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Chemical Compositions of Essential Oils of *Artemisia dracunculus* L. and Endemic *Matricaria chamomilla* L. and an Evaluation of their Antioxidative Effects

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ABSTRACT

The present study explores the chemical constitution and antioxidant activity of the essential oils of the aerial parts of *Artemisia dracunculus* L. and the flower heads of *Matricaria chamomilla* L. GC-MS analysis revealed the presence of (*Z*)-anethole (51.72%), (*Z*)-*β*-ocimene (8.32%), methyleugenol (8.06%), limonene (4.94%) and linalool (4.41%) in *Artemisia dracunculus* and (*E*)-*β*-farnesene (24.19%), guaiazulene (10.57%), *α*-bisabolol oxide A (10.21%), α-farnesene (8.7%) and α-bisabolol (7.27%) in *M. chamomilla* L.. The antioxidant activity (AOA) of the essential oils was investigated using DPPH˙ (2, 2′-diphenyl 1-picrylhydrazyl) free radical scavenging and *β*-carotene/linoleic acid methods. The essential oil EC_{50} values were determined as 3.19±0.13 and 5.63±0.20 mg ml^{-1} for *A. dracunculus* and *M. chamomilla*, respectively. Further, the *A. dracunculus* L. essential oil (ADEO) and *M. chamomilla* L. essential oil (MCEO) were able to reduce the oxidation rate of soybean oil under accelerated conditions at 60 °C (oven test).

Keywords: Antioxidant activity, *Artemisia dracunculus* L., *β*-carotene bleaching, DPPH˙, Essential oil, *Matricaria chamomilla* L.

INTRODUCTION

Lipid oxidation has been reported to be responsible for toxic compound formation and to be the cause of many diseases (Barlow, 1990). Antioxidants can prevent the reaction of free radicals with biomolecules in the human body and reduce cell injury and death, chronic and cardiovascular diseases and etc. (Leong and Shui, 2002; Tepe *et al.,* 2005). Antioxidants have also been used in the food industry to increase the shelf life of foods, especially those rich in polyunsaturated fats. Polyunsaturated fats in food are readily oxidized by molecular oxygen and are a major cause of oxidative deterioration, nutritional losses, off flavour development and discoloration (Singh *et al.,* 2007). The synthetic antioxidants, such as propyl gallate (PG), butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and tertiary butyl hydroquinone (TBHQ) have been greatly used to control lipid oxidation in foods; however, recent reports reveal that these compounds may be implicated in many health risks, including cancer and carcinogenesis (Hou, 2003). Today, growing interest has been focused on the use of extensive groups of medicinal plants and their aromatic components as natural sources, due to their well-known abilities to scavenge free radicals. Essential oils are complex mixtures of different volatile principles deriving from secondary plant metabolism. They are extracted from

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specific plant organs (e.g. leaves, peels) and possess different pharmacological activities. In fact, the antimicrobial, antioxidant, anti-inflammatory, antispasmodic and relaxing properties of essential oils have been described both in animals and humans (Burt, 2004).

"Tarkhun" and "Babune Shirazi" are the Persian names for *Artemisia dracunculus* L. and *Matricaria chamomilla* L., respectively, belonging to the family Asteraceae. These plants are two well-known, valuable and native medicinal plants used in Iranian traditional medicine. "Tarkhun" is used traditionally in foods as a flavouring and for producing different kinds of sauces. The essential oil of this plant is said to be a neuromuscular antispasmodic, anti-inflammatory, is used in aerophagy and spasmodic colitis, antibacterial and antifungal. In addition, it is advised in cases of difficult digestion, intestinal bloating, nausea, windy colic, flatulence, aerophagy, stomachic, carminative, emmenagogic conditions and a desire to vomit (Kordali *et al.,* 2005).

M. chamomilla L. is an important medicinal and aromatic plant in both traditional and modern medicine. There are also commercial pharmaceuticals with formulae based on MCEO. This oil has been used commonly in medicine as an anti-inflammatory, anti-spasmodic, anti-intestinal bloating, anti-peptic ulcer, anti-bacterial and anti-fungal agent. Also, it is advised in the treatment of malaria and parasitic worm infections, cystitis, colds, flu, skin inflammation and healing wounds (Sashidhara *et al.,* 2006; Owlia *et al.,* 2007). To the best of our knowledge, the essential oil composition of *M. chamomilla* L. originating from Golestan in Iran has not been reported before and, therefore, our results can be evaluated as the first report about the composition of the essential oil of this unique and endemic species.

