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In Vitro Antioxidant Activities, Total Phenolic Contents and Main Phenolic Compounds of Essential Oil Blend and Grape Seed Extract

Esansiyel Yağ Karışımı ve Üzüm Çekirdeği Ekstraktının *In vitro* Antioksidan Aktiviteleri, Toplam Fenolik Madde İçerikleri ve Başlıca Fenolik Bileşenleri

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Key Words:

Essential oil, Grape seed, DPPH, TEAC, Total phenolic content, Phenolic compounds

ABSTRACT

Objective: This study was conducted to assess antioxidant activities, total phenolic contents and main phenolic compounds of essential oil blend (EOB) and grape seed extract (GSE).

Material and Methods: The antioxidant activities of EOB (composed of oregano, clove and cumin essential oils) and GSE were determined by *in vitro* methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and trolox equivalent antioxidant capacity (TEAC). The total phenolic contents of EOB and GSE were determined by the Folin-Ciocalteu method and calculated as gallic acid equivalents (GAE). The main phenolic compounds of EOB were calculated from the individual essential oils compounds analyzed by GC/MS. The condensed tannin concentration of GSE was measured by the butanol/HCl method.

Results: The antioxidant activities of EOB and GSE were determined by two different *in vitro* methods provided the values of 79.0 % and 74.7 % for DPPH, and 276.51 µM/100g and 83.0 µM/100g for TEAC, respectively. The total phenolic contents of EOB and GSE were 437.84 mg GAE/g and 175.50 mg GAE/g, respectively. The main phenolic compounds of the EOB were carvacrol (42.08 %), thymol (4.17 %), eugenol (22.38 %), cuminaldhyde (5.04 %) and safranal (2.69 %). The condensed tannin concentration in GSE was 45.88 g/100g.

Conclusion: According to results obtained this study, EOB and GSE have antioxidant potential. However, EOB showed higher total phenolic content and antioxidant activity determined by two methods (DPPH radical scavenging activity and trolox equivalent antioxidant capacity) than GSE. The results obtained by both methods are compatible and quite similar. It is necessary to support these *in vitro* results with *in vivo* studies.

Anahtar Kelimeler:

Esansiyel yağ, Üzüm çekirdeği, DPPH, TEAC, Toplam fenolik madde içeriği, Fenolik bileşenler

ÖZ

Amaç: Bu çalışma esansiyel yağ karışımı (EYK) ve üzüm çekirdeği ekstraktının (ÜÇE) iki farklı yöntemle antioksidan aktivitelerini, toplam fenolik madde içerikleri ile başlıca fenolik bileşenlerini saptamak amacıyla yürütülmüştür.

Materyal ve Metot: EYK (kekik, karanfil, kimyon karışımı) ve ÜÇE'nin antioksidan aktiviteleri 2.2-difenil-1-pikrilhidrazi hidrat (DPPH) radikal süpürme aktivitesi ve troloks eşdeğeri antioksidan kapasitesi (TEAC) olmak üzere *in vitro* yöntemlerle belirlenmiştir. EYK ve ÜÇE'nin toplam fenolik madde içerikleri Folin-Ciocaltaeu yöntemine göre saptanmıştır ve gallik asit eş değeri (GAE) olarak hesaplanmıştır. EYK'nın başlıca fenolik bileşenleri her bir esansiyel yağın GC/MS verileri esas alınarak hesaplanmıştır. ÜÇE'nin kondanse tanen konsantrasyonu butanol-HCl yöntemi ile saptanmıştır.

Bulgