

# Antioxidative Activity of Purple Peril (*Perilla frutescens* L.), Moldavian Dragonhead (*Dracocephalum moldavica* L.), and Roman Chamomile (*Anthemis nobilis* L.) Extracts in Rapeseed Oil

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**ABSTRACT:** The antioxidant activities (AA) of acetone oleoresins (AO) and deodorized acetone extracts (DAE) of Roman chamomile (*Anthemis nobilis* L.), purple peril (*Perilla frutescens* L.), and Moldavian dragonhead (*Dracocephalum moldavica* L.) were tested in refined, bleached, and deodorized rapeseed oil by the Schaal oven test at 50°C. The addition of 1,000 ppm of AO and DAE of moldavian dragonhead and Roman chamomile significantly stabilized rapeseed oil. Their AA at the used concentration were higher than AA of a synthetic antioxidant, butylated hydroxytoluene (200 ppm), in reducing the rate of peroxide value increase to 20 meq/kg. AA of AO of purple peril was not significant, while DAE of this plant increased autoxidation induction period by 22%. It is also worthy of notice that AA of DAE from all investigated plants was slightly higher than AA of AO obtained from the same plants.

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**KEY WORDS:** Antioxidant activity, extracts, Moldavian dragonhead, purple peril, rapeseed oil, Roman chamomile.

Autoxidation of lipids, which can be induced by light, temperature, oxygen and some other factors, significantly decreases the quality of food containing fat. It is also known that consumption of such food is associated with aging, heart diseases, stroke, and cancer; therefore, antioxidants are widely used in foods. Because of the possible toxicity of synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), a search for and development of natural substitutes has been very intensive over the past 10 yr (1).

Recently, many antioxidants were isolated from the Labiatae and the Compositae plant families (2–6). Among the isolated natural products, the extracts of some herbs, particularly sage and rosemary, have been shown to possess very strong antioxidant compounds. Reported data (4) indicate that the most effective compounds of sage extracts are carnosol,

carnosic and rosmarinic acids, rosmadiol, rosmanol, epirosmanol, and methyl carnosate. A better knowledge was obtained through synergism and antagonism of phenolic acids, diterpenoids, and flavones present together in the extract (7). The extracts from various aromatic plants growing in Lithuania were also analyzed, and some of them were found to possess antioxidative activity (8). However, there are still many plants which have not been thoroughly investigated in terms of their possible antioxidant properties. Purple peril (*Perilla frutescens* L.), Moldavian dragonhead (*Dracocephalum moldavica* L.), and Roman chamomile (*Anthemis nobilis* L.) are among such plants.

Purple peril is found from the Himalayas to Japan and is naturalized in some parts of North America; the plant also grows in the Ukraine (9). It is widely grown as a culinary herb in eastern Asia and is becoming increasingly popular as an ornamental for summer bedding (10). Rosmarinic acid, 3,4-dihydroxybenzaldehyde, methyl 3,4-dihydroxybenzoate, methyl caffeate, 3',4',5,7-tetrahydroxyflavone, caffeic acid, 6,7-dihydroxycoumarin, vinyl caffeate and *trans-p*-menth-8-en-7-yl caffeate, 5-(3,4-dihydroxyphenyl-methyl) oxazolidine-2,4-dione, and 3-(3,4-dihydroxyphenyl) lactamide have been isolated from the seeds of *P. frutescens* (5,11). The seeds are rich in lipids (34–49%), consisting mainly of oleic (4%), linoleic (53%), and linolenic acids (23%) (12). Rosmarinic acid, quercetin, kaempferol, myricetin, luteolin, apigenin, and scutellarein were isolated and identified in the leaves of peril (12,13). The volatile oil in the leaves of *P. frutescens* contains perillaldehyde, which is 2,000 times sweeter than sucrose. Purple peril has been used as a medicinal herb in China since about 500 A.D. (14).