The aims of this work were: (i) to determine the chemical compositions of ADEO and MCEO by using GC–MS, (ii) to evaluate the AOA by using the 2, 2′-diphenyl 1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging and β-carotene bleaching (BCB) methods, (iii) and to determine the AOA of ADEO and MCEO in crude soybean oil by measuring peroxide and thiobarbituric acid values (oven test).

MATERIAL AND METHODS

Materials

All chemicals were analytical grade with the highest purity available and used without further purification. Crude soybean oil was purchased from Margarin Factory in Varamin (Tehran, Iran). The *M. chamomilla* L. plant was obtained from a research farm of the Institute of Medicinal Plants and Natural Products Research in Golestan, Iran. The *A. dracunculus* L. plant was obtained from the Neyshabur in Iran. The dried parts of the two species (100 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 hours (Anonymous, 1988). The oils were dried over anhydrous sodium sulphate and kept at 4°C until it was used.

GC-MS Analysis of Essential Oils

GC analysis was carried out on an Agilent Technologies 6890 gas chromatograph equipped with Flame Ionization Detector (FID) and a HP-5 capillary column (30 m×0.25 mm; 0.25 μm film thickness). The oven temperature was held at 50ºC for 5 minutes, and then programmed at 3ºC min^{-1} to 240ºC and after that programmed at 15ºC min^{-1} to 300ºC (held for 3 minutes) and, finally, reached 340°C (at 3°C min^{-1}). Other operating conditions were: carrier gas, He with a flow rate of 0.8 mL min^{-1}; injector and detector temperatures was 290ºC, and split ratio, 1:10. GC/MS analysis was performed on a GC mentioned above coupled with a Agilent Technologies 5973 Mass system. The other operating conditions were the same conditions as described above, mass spectra were taken at 70 eV. Mass range was from m/z 35–375 amu.

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Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the essential oils were identified by comparison of their mass spectra and retention indices with those published in the literature (Adams, 1995; Swigar and Silverstein, 1981) and presented in the MS computer library.

DPPH$^{\bullet}$ Free Radical Scavenging Assay

The hydrogen atom or electron-donation ability of the BPEO was measured from the bleaching of the purple-colored ethyl acetate solution of DPPH$^{\bullet}$. This spectrophotometric assay uses stable
2, 2'-diphenyl 1-picrylhydrazyl radical (DPPH$^{\bullet}$) as a reagent. Two mL of various concentrations of the samples (0.045-0.45% w/v) in ethyl acetate were added to 1 mL of a 2×10^{-4} M solution of DPPH$^{\bullet}$. The decrease in absorbancy at 517 nm was determined by a Scinco spectrophotometer (Seoul, South Korea) after 1 hour for all samples. Ethyl acetate was used as a blank. The absorbance of the ethyl acetate solution DPPH$^{\bullet}$ radical without antioxidant was measured as a control. All determinations were performed in triplicate and the results were averaged. The percentage inhibition of the DPPH$^{\bullet}$ radical by the sample was calculated according to the following formula (Kulisic *et al.,* 2004):

$$\% \text{ Inhibition} = ((A_{C(0)} - A_{S(t)})/A_{C(0)})100$$

where $A_{C(0)}$ is the absorbance of the control at $t = 0$ minute and $A_{S(t)}$ is the absorbance of the sample at t. Essential oil concentration providing 50% inhibition (EC_{50}) was calculated from the graph plotting percentage of remaining DPPH$^{\bullet}$ against essential oils concentrations.

