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Chemical Compositions and Antioxidant Activities of The Essential Oils of Some Medicinal and Aromatic Plants

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Abstract

The present study was conducted to determine the chemical compositions and antioxidant activities of some essential oils of some medicinal and aromatic plants widely used in feed or food industry. The major compounds of the essential oils of cinnamon, cumin, laurel, mint, oregano, rosemary and sage are cinnamaldehyde propylene glycol acetal (41.50%), cuminaldehyde (44.01%), 1,8 cineole (39.55), (+) pullegon (67.80%), carvacrol (59.03%), 1,8 cineole (30.12%) and (+) camphor (17.15%), respectively. There were significant differences in the antioxidant activities of these essential oils (P<0.01). In terms of the 2,2-diphenyl-1-picryhydrazyl (DPPH) assay, laurel essential oil (79.00%) demonstrated the highest antioxidant activity, followed by that from cumin (75.98%), oregano (75.81%), mint (69.49%), sage (69.01%), cinnamon (68.83%) and rosemary (63.88%). To conclude, the DPPH free radical scavenging activities of all essential oils from some medicinal and aromatic plant species are significantly greater than those of vitamin E and Trolox (P<0.01).

Keywords: Essential oil, chemical composition, antioxidant activity, aromatic plants, spices.

Bazı Tıbbi ve Aromatik Bitkilerden Elde Edilen Uçucu Yağların Kimyasal Kompozisyonları ve Antioksidan Aktiviteleri

Öz

Bu çalışma yem ve gıda endüstrisinde yaygın olarak kullanılan bazı uçucu yağların kimyasal kompozisyonlarının ve antioksidan aktivitelerinin belirlenmesi amacıyla yürütülmüştür. Tarçın, kimyon, defne, nane, kekik, biberiye ve adaçayı uçucu yağlarının başlıca bileşenleri sırasıyla cinnamaldehyde propilene glycol acetat (%41.50), cuminaldehyde (%44.01), 1,8 cineole (%39.55), (+) pullegon (%67.80), carvacrol (%59.03), 1,8 cineole (%30.12) ve (+) camphor (%17.15)'dur. Bu uçucu yağların antioksidan aktivitelerinde önemli düzeyde farklılıklar bulunmuştur (P<0.01). DPPH yöntemine göre en yüksek antioksidan aktiviteyi defne uçucu yağı (%79.00) göstermiştir. Bunu, kimyon (%75.98), kekik (%75.81), nane (%69.49), ada çayı (%69.01), tarçın (%68.83) ve biberiye (%63.88) uçucu yağları izlemiştir. Sonuç olarak, bazı tıbbi ve aromatik bitkilerden elde edilen uçucu yağların DPPH serbest radikal yakalama aktivitelerinin Vitamin E ve Trolox'dan önemli düzeyde yüksek olduğu belirlenmiştir (P<0.01).

Anahtar kelimeler: Uçucu yağ, kimyasal kompozisyon, antioksidan aktivite, DPPH

Introduction

In feed or food industry, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) have been widely used to prevent or delay chemical deterioration caused by lipid oxidation. These additives are preferred due to their low cost and high stability over many years. However, consumers are now concerned about the health risks attributed to some of these synthetic antioxidants (namely, BHT and BHA) (Prasad et al., 2009). Thus, natural antioxidants are in high demand because of their health-enhancing and disease-risk-preventing properties (Su et al., 2007).

Due to their antiviral, antibacterial, antifungal and antioxidant properties, plant essential oils are multifunctional (García et al., 2003; Matan et al., 2006; Sukutta et al., 2008; Reichling et al., 2009). Globally, essential oils are increasingly used as natural antioxidants that limit oxidative degradation of lipids. The antioxidant activity of plant essential oils might be related to the presence of hydroxyl groups on their phenolic compounds (Shahidi and Naczk, 2004). The effectiveness of phenolic compounds in retarding lipid oxidation is mainly due to their free radical scavenging

activity, transition metal-chelating potential and/or singlet oxygen-quenching capacity (Shahidi and Naczk, 2004; Carmona-Jimenez et al., 2014).