Moldavian dragonhead is a hardy plant, native to regions from Eastern Europe to Siberia. It has also been naturalized in other parts of the world (9). The annual Moldavian dragonhead is grown in Russia as a honey-bearing plant (15,16). The lemon-scented leaves have been used instead of lemon balm in eastern Europe to flavor fish dishes. The composition of the essential oil in dragonhead, grown in Lithuania, has been examined and 62 components have been identified (17). However, data about determination of the antioxidant effect of dragonhead were not found in available literature.

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Native to western Europe, Roman chamomile is now cultivated across Europe and in other temperate climate regions (9). As a homeopathic remedy, Roman chamomile is often used against nausea, vomiting, indigestion, and loss of appetite (18). Roman chamomile contains up to 1.75% volatile oil, consisting mainly of tiglic and angelic acid esters and chamazulene (14). The antioxidant and antimicrobial properties of essential oil in *A. nobilis* from Italy were investigated together with some other plants, and it was found that the volatile oil from Roman chamomile possessed the highest antioxidant activity (AA) (6). Flavonoids (apigenin, apiin, quercetin), coumarins (scopolin, umbelliferone, herniarine), phenolic acids (caffeic, ferrulic, anthenobilic), procyanidins, and sesquiterpene lactones have been reported in the flowering parts of Roman chamomile (14,19).

Various solvents have been used for the isolation of active antioxidative substances, including polar compounds such as ethanol (1,20) and methanol (21), and nonpolar ones, mainly hexane (21,22). Cuvelier *et al.* (1) investigated 32 pilot-plant and commercial extracts from rosemary and sage, isolated with hexane, CO<sub>2</sub>, and ethanol, and found significant differences in AA of the extracts obtained, even the ones obtained by the same solvent. The authors concluded that these differences could be caused by synergism and antagonism between extracted phenolic acids, diterpenoids, and flavones present together in the extracts. However, in most cases, acetone was the most efficient solvent for the extraction of antioxidative substances (23,24). Economou *et al.* (21), concluded that acetone was the most efficient solvent for the extraction of the antioxidative compounds from sage and rosemary. Similarly, other reports also show acetone to be an efficient solvent for the extraction of antioxidative substances from sage, rosemary, and some other herbs (8,23,24). In general the use of different polarity substances can provide more exhaustive information on the properties of the extracts; however, in this study, acetone was selected as a medium-polarity solvent.

## EXPERIMENTAL PROCEDURES

**Materials.** The first-year vegetation of purple peril and Moldavian dragonhead was harvested from the collection of aromatic herbs at the Lithuanian Institute of Horticulture in July, 1998. Second-year vegetation of Roman chamomile was harvested from the collection of medicinal herbs at the Kaunas Botanical Garden in August, 1997, during its full flowering phase. Plants were dried at 30 ± 2°C in a ventilated oven ("Vasara," Utena, Lithuania). Joint stock company, "Obeliu, Aliejus," in Lithuania, donated fresh, fully refined, deodorized rapeseed oil without any additives. The rapeseed oil had an initial peroxide value (PV) of 2.88 meq/kg, an erucic acid content of 0.56%, a linolenic acid content of 9.8%, and a total natural tocopherols content of 769 mg/kg.

**Determination of total phenolic compounds.** Phenolic compounds were extracted from 0.5 g of ground material with 3 × 30 mL portions of 80% ethanol on a heating stove (LTHS-1000, Druteva Brnenska, Czech Republic) at 50°C for 1 h.

After each extraction, the extract was filtered and diluted with ethanol. The total amount of phenolic compounds was measured with Folin-Ciocalteu (using gallic acid as a standard) and Folin-Denis (using chlorogenic acid as a standard) reagents. The standard Folin-Ciocalteu reagent (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was diluted with distilled water (1:10) and 4 mL was added to 1 mL of ethanolic plant extract. The color was developed by adding 5 mL of 7.5% sodium carbonate (Reachim, Riga, Latvia) solution in distilled water. The absorbance was read at 765 nm after 30 min on a SPECORD M 40 spectrophotometer (Carl Zeiss, Jena, Germany).