β-carotene Bleaching Assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.2 mg of β-carotene was dissolved in 10 mL of chloroform and 1 mL was added to 20 mg linoleic acid and 200 mg of Tween 40. Chloroform was gently removed under a stream of nitrogen gas. Then, 50 mL of distilled water, saturated with oxygen (30 minutes, 100 mL min^{-1}), was added with vigorous shaking. 200 µl of ethanolic stock solution of sample and BHT were separately mixed with 5 mL emulsion. Readings of all samples were taken immediately by spectrophotometer at $t = 0$ minute at 470 nm. The cuvettes were incubated in a water bath at 50ºC for 30 minutes. Then, absorbance of samples at 470 nm were determined by spectrophotometer (Zhang, *et al.,* 2006). All determinations were performed in triplicate and the results were averaged. The percentage inhibition was calculated using the following equation:

$$\%\text{Inhibition} = (A_{\text{sample(t)}} - A_{\text{control(t)}})/(A_{\text{control(0)}} - A_{\text{control(t)}})\times100$$

where $A_{sample(t)}$ and $A_{control(t)}$ are the absorbance of the sample and control at t, respectively, and $A_{control(0)}$ is absorbance of the control at $t = 0$ minute.

Effect of ADEO and MCEO on Soybean Oil Oxidation

The ADEO and MCEO were added to crude soybean oil at 0.2, 0.4, 0.6 and 0.8 mg ml^{-1}. AOA of essential oils were compared with synthetic antioxidants including BHA, BHT at 0.1 and 0.2 mg ml^{-1}. The oven test method at 60°C was used to check crude oil stability. Oxidation was periodically assessed by the measurement of peroxide (PV) and thiobarbituric acid (TBA) values at 0th, 8th, 16th, 24th and 32nd days of storage according to the AOCS method (AOCS, 1989) and (Sidewell *et al.*). All experiments were performed in triplicate and results were averaged.

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Statistical Analysis

Data were analyzed statistically using analysis of variance (ANOVA) and differences among the means were determined for significance at $P \leq 0.001$ using the least significant differences (LSD) test (by SAS software). The data are presented as mean±standard deviation of the three replicates.

RESULTS AND DISCUSSION

GC/MS Analysis of Essential Oils

The chemical components of the Iranian ADEO and MCEO are presented in Table 1. The essential oil composition of tarragon grown in Cuba (Pino *et al.,* 1996), Albany (Meepagala *et al.,* 2002), and Turkey (Kordali *et al.,* 2005) have been previously reported. The essential oil of tarragon of Cuban origin contains elemicin (53.0%) and methyleugenol (17.6%) as major components (Pino *et al.,* 1996). In the present study, the relative amount of methyleugenol was found to be 8.06%, whereas elemicin was not detected. The essential oil of Albany tarragon contains terpinolene (25.4%), (*Z*)-*β*-ocimene (22.2%), 5-phenyl-1, 3-pentadiyne (11.7%), and capillene (6-phenyl-2, 4-hexadiyne) (4.8%), which are two unusual and rarely occurring alkynes (Meepagala *et al.,* 2002), whereas these alkynes were not found in Iranian tarragon essential oil in the present study. The chemical composition of Turkish tarragon essential oil contained (*Z*)-anethole (81.0%), (*Z*)-*β*-ocimene (6.5%), (*E*)-*β*-ocimene (3.1%), limonene (3.1%), and methyleugenol (1.8%) (Kordali *et al.,* 2005), whereas these components are found in ADEO but with different amounts.

To the best of our knowledge, the essential oil composition of *M. chamomilla* L. from Golestan in Iran has not been reported before and so our results can be regarded as the first report of the composition of the essential oil of this unique and endemic species. Jaimand and Rezaee (2003) have analyzed MCEO originating from Kazeroon, Hamedan and Tehran in Iran. The main components of sample from Kazeroon were as follows: *α*-bisabolol (7.27%), (*Z, Z*)-farnesol (17.00%), *cis-β*-farnesene (11.50%), guaiazulene (10.57%) and chamazulene (2.60%), whereas chamazulene was not detected in the present study; in the sample from Hamedan as: (*Z, Z*)-farnesol (39.70%), *α*-bisabolol oxide B (18.50%), guaiazulene (17.00%) and *cis-β*-farnesene (6.90%); and in the samplefrom Tehran as: (*Z, Z*)-farnesol (66.00%), guaiazulene (16.20%), *α*-bisabolol oxide A (11.00%) and *cis-β*-farnesene (4.40%). Differences among chemical compositions of the essential oils widely depend on production conditions such as climate, soil, harvest date, storage time, variety and cultivar factors (Blair *et al.,* 2001). Totally, ADEO contains 30.51% hydrocarbons (CH), 54.55% oxygenated compounds (CHO) and 14.81% phenolic (OH) fractions. In addition, MCEO contains CH (54.10%), CHO (10.54%) and OH (28.22%) fractions. Therefore, it is revealing that the amount of oxygenated and phenolic compounds is higher in ADEO than MCEO.

