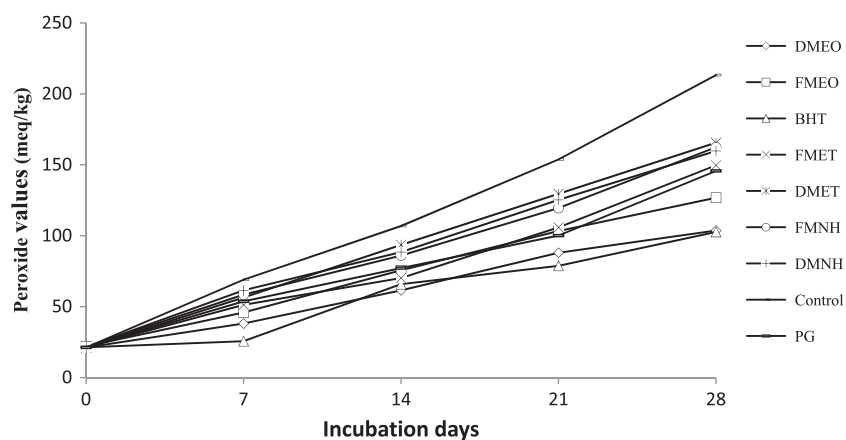


Figure 4. Inhibitory effect of *Mentha longifolia* essential oils and its oleoresins on the primary oxidation of mustard oil measured using the peroxide value method.

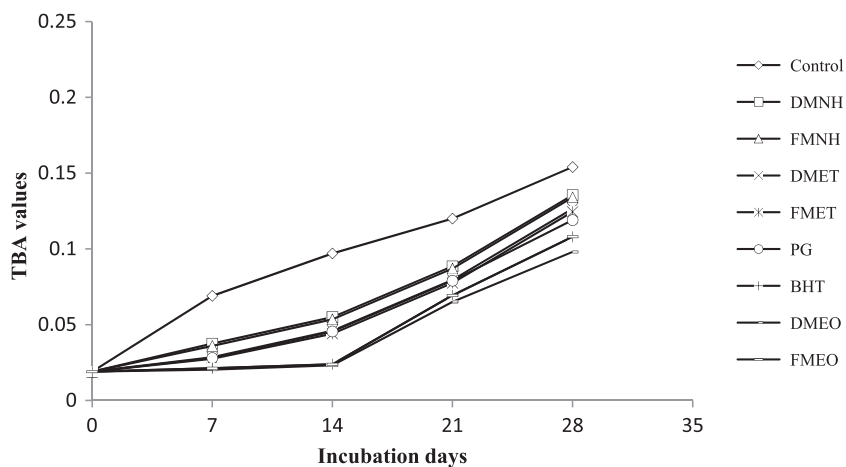


Figure 5. Inhibitory effect of *Mentha longifolia* essential oils and its oleoresins on the primary oxidation of mustard oil measured using the thiobarbituric acid (TBA) value method.

comparing the antioxidant activity measured by different methods and the relationship between the chemical composition and antioxidant activity, it was seen that DMEO, FMEO and FMET possess good capacity to scavenge free radical and to prevent lipid peroxidation, which can be attributed to the presence of oxygenated sesquiterpenes and monoterpenes. In recent years, phenolic compounds, terpenes and their oxygenated relatives have been reported to have enormous antioxidant and free radical scavenging capacity (31). Tables 1 and 2 show that essential oils and oleoresins of *M. longifolia* are markedly rich in oxygenated terpenes, which may act as radical scavenging agents. Also Tepe et al. (32) indicated that essential oils containing oxygenated monoterpenes and/or sesquiterpenes have greater antioxidative properties. On the other hand, Foti and Ingold (33) showed that γ -terpinene, a monoterpene hydrocarbon, has antioxidant activity comparable with that of phenolic compounds. This compound breaks free-radical chain reactions, which could be accompanied by its irreversible oxidation into inert compounds. The drying process has a significant influence on the yield of extracted substances as well as on the chemical composition and it may be the reason for the better activity of DMEO for antioxidant potentials (34). It has also been reported that natural antioxidant compounds often work synergistically to produce a broad spectrum of activities that creates an effective defense system against free radical attack (35). Generally, Labiatae species are rich sources of terpenoids and phenolic compounds (36). Therefore, it is likely that phenolic constituents present in *Mentha* species are, at least in part, responsible for the antioxidant and free radical scavenging activities.

Conclusion

The results of this study indicate that the essential oils and oleoresins of *M. longifolia* L. are favorable free radical scavengers as well as primary antioxidants that may react with free radicals and limit reactive oxygen species attack on food systems. The investigated essential oils may be used for the preservation of processed foods. Further study under *in vivo* conditions is recommended to elaborate the antioxidant potentials of these essential oils and oleoresins.

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