Folin-Denis reagent was prepared by mixing 100 g of Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O with 20 g of phosphate molybdenic acid and 50 mL of 85% H<sub>3</sub>PO<sub>4</sub> and diluting in 750 mL of distilled water (all chemicals were of analytical grade and obtained from Reachim). The solution was heated for 2 h with reflux, filtered, and diluted with distilled water up to 1 L (25).

Freshly prepared Folin-Denis reagent (0.5 mL) was added to 1 mL of ethanolic plant extract in a 10 mL volumetric flask, diluted with 6 mL of distilled water, and held for 3 min. The color was developed by adding a 20% sodium carbonate solution in distilled water to make the volume 10 mL. The absorbance was read at 725 nm after 60 min, and the total amount of phenolic compounds was calculated by the following formula:

$$C = \frac{c \cdot V}{m} \quad [1]$$

where *C* is the concentration of the total phenolics in gallic (GAE) or chlorogenic acid equivalents (CHAE); *c* is the concentration of gallic or chlorogenic acid, mg mL<sup>-1</sup>; *V* is the volume of plants extracts, 100 mL; and *m* is the weight of plant materials, in g. Three replicates were analyzed for every sample.

**Preparation of herb oleoresins and deodorized extracts.** Acetone oleoresins (AO) were obtained by extracting 50 g of freshly crushed leaves and flowering parts with 2,000 mL of acetone for 6 h in a Soxhlet apparatus. The extracts were concentrated at 60°C to 20 mL in a R114 vacuum rotavapor equipped with a B480 water bath and a B169 vacuum pump (Büchi, Switzerland). The remaining acetone was evaporated to dryness by placing the samples in a SPT200 vacuum drier (Horizont, Poland) at 50°C and 0.08 MPa. Dry extracts were stored in a freezer until use. The synthetic antioxidant BHT and a well-known strong natural antioxidant, sage AO, were used as reference substances for the comparison purposes. Sage oleoresin was prepared in the same way as all other plant extracts used in the present study.

For the preparation of deodorized acetone extracts (DAE), the essential oils were removed by hydrodistillation of comminuted herbs for 3 h, the liquid was decanted, and the residue was dried at 30°C in a ventilated drying oven ("Vasara"). Dry residue was extracted with acetone as described above.

**Preparation of the samples.** Rapeseed oil stability and consequently AA of the extracts were assessed with Shaal oven

test by storing the samples protected against direct light at 50°C (21). The extract was added directly into 80.0 g of rapeseed oil at the concentration of 1,000 ppm (0.1% of the oil mass) and dissolved by using an ultrasonic bath (ASTRASON™, model 9HT, 50/60 Hz; Heat Systems Ultrasonics, NY) at 50°C for 60 min. Afterward, three aliquots, 25.0 g each, were weighed into 150-mL glass beakers for further analysis. Sage AO and BHT, at concentrations of 1,000 (0.1%) and 200 ppm (0.02%), respectively, were used for comparison purposes. The samples were stored in a thermostatic oven KC-65 (PREMED, Poland) at 50°C for 200 h. A blank sample was prepared under the same conditions without any additive.

**Evaluation of antioxidant activity.** PV was determined by method Cd 8-53 of the American Oil Chemists' Society (26). Induction period (IP) values were expressed as the time when PV reaches 20 meq kg<sup>-1</sup> (27). Ultraviolet (UV) absorption was measured by the International Union of Pure and Applied Chemistry 2.505, International Standard Organisation 3656 method (28).

Absorption of oxygen was measured by the sample weight increase (24). IP was calculated when the weight increased by 0.05%. Protection factors (PF) and AA were calculated by using the same formula as was used to calculate PV (29).

PF and AA were calculated by using the values of measured parameters and the following formulae (29):

$$PF = \frac{IP_X}{IP_K} \quad [2]$$

$$AA = \frac{IP_X - IP_K}{IP_{BHT} - IP_K}$$

where  $IP_X$  is the induction period of sample with additive (h),  $IP_K$  is the induction period of sample without additive (h), and  $IP_{BHT}$  is the induction period of sample with BHT (h). Three replicates were analyzed for every sample and the standard deviation in all cases was in the range of 0.5 to 3%.

## RESULTS AND DISCUSSION

The total yield of the extracts was: peril AO, 11%; peril DAE, 12%; dragonhead AO, 8%; dragonhead DAE, 9%; Roman chamomile AO and DAE, 10% each. The amounts of AO and DAE were quite similar. The yield of DAE from purple peril and dragonhead was slightly higher than that of their AO, possibly due to the hydrolysis of some insoluble components during hydrodistillation. The amounts of the total phenolic compounds in dried herbs are presented in Table 1. The results obtained show that the highest concentration of phenolics was determined in peril leaves using both reagents. The amount of phenolics in dragonhead was slightly lower, whereas Roman chamomile possessed approximately two times less of these constituents as compared with the other two herbs. Both reagents gave comparable and reproducible results.

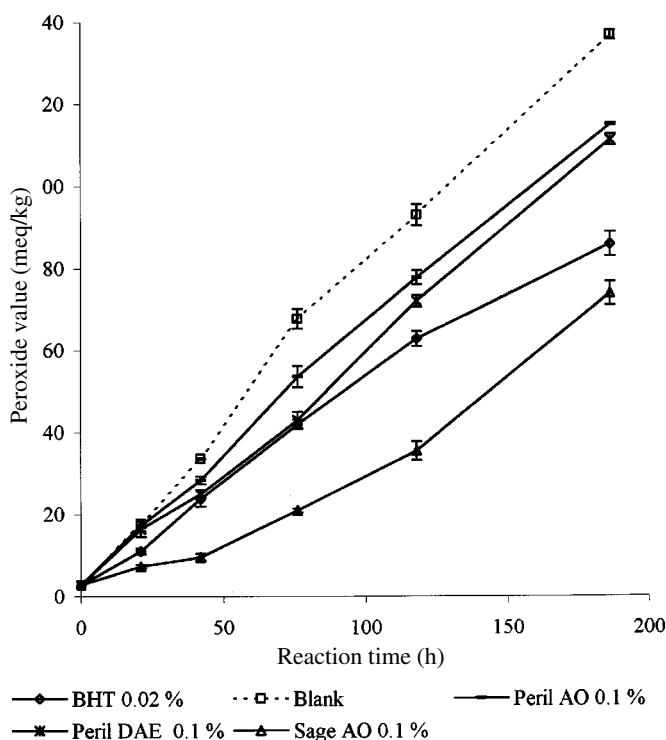
The changes of PV in rapeseed oil with different extracts

**TABLE 1**  
Total Amount of Phenolic Compounds in the Plants Used for the Oxidation Experiments, in Gallic Acid Equivalents (GAE) and Chlorogenic Acid Equivalents (CHAE) in 1 g of Herb

Plants	GAE (g <sup>-1</sup> )	CHAE (g <sup>-1</sup> )
Peril	122.4 ± 0.70	140.0 ± 0.48
Dragonhead	109.4 ± 0.58	112.6 ± 0.42
Roman chamomile	49.6 ± 0.42	59.6 ± 0.34

are given in Figures 1, 2, and 3, and the correlating characteristics (IP, PF, and AA) of the extracts are presented in Table 2. Rapeseed oil oxidation was measured at timed periods during 186 h of storage. During this time, PV increased in the blank sample to 137 meq kg<sup>-1</sup>.

The curves in Figures 1–3 show that all extracts reduced the rate of oxidation in terms of formation of peroxides. However, the AA of sage AO was most effective and considerably exceeded the effect of BHT and other herbal extracts. However, AO and DAE of Roman chamomile and dragonhead possessed a slightly higher positive effect on such characteristics as IP, PF, and AA as compared with BHT and perils (Table 2). For instance, a 0.1% addition of Roman chamomile DAE and AO, and dragonhead DAE and AO prolonged the induction period by 71 and 59, and 57 and 53%, respectively, as compared with the blank samples. The reduction in the rate of formation of peroxides in rapeseed oil after the addition of AO and DAE of perils was not considerable, e.g., IP of the sample with 0.1% of peril AO was almost equal to IP of the



**FIG. 1.** Effect of purple peril extracts on the formation of peroxides in rapeseed oil at 50°C. BHT, butylated hydroxytoluene; AO, acetone oleoresin; DAE, deodorized acetone extract.

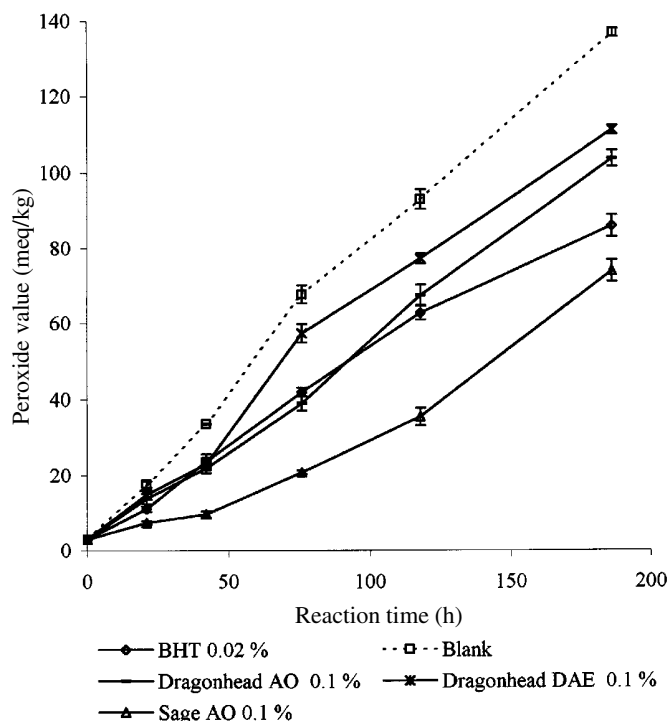


FIG. 2. Effect of Moldavian dragonhead extracts on the formation of peroxides in rapeseed oil at 50°C. See Figure 1 for abbreviations. Compounds with varied levels of activity; therefore, it is not surprising that it took five times more extract to equal the effect of BHT.

The effect of the extracts on the formation of primary and secondary oxidation products was assessed by measuring the changes of UV absorption values at 232 and 268 nm during

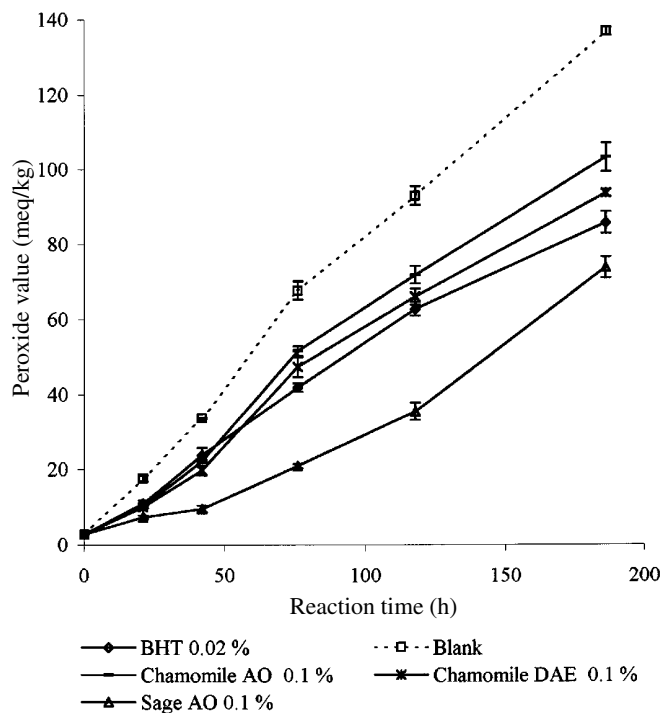


FIG. 3. Effect of Roman chamomile extracts on the formation of peroxides in rapeseed oil at 50°C. See Figure 1 for abbreviations.