DPPH and *β*-carotene Bleaching Assay

Figures 1A and B show the effect of different concentrations of ADEO and MCEO on the scavenging rate of DPPH, respectively. The kinetic behaviors of essential oils show that the reaction approaches an almost steady state in 60 minutes. Free radical scavenging activity of the essential oil depends on its concentration and there is a direct correlation between these. A lower EC_{50} value reflects a better protective action. EC_{50} values of the ADEO and MCEO have been compared with other essential oils, BHT and α-tocopherol. The free radical-scavenging activity of ADEO (EC_{50}= 3.19±0.13 mg ml^{-1}) was superior to the MCEO (EC_{50} value= 5.63±0.20 mg ml^{-1}).

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Table 1. Chemical composition of ADEO and MCEO determined by GC/MS.

Artemisia dracunculus L.			*Matricaria chamomilla* L.		
Components	Kovat's R. I.[a]	Amount (%)	Components	Kovat's R. I.	Amount (%)
α-pinene	933	1.02	α-pinene	933	0.32
sabinene	973	tr[b]	benzaldehyde	959	0.10
-pineneβ	977	0.15	sabinene	973	0.14
myrceneβ–	989	0.77	myrcene	989	tr
-phellandreneα	1004	0.85	*p*-cymene	1024	0.36
3-careneδ–	1010	tr	limonene	1028	0.16
α-terpinene	1018	0.27	1,8-cineole	1031	0.13
p-cymene	1025	0.34	(*Z*)-β-ocimene	1036	0.12
limonene	1030	4.94	(*E*)-β-ocimene	1046	0. 89
(*Z*)-β-ocimene	1038	8.32	artemisia ketone	1059	1.05
(*E*)-β-ocimene	1048	2.37	linalool	1098	0.27
terpineneγ–	1059	0.16	*n*-nonanal	1101	0.32
terpinoleneα–	1090	3.83	α -thujone	1107	0.21
linalool	1098	4.41	β -thujone	1118	3.09
allo-ocimene	1128	3.28	terpinene-4-ol	1180	0.13
neo-allo-ocimene	1141	0.18	carvacrol methyl ether	1277	tr
borneol	1146	0.67	thymol	1292	0.51
(*Z*)-anethole	1251	51.72	*n*-tridecane	1295	0.10
(*E*)-carvone oxide	1262	0.19	carvacrol	1303	1.07
bornyl acetate	1289	0.15	methyl granate	1320	0.11
thymol	1293	0.29	δ–elemene	1342	0.63
elemeneδ–	1342	0.66	granyl acetate	1382	0.16
methyleugenol	1404	8.06	β–elemene	1389	0.13
(*E*)-β-caryophyllene	1429	0.26	β–caryophyllene	1418	1.70
humuleneα–	1455	0.33	aromadendrene	1437	0.32
decaluctoneγ–	1463	0.39	(*E*)-β-farnesene	1457	24.19
bicyclogermacrene	1490	2.64	γ–muurolene	1471	0.34
farnesene–α–(*E,E*)	1505	0.22	Germacrene D	1484	3.82
-sesquiphellandreneβ	1525	tr	α -selinene	1497	0.37
elemicin	1554	0.14	α -farnesene	1508	8.70
gleenol	1589	0.50	δ–cadinene	1529	0.36
dillapiole	1630	2.35	Germacrene B	1553	0.10
Epi-α-cadinol	1649	0.11	(*E*)-nerolidol	1564	0.81
phytol[c]	1934	0.30	spathulenol	1574	0.42
			caryophyllene oxide	1585	1.44
			viridiflorol	1590	1.67
			10-*epi*-γ-eudesmol	1621	0.61
			γ-eudesmol	1626	0.16
			Epi-α-cadinole	1634	0.52
			α-bisabolol oxide B	1651	4.57
			α-bisabolone oxide A	1672	0.18
			α-bsabolol	1690	7.27
			α-bisabolol oxide A	1745	10.21
			guaiazulene	1762	10.57
			cis-en-yn-dicycloether	1891	3.36
			trans-en-yn-dicycloether	1901	0.40
			n-tricosane	2294	0.78
			ix-2D/no chemical name	2359	5.34

[a] Retention index; [b] Trace, [c] Correct isomer was not determined.

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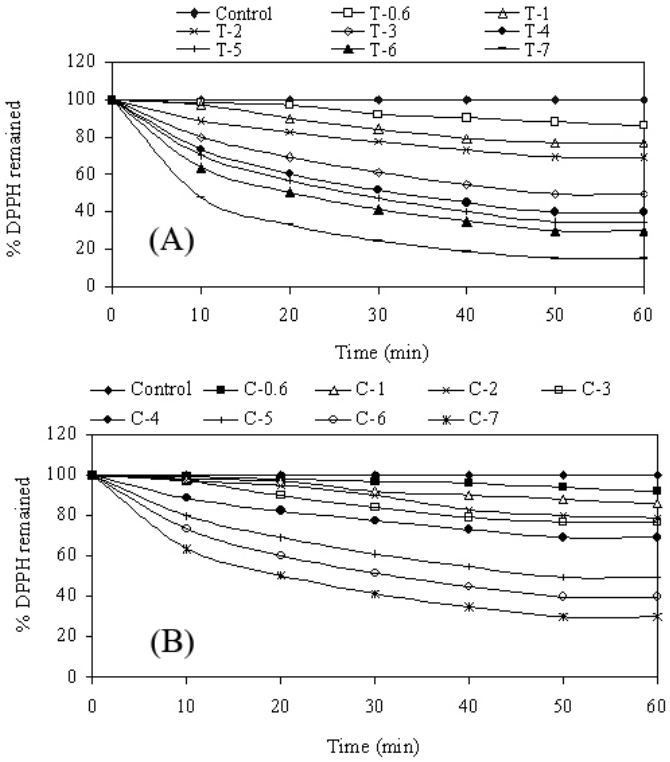

Figure 1. Effect of ADEO and MCEO concentrations on DPPH$^{\bullet}$ (A and B, respectively). T-0.6 to T-7 and C-0.6 to C-7 represents the concentrations of ADEO and MCEO from 0.6 to 7 mg ml^{-1}.

However, radical scavenging power of ADEO and MCEO are higher than activities reported by other researchers (Singh *et al.,* 2007; Fazel *et al.,* 2008; Demirci *et al.,* 2007). In addition, AOA of BHT as positive control, was compared in a parallel experiment that showed higher AOA with EC_{50}= *0.038±0.001* mg ml^{-1}. The higher AOA of the ADEO in comparison with MCEO may be due to its higher amounts of the oxygenated and phenolic compounds. In addition, a possible synergistic effect among oxygenated compounds can be suggested too (Kulisic *et al.,* 2004). Other authors have already reported that the CHO and phenolic compounds were the most potent antioxidants (Demirci *et al.,* 2007).

The rate of *β*-carotene bleaching can be slowed down in the presence of antioxidants (Tepe *et al.,* 2005; Kulisic *et al.,* 2004). So, antioxidant activities of the ADEO and MCEO in comparison with synthetic antioxidants, namely BHT and BHA, were evaluated (Figure 2). The AOA of ADEO at a concentration of 5 and 7 mg ml^{-1} was similar to BHT at a concentration of 0.1 mg ml^{-1} and the essential oil at a concentration of 9 mg ml^{-1} was similar to BHT at a concentration of 0.2 mg ml^{-1}. The AOA of MCEO (at 9 mg ml^{-1}) was similar to BHT (at 0.1 mg ml^{-1}). In addition, ADEO is more effective as an antioxidant than MCEO, which can be attributed to the CHO (such as anethole) and OH (such as methyleugenol and linalool) fractions in the oil as compared with MCEO oil, which contains CH fraction (about 54.1%, namely (*E*)-*β*-farnesene and guaiazulene). In the literature, there are many reports indicating the antioxidant potential of methyleugenol, linalool, thymol and carvacrol (Fazel *et al.,* 2008; Ruberto and Baratta, 2000). So, the key role of phenolic compounds as scavengers of free radicals is emphasized in several reports.