In recent years, several *in vitro* and *in vivo* studies have been conducted to determine the antioxidant effects of aromatic plants and spices. Extensive *in vitro* studies on different rosemary, oregano, sage and cumin have been performed (Stefanovits-Bányai et al., 2003; Embuscado 2015). Due to the use of plant extracts in most of these studies, information about the antioxidant activities of plant essential oils is limited.

Various *in vitro* methods have been used to measure and compare the antioxidant activities of aromatic plants and spices. 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity is a rapid, simple and inexpensive method and can be applicable to both solid and liquid samples. In addition, this method is not specific to any particular antioxidant component and measures the overall antioxidant capacity of the samples (Carmona-Jimenez et al., 2014).

The present study was conducted to determine the chemical composition and antioxidant activities of cinnamon, cumin, laurel, mint, oregano, rosemary and sage essential oils. The DPPH free radical scavenging activities of these oils were also compared to those of vitamin E and trolox, which were used as reference antioxidants.

Material and Method

Material

In this study, essential oils obtained from cinnamon (*Cinnamomum zeylanicum* L.), cumin (*Cuminum cyminum* L.), laurel (*Laurus nobilis* L.), mint (*Mentha pulegium* L.), oregano (*Origanum onites* L.), rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia triloba* L.) were used. These essential oils were obtained from Ege Lokman Botanical Plant Industry Trade Ltd. Company (Kırkağaç, Manisa, Turkey). The herbs used for steam-distilled essential oils were detailed in Table 1.

Method

Tris hydroxymethyl-aminomethane was purchased from Riedel-de Haën (Steinheim, Germany). Hydrochloric acid (HCl), absolute ethanol, Tween-20 and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Fluka Chemie AG (Steinheim, Germany). Water was purified using the Easy Pure RF UV Small Batch Water System (Barnsteid, USA).

Components of the essential oil were separated on an HP Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer. An HP-Agilent (Nr:19091N-116 Innowax) capillary column (60 m x 0.32 mm, 0.25 µm film thickness) was used directly into the ion source of the MSD. The carrier gas was helium at a constant pressure of 9.05 psi. The owen programming was conducted with an initial temperature of 70°C, a rate of 7°C/minute, a final temperature of 210°C and an injection volume of 1 µl.

In the present study, the chemical compositions of the essential oils of cinnamon, cumin, laurel, mint, oregano, rosemary and sage were identified using gas chromatography–mass spectrometry (GC/MS, HP 6890 GC/5973 MSD) at the Ege University Centre R & D and Pharmacokinetic Applications-Environmental & Food Analysis Laboratories-Food Control Laboratory (Bornova, İzmir, Turkey) according to the United States Pharmacopeia and the National Formulary (USP, 1995).

The free radical scavenging activities of seven essential oils, vitamin E and trolox were determined in accordance with the procedures recommended by Sacchetti et al. (2005). This method involves measuring the scavenging ability of free radicals. This spectrophotometric assay uses the stable radical DPPH as a reagent. DPPH was added into the tubes for analysis as follows: 900 µL of buffer (100 mM Tris-HCl, pH 7.4), 40 µL of solvent (ethanol), 50 µL of emulsifying agent (Tween-20, 0.5%) and 1000 µL of

Table 1. Botanical characteristics of some medicinal and aromatic plants used in the study.

Herbs	Family	Botanical name	Plant part	Collection site
Cinnamon	Lauraceae	*Cinnamomum zeylanicum*	Cortex cinnamomi zeylanici	Seylan
Cumin	Apiaceae	*Cuminum cyminum*	Fructus cumini cymini	Aksehir
Laurel	Lauraceae	*Laurus nobilis*	Folium lauri nobili	Hatay
Mint	Lamiaceae	*Mentha pulegium*	Folium pulegi	Mugla
Oregano	Lamiaceae	*Origanum onites*	Folium origani	Izmir
Rosemary	Lamiaceae	*Rosmarinus officinalis*	Folium rosmarini	Mersin
Sage	Lamiaceae	*Salvia triloba*	Folium salviae trilobae	Izmir