TABLE 2  
Antioxidant Characteristics of the Plant Extracts Calculated on the Basis of Peroxide Values in Rapeseed Oil<sup>a</sup>

Additive	Conc. (%)	IP (h)	PF	AA
Without additives (blank)	—	24.5	—	—
BHT	0.02	36.0	1.47	1.00
Sage AO	0.10	73.0	2.98	4.22
Peril AO	0.10	26.0	1.06	0.13
Peril DAE	0.10	30.0	1.22	0.47
Dragonhead AO	0.10	37.5	1.53	1.13
Dragonhead DAE	0.10	38.5	1.57	1.22
Roman chamomile AO	0.10	39.0	1.59	1.26
Roman chamomile DAE	0.10	42.0	1.71	1.52

<sup>a</sup>BHT, butylated hydroxytoluene.

Abbreviations: IP, induction period; PF, protection factor; AA, antioxidant

storage of rapeseed oil (28). The changes of UV absorption at 232 nm were similar to the changes of peroxides, and clear linear dependence was determined between these two characteristics during the rapeseed oil oxidation in the samples (correlation coefficient = 0.99).

The formation of secondary oxidation products, such as aldehydes, correlates with UV absorption at 268 nm (28). The bars in Figure 4 show that all measured samples absorb more intensively after storage, which indicates the formation of secondary degradation products in the rapeseed oil. However, this process seems to be more complicated in terms of assessing the effect of the storage time and extracts as compared

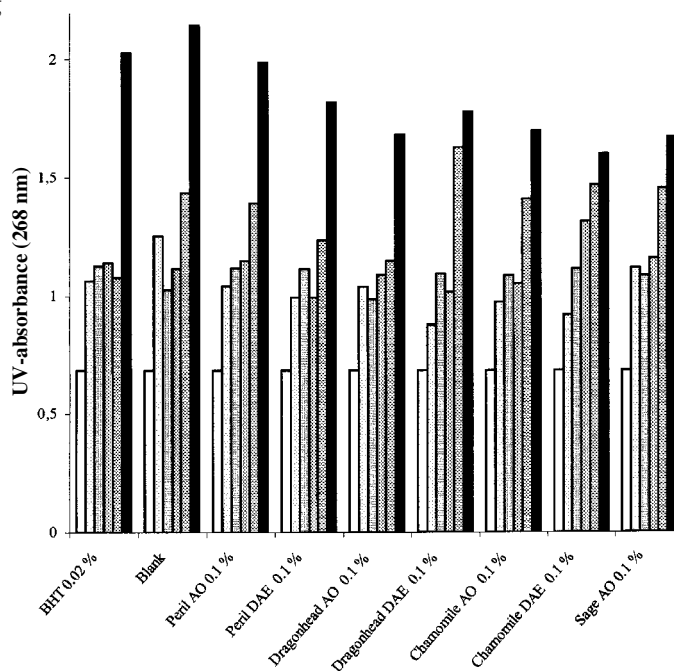


FIG. 4. Effect of plant extracts on the formation of aldehydes and ketones (ultraviolet (UV) absorbance at 268 nm wavelength) in rapeseed oil. For each of the nine entities tested, formation of aldehydes and ketones was determined (left to right) after 0, 21, 42, 76, 118, and 186 h. See Figure 1 for abbreviations.

with peroxides and conjugated dienes. Slightly lower absorbance values can be observed in the samples with 1,000 ppm of dragonhead, chamomile, and sage extracts at the end of the storage (186 h); however, these values in the same samples (except for dragonhead AO) were higher after 118 h of storage as compared with the samples with 200 ppm of BHT. Therefore, UV measurements at 268 nm cannot give a clear answer about the effect of the test extracts on the kinetics of the formation of secondary oxidation products in rapeseed oil.