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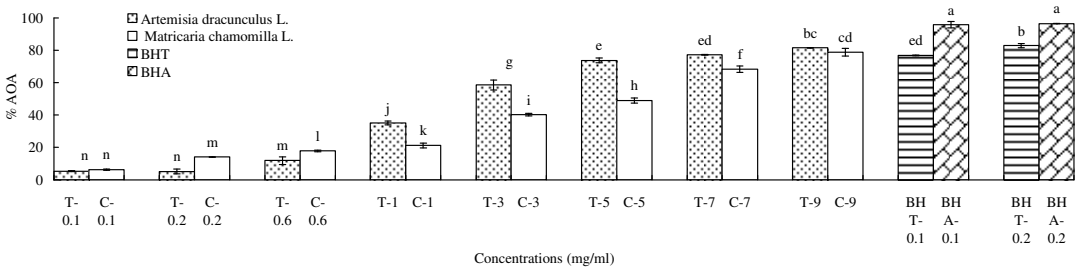

Figure 2. Antioxidant activities of ADEO, MCEO, BHA and BHT as determined by β-carotene bleaching method.

Effect of ADEO and MCEO on Soybean Oil Oxidation

Figures 3A and C show peroxide value changes in soybean oil for all investigated samples at 60°C. PV is a commonly used measurement of the primary lipid oxidation, indicating the amount of peroxides formed in the fats and oils during oxidation. Soybean oil oxidation was measured during 32 days of storage at 8-day intervals. Primary oxidation products are unsteady compounds that produce a number of secondary products, e.g. malonaldehyde, which may smell badly at low threshold values. Hence, simultaneously with the measurements of peroxide value, the changes of secondary oxidation products such as malonaldehyde, which are measured by TBA, were also determined after every 8 days (Figures 3B and D).

All samples containing 0.2–0.8 mg $ml^{-1}$of ADEO were more stable on heating at 60°C than the control when assessed by the change in peroxide (Figure 3A) and TBA (Figure 3B) values. The AOA of ADEO was comparable to BHT and lower than BHA. The antioxidative effect of ADEO showed a

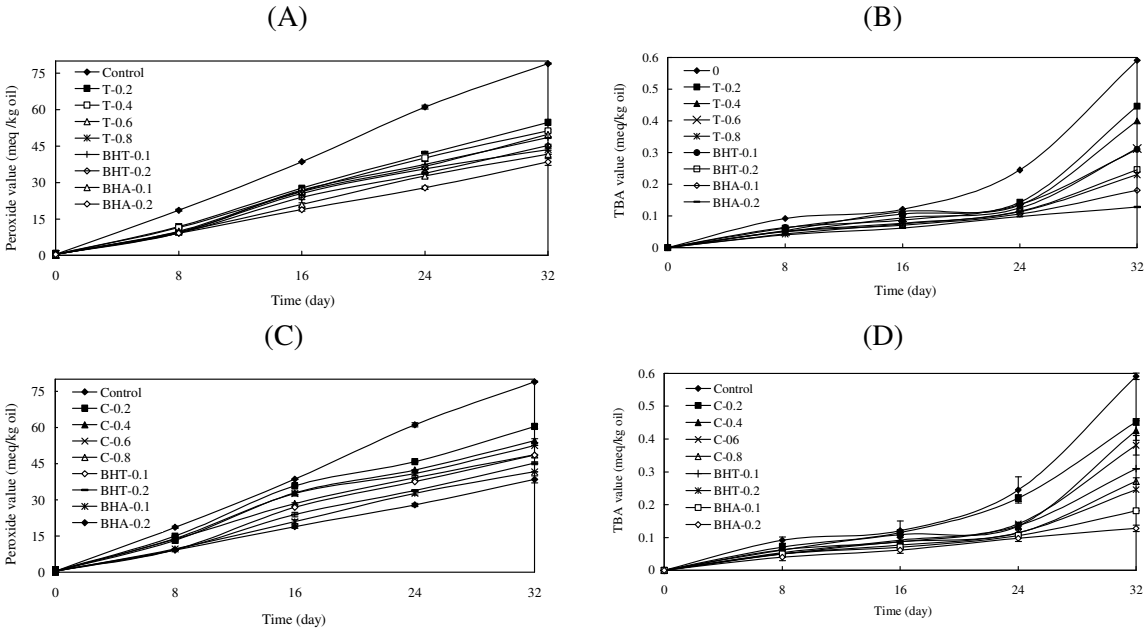

Figure 3. Effect of ADEO (T-0.2 to T-0.8) and MCEO (C-0.2 to C-0.8) on soybean oil oxidation expressed as peroxide (A and C, respectively) and thiobarbituric acid (B and D, respectively) values during storage at 60°C.