colour compound (0.5 mM DPPH). Next, 10 µL of type 1 pure water (resistivity > 0.1 megohm and conductivity <1 µS) was added into the blank tube, 50 µL of trolox was added into the trolox tube, and 10 µL of each essential oil was added into the test tubes. After 70 minutes of incubation in the dark at room temperature, the absorbance at 517 nm was measured against the blank with a visible spectrophotometer. Vitamin E and trolox were used as positive controls. The values are presented as the mean of five analyses. The radical-scavenging activities of the samples, expressed as per cent inhibition of DPPH (*I%*) were calculated from the absorbance of the blank (A_B) and of the sample (A_A) by following the equation from Carmona-Jimenez (2014).

$I\% = [(A_B - A_A) / A_B] \times 100$

where A_B and A_A are the absorbance values of the blank and essential oil solutions checked after 70 minutes, respectively.

The results are expressed as mean ± standard error (SE) (n=5). Free radical scavenging activities of different essential oils were analysed by one-way ANOVA using the General Linear Model Procedure of SAS (1989). Means were compared using and Dunnett's means test at the 5% significance level.

Results and Discussion

Chemical composition of essential oils

The chemical compositions of the essential oils of cinnamon, cumin, laurel, mint, oregano, rosemary and sage are presented in Table 2.

In our study, it was shown that the major compounds in the essential oil of cinnamon are cinnamaldehyde propylene glycol, cinnamaldehyde and propylene, which amounted to 41.50%, 35.28% and 2.76% of the contents, respectively. Tomaino et al. (2005) determined that the essential oil of cinnamon contains cinnamic aldehyde (67.9%), eugenol (6.72%) and β-caryophyllene (5.03%) at room temperature. In another study, Ünlü et al. (2010) found out that the essential oil from *C. zeylanicum* bark contains (E)-cinnamaldehyde (68.95%), benzaldehyde (9.94%), (E)-cinnamyl acetate (7.44%), limonene (4.42%) and eugenol (2.77%). The level of cinnamaldehyde reported in the present study is lower than that found by Ünlü et al. (2010).

The major compounds in the essential oil of cumin were 44.01% cuminaldehyde, 23.85% *p*-cymene, 8.25% safranal and 8.11% β-pinene. Gachkar et al. (2007) identified the major compounds of the essential oil of *C. cyminum* as α-pinene, limonene, 1,8-cineole and linalool (29.1%, 21.5%, 17.9% and 10.4 %, respectively). The cuminaldehyde content of the essential oil of cumin defined in the present study is lower than reported by Soycan-Onenç and Akkan (2009).

In Table 2, it was shown that the essential oil of laurel is especially rich in 1,8-cineole (39.55%), α-terpinyl acetate (21.72%) and terpinen-4-ol (4.38%). Sangun et al. (2007) determined that the essential oil of *L. nobilis* leaves contains 1,8-cineole (46.61-59.94%), α-terpinyl acetate (11.94-25.70%) and terpinen-4-ol (1.82-2.20%). The current level of 1,8-cineole in laurel was lower than that of Sangun et al. (2007) while α-terpinyl acetate content was in line what Sangun et al. (2007) determined. However, the present terpinen-4-ol content was higher than that of Sangun et al. (2007).

In our study, it was found that the essential oil of mint contains (+)-pulegone (67.80%), isomenthone (15.77%), piperitenone (3.91%), piperitone oxide (1.44%) and menthol (1.43%) as the main compounds. The pulegone content of *M. Pulegium* was reported by Stoyanova et al. (2005) as 42.9-45.4% and by El-Ghorab (2006) as 43.5% and piperitone content of the latter study 12.2% as the major compounds. The pulegone content in mint essential oil determined in the present study is higher than that found by Stoyanova et al. (2005) and El-Ghorab (2006). The level of piperitone was lower than that reported by El-Ghorab (2006).

Tomaino et al. (2005) reported that the major components in oregano oil (*Origanum floribundum* Munby) kept at room temperature are carvacrol (48.9%), thymol (5.03%) and *p*-cymene (11.77%). The major components of the essential oil of oregano in this study were carvacrol, thymol, and *p*-cymene (59.03%, 12.04% and 6.37%) respectively. The carvacrol content reported in this study is higher than that reported by Tomaino et al. (2005).