Oxygen absorption method also proved that herbal extracts possess some AA (Table 3). Oxygen absorption was determined by weighing the samples at timed periods during storage. Actually, the curves of oxidation kinetics obtained by this method were comparable with the curves of PV increase (Figs. 1–3). The weight increase, and consequent absorption of oxygen, was very low in the samples with 1000 ppm of sage AO. BHT was less effective at the 200 ppm dose used; however, the rate of weight increase of the samples with this synthetic compound was lower as compared with all other herbal extracts. The differences between the effects of AO and DAE were also negligible. In comparing the results obtained by PV determination, the AA of Roman chamomile and dragonhead extracts was slightly higher than that of BHT, while the AA of the same herbal extracts assessed on the basis of weight increase measurement was significantly lower than the AA of BHT. The process of lipid oxidation is known to be very complex, and the use of different methods for its assessment can give more comprehensive information, especially when the effectiveness of multicomponent natural extracts is investigated.

Summarizing the results obtained by all three methods, we see that all herbal extracts exhibited some AA by stabilizing rapeseed oil during storage. In terms of effectiveness, the extracts at 0.1% concentration can be put into the following succession: sage AO > Roman chamomile DAE > Roman chamomile AO > dragonhead DAE > dragonhead AO > peril DAE > peril AO. It should be emphasized that no positive correlation was found between the total amount of phenolic compounds and AA. For instance, perils, which were rich in total phenolics, possessed the lowest AA as compared with Roman chamomile (low amount of phenolics and the highest AA) and

dragonhead (high amount of phenolics, medium AA). At this point it should be noted that different solvents were used for the isolation of phenolic compounds (ethanol) and plant extracts (acetone). However, we believe that the solvent in this case was not an important factor, since the procedure of extraction in a Soxhlet apparatus is sufficiently efficient to isolate herb phenolics with acetone. The absence of a correlation between the amount of total phenolics and AA of the extracts can be explained by the well-known fact that the AA of individual phenolic compounds can vary widely. Therefore, to establish any correlation between AA and the concentration of various compounds, the examination of the composition and chemical structures of separate constituents would be needed, which was beyond the goals of this preliminary study.

Although the differences in the AA between AO and DAE obtained from the same herbal material were not significant, some tendency can be observed in favor of DAE as compared with AO. It is known that most of the essential oils consisting mainly of terpenes and sesquiterpenes do not retard the oxidation of unsaturated acids (8). Therefore, it is not likely that the essential oils extracted into the AO of the tested herbs could increase the AA of the extracts. Reported data on the AA of the essential oil from Roman chamomile from Italy were obtained by using a model  $\beta$ -carotene bleaching system and cannot be directly compared with our results (6). It should also be noted that after the preparation of AO, the essential oil phases in the extracts of peril and dragonhead AO were clearly separated on the top of the extracts. Therefore, before the addition, these extracts were homogenized. Roman chamomile AO was obtained as homogeneous, most likely due to the low content (0.2%) of the essential oil (30). On the other hand, during hydrodistillation of the essential oils some hydrolysis of complex compounds, e.g., glycosides, could take place by releasing active constituents, which could participate in the lipid oxidation processes.

AA of peril, dragonhead, and Roman chamomile extracts could be due to phenolic compounds that are present in these plants and have been mentioned in the introduction. It is well established that rosmarinic and caffeic acids are remarkable for their AA, as well as most of the mentioned flavonoids (22).

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**TABLE 3**  
Antioxidant Characteristics of the Plant Extracts Calculated on the Basis of Oxygen Absorption in Rapeseed Oil<sup>a</sup>

Additive	Conc. (%)	IP (h)	PF	AA
Without additives (blank)	—	5.6	—	—
BHT	0.02	13.6	2.43	1.00
Sage AO	0.10	37.8	6.75	4.02
Peril AO	0.10	7.3	1.30	0.21
Peril DAE	0.10	7.6	1.38	0.25
Dragonhead AO	0.10	7.9	1.41	0.29
Dragonhead DAE	0.10	8.3	1.48	0.34
Roman chamomile AO	0.10	9.3	1.66	0.46
Roman chamomile DAE	0.10	9.8	1.75	0.53

<sup>a</sup>See Table 2 for abbreviations.

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