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direct correlation with concentration and, at a concentration of 0.6 and 0.8 mg ml^{-1}, the AOA was not significantly different from that of the BHT at 0.1 and 0.2 mg ml^{-1}.

All treatments with MCEO added at 0.2–0.8 mg ml^{-1} were more stable on heating at 60°C than the control, when assessed by the change in peroxide (Figure 3C) and TBA (Figure 3D) values. The AOA of MCEO (same as ADEO) was comparable to BHT and lower than BHA. The antioxidative effect of MCEO showed a direct correlation with concentration and, at a concentration of 0.8 mg ml^{-1}, was almost equal to BHT at 0.1 mg ml^{-1}. These results showed a good correlation between the peroxide and TBA values. However, ADEO had a higher AOA than MCEO in soybean oil. Therefore, our results showed good antioxidant activities for ADEO and MCEO in a food system (crude soybean oil at $P< 0.001$).

These findings are consistent with the results of a study on the AOA of *Carum nigrum* (seed) essential oil in mustard oil and showed that the effect of 0.1 mg ml^{-1} of essential oil was comparable to that of 0.2 mg ml^{-1} BHA (Singh and Marimuthu, 2006). Shahsavari *et al.* (2008) compared the AOA of essential oil of *Bunium persicum* in soybean oil with synthetic antioxidant (BHA) and reported that there was no distinct difference between synthetic antioxidants (0.02%) and essential oil (0.06%) in the inhibition of soybean oil peroxidation.

CONCLUSIONS

This study concludes that ADEO and MCEO offer antioxidant properties. Unlike synthetic antioxidants, ADEO and MCEO can be added in larger quantities to get optimal effects (addition of synthetic antioxidants is limited under food laws and regulations). Therefore, lipid peroxidation can be reduced by the required amount of ADEO and MCEO. However, further investigation (such as other antioxidant testing methods, toxicological testing etc.) and the antioxidant activity mechanism are warranted. These studies can be useful as a starting point for further application of ADEO and MCEO in food preparations.

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ترکیبات شیمیایی اسانس‌های گیاهان ترخون (*Artemisia dracunculus* L.) و بابونه‌ی شیرازی (*Matricaria chamomilla* L.) و ارزیابی خاصیت آنتی‌اکسیدانی آنها

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چکیده

مطالعه‌ی حاضر به بررسی ترکیبات شیمیایی و خاصیت آنتی‌اکسیدانی اسانس‌های حاصل از سرشاخه گلدار گیاه ترخون و گل گیاه بابونه‌ی شیرازی توسط تکنیک GC-MS می‌پردازد. تجزیه‌ی با GC-MS حضور ترکیباتی همچون سیس-آنتول (۵۱/۷۲٪)، سیس-بتااوسیمن (۸/۳۲٪)، متیل‌ائوژنول (۸/۰۶٪)، لیمونن (۴/۹۴٪) و لینالول (۴/۴۱٪) در اسانس ترخون ، و *E*-بتا-فارنزن (۲۴/۱۹٪)، گوآزولن (۱۰/۵۷٪)، آلفا-اکسید بیزابولول A (۱۰/۲۱٪)، آلفا-فارنزن (۸/۷٪) و آلفا-بیزابولول (۷/۲۷٪) در اسانس بابونه‌ی شیرازی به اثبات رساند. به منظور بررسی فعالیت آنتی‌اکسیدانی اسانس‌ها، از دو آزمون رادیکال ۲ و '۲-دی فنیل ۱- پیکریل هیدرازیل ($DPPH^{\bullet}$) و سامانه‌ی بتاکاروتن/ لینولئیک اسید استفاده شد. مقدارEC_{50} به دست آمده برای دو اسانس ترخون و بابونه‌ی شیرازی به ترتیب ۰/۱۳±۳/۱۹ و ۰/۲۰±۵/۶۳ mg/ml بود. به‌علاوه، دو اسانس ترخون و بابونه شیرازی توانایی کاهش سرعت اکسیداسیون روغن سویای خام را در شرایط دمایی تسریع شده (دمای ۶۰ درجه‌ی سانتی‌گراد، آزمون آون) داشتند.

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