The major components of the essential oil of rosemary were characterised by Gachkar et al. (2007) as piperitone, α-pinene, linalool, 1,8-cineole, camphor, borneol, camphene and bornyl acetate; and by Verma et al. (2010) as camphor, α-pinene,1,8-cineole, borneol, verbenone, linalool, limonene, *exo*-bornyl acetate, terpinen-4-ol and camphene. Soycan-Onenç and Akkan (2009) reported that the major components in rosemary essential oil are 1,8-cineole (15-30 and 14.34%), camphor (5-10 and 23.54%) and borneol (10-20 and 26.16%). In our study, the essential oil of rosemary

Table 2. Chemical composition of the essential oils obtained from some medicinal and aromatic plants.

Cinnamon		**Cumin**		**Laurel**		**Mint**	
Compounds	%	Compounds	%	Compounds	%	Compounds	%
Cinnamaldehyde propylene glycol acetal	41.50	Cuminaldehyde	44.01	1,8 Cineole	39.55	(+) Pulegon	67.80
Cinnamaldehyde	35.28	p- Cymene	23.85	α- Terpinyl acetate	21.72	Isomenthon	15.77
Propylene glycol	2.76	Safranal	8.25	Terpinen-4-ol	4.38	Piperitenone	3.91
Unidentified	19.66	β- Pinene	8.11	β-Phellandrene	3.70	Piperitone Oxide	1.44
		γ-Terpinene	2.11	p-Cymene	3.43	Menthol	1.43
		Carotol	2.00	α- Pinene	3.16	α- Pinene	1.05
		(-)α- Cedren	1.93	Eugenol metyl ether	3.01	β- Pinene	1.00
		Carvacrol	1.86	β- Pinene	2.80	Piperitone	0.77
		Anethol	1.65	α-Terpineole	2.05	Neomenthol	0.74
		Cuminic Alcohol	1.52	Linalool	1.99	Limonen	0.73
		α- Thujen	0.75	Euganol	1.91	Amyl Vinyl Carbinol	0.55
		Limonen	0.58	Limonen	1.63	Spathulenol	0.49
		α- Terpineole	0.57	Gamma Terpinen	1.62	α-Terpineole	0.34
		p- Cymene-8- ol	0.41	Cis α-Bisabolene	1.09	β-Phellandrene	0.33
		Others	1.24	Others	5.80	Others	0.73
		Unidentified	1.15	Unidentified	2.16	Unidentified	2.91
Total	100		100		100		100

Oregano		**Rosemary**		**Sage**	
Compounds	%	Compounds	%	Compounds	%
Carvacrol	59.03	1,8 Cineole	30.12	(+) Camphor	17.15
Thymol	12.04	α- Pinene	12.80	1,8 Cineole	14.84
p-Cymene	6.37	(+)Campher	12.75	β-Thujon	8.01
Γ- Terpinen	3.86	(+) Borneol	6.38	Caryophyllene	6.53
Linalool	2.73	Caryophyllene	5.48	α- Humulene	5.98
(+) Borneol	2.09	Camphene	4.06	(+) Borneol	5.34
Terpinen-4-ol	1.83	Terpineole	3.99	α- Pinene	5.01
B-Caryophyllene	1.67	p-Cymene	2.81	Carvacrol	3.71
B- Bisabolene	1.58	(+)-Limonen	2.33	Camphene	3.60
A-Terpinen	1.24	L-Bornylester	1.79	Viridiflorol	3.37
Sabinen	1.03	Carvacrol	1.61	L-Bornylester	3.26
α- Humulene	0.83	Linalool	1.49	α- Thujon	3.10
α- Thujon	0.74	β- Pinene	1.16	α- Pinene	2.20
α- Terpineol	0.65	Myrcen	1.04	p-Cymene	2.12
Others	4.47	Others	8.42	Others	10.957
Unidentified	0.32	Unidentified	3.43	Unidentified	4.77
Total	100		100		100

mainly comprised 1,8-cineole (30.12%), α-pinene (12.80%), (+) camphor (12.75%), (+) borneol (6.38%) and caryophyllene (5.48%), respectively. Similar to our findings, Fu et al. (2007) reported that the major components of the essential oil of this plant are 1,8-cineole (48.5 and 27.23%), α-pinene (15.4 and 19.43%) and camphor (10 and 14.26%). As shown in Table 2, (+) camphor, 1,8-cineole, β-thujone, caryophyllene, α-humulene, (+) borneol and α-pinene are the major compounds in the essential oil of sage, at 17.15, 14.84, 8.01, 6.53, 5.98, 5.34 and 5.01%, respectively. Soycan-Onenç and Akkan (2009) reported that the major components of the essential oil of *S. triloba* are 1,8-cineole (34.40%), camphor (18.40%), camphene

(8.80%), α-pinene (7.90%), borneol, α-terpinyl acetate (4.80%) and β-pinene (4.40%).

Antioxidant activity of essential oils and vitamin E

The DPPH free radical scavenging activities in the essential oils of cinnamon, cumin, laurel, mint, oregano, rosemary and sage are shown in Table 3.

Table 3. DPPH-free radical scavening activities of the essential oils and vitamin E (X±SE).

The source of essential oils	Inhibition, %
Medicinal and aromatic plants	
Sage	69.01 ± 0.30^{c}
Rosemary	63.88 ± 1.29^{d}
Laurel	79.00 ± 0.54^{a}
Oregano	75.81 ± 1.65^{b}
Cumin	75.98 ± 1.16^{b}
Mint	69.49 ± 1.47^{c}
Cinnamon	68.83 ± 0.88^{c}
Control substances	
Vitamini E	22.08 ± 0.85^{f}
Trolox	28.79 ± 0.57^{e}
Probablitiy (*P value*)	0.0001

[a-f]:Means within a column with different superscripts differ significantly (P<0.05).

There were significant differences in the free radical scavenging activities between essential oils investigated in this study (P<0.01). Among the investigated essential oils, laurel had the highest free radical scavenging activity at 79±0.54%, while rosemary essential oil showed the lowest free radical scavenging activity at 63.88±1.29%. The difference in inhibition between the essential oil of cumin (75.98±1.16%) and that of oregano (75.81±1.65%) was not statistically significant. A similar situation was found for the essential oils of mint, sage and cinnamon; inhibition levels of these essential oils were 69.49±1.47%, 69.01±0.30% and 68.83±0.88%, respectively. The free radical scavenging activities of trolox and vitamin E were determined to be 28.79±0.57% and 22.08±0.85%.

In this study, the highest DPPH free scavenging activity was found for the essential oil of laurel, which contains approximately 40% 1,8-cineole. In accord with our findings, Politeo et al. (2006) also found that the essential oil of *L. nobilis* (with a major component of 1,8-cineole) showed strong antioxidant activity.

The essential oil of cumin (75.98±1.16%) demonstrated the next highest antioxidant activity (Table 3). Lu et al. (2011) found that *C. cyminum* extract has low antioxidant capacity (18.12%). This result was probably due to the low total phenolic content of the cumin extract used (9.00±0.15 mg GAE/g DW) in their study.

While the essential oil of oregano had similar DPPH inhibition levels to the essential oil of cumin, showing significantly higher antioxidant activity than those of mint, sage, cinnamon and rosemary.

DPPH inhibition of the essential oil of mint was significantly higher than that of the essential oil of rosemary only (Table 3). In a previous study, Mata et al. (2007) determined that water extracts of *M. spicata* and *M. pulegium* (IC_{50}=5.7 and 8.9 μg/ml, respectively) have higher DPPH radical scavenging activities than the ethanol extracts of these species (IC_{50}=65.2 and 24.9 μg/ml, respectively), the water and ethanol extracts of *R. officinalis* (IC_{50}=37.3 and 36.0 μg/ml respectively) and BHT (IC_{50}=15.7 μg/ml). Kamkar et al. (2010) found that water and methanol extracts from *M. pulegium* have similar free radical scavenging activities to BHT but higher free radical scavenging activities than the essential oil of *M. pulegium*. These researchers noted that the lower antioxidant activity in the essential oil of *M. pulegium* could be due to the lower rate of presence of different antioxidants in the essential oil.

In our study, it was found that the essential oil of sage had a significantly higher DPPH inhibition level than the essential oil of rosemary. Dorman et al. (2003) determined that there was no difference in the DPPH radical scavenging activities between the aqueous extracts of *R. officinalis* and *S. officinalis* (IC_{50}=236.5 μg/ml and 265.8 μg/ml, respectively). These plants' extracts showed greater anti-radical effects than that from the aqueous extract of *O. vulgare* (IC_{50}=335 μg/ml).

This study showed that the highest anti-radical activity was in the essential oil of laurel, which contains approximately 40% 1,8-cineole. Many essential oils isolated from various plant species belonging to different genera contain relatively high amounts of oxygenated monoterpenes such as borneol, borneol acetate, camphor, carvone, 1,8-cineole, linalool, linalool acetate, limonene oxide, menthol, menthone, terpinen-4-ol, *α*-terpineol (Kotan et al. 2007), which has demonstrated poor ability of inhibiting oxidation (Cherrat et al. 2014). This high antioxidant activity in the essential oil of laurel can be attributed to the presence of monoterpene hydrocarbons, which was the α-terpinyl acetate (21.72%).

Prasad et al. (2009) reported that the DPPH radical scavenging activities of cinnamon leaf extracts from

five species increased with increasing concentration, and the highest DPPH radical scavenging activity was observed in *C. zeylanica* (92.1%) at 100 µg/ml. Moreover, in the studies carried out by Muchuweti et al. (2007), Dudonné et al. (2009) and Lu et al. (2011) *C. zeylanica* extract showed a high antioxidant capacity, with mean values of 84.43, 92 and 87.45%, respectively, for DPPH inhibition. It was hypothesised that the DPPH radical scavenging activities of cinnamon species may result from the hydrogen-donating ability of phenols and flavonoids (Prasad et al., 2009). The DPPH inhibition levels for cinnamon reported by these authors are greater than we determined in our study. The discrepancy in the results may be due to the use of extracts instead of essential oils in these four studies. In addition, the type of extracting solvent might affect the antioxidant activities of spices and herbs, as reported by Su et al. (2007).

Among the examined essential oils, it was determined that the lowest antioxidant capacity was found for the essential oil of rosemary (Table 3). In an earlier study carried out by Sacchetti et al. (2005), it was observed that the DPPH free radical scavenging activity of the essential oil of *R. officinalis* was lower than that of the essential oil of *T. vulgaris* and approximately two times higher than that of trolox. The inhibition values for the essential oil of rosemary essential oil were similar to each other.

The major components of the essential oil of rosemary (1,8-cineole, camphor, camphene, borneol, borneol acetate, linalool, limonene, α-pinene, piperitone, verbenone) were different from those of rosemary extracts (carnosol, carnosic acid, rosmanol, rosmarinic acid, naringin, hispidulin, cirsimaritin, caffeic acid, vanillic acid, apigenin) (Luis and Johnson, 2005). Carnosol, carnosic acid and rosmarinic acid were the most active antioxidant components (Wei and Ho, 2006), while 1,8-cineol, α-pinene, camphor and verbenone are the most active antimicrobial components (Moghtader and Afzali, 2009) in rosemary. In our study, the essential oil of rosemary rich in 1,8-cineole (30.12%) content exhibited the lowest antioxidant activity, which supports the above statements.

Conclusion

In this study, chemical compositions and *in vitro* antioxidant activities of essential oils in some medicinal and aromatic plants widely used in food or feed industry were investigated. The DPPH radical scavenging activities of the essential oils were ranked as followings: laurel > cumin ≈ oregano > mint ≈ sage ≈ cinnamon > rosemary. It is important that these results sould be checked by further *in vivo* studies, especially for the feed industry, in terms of defining the availability of essential oils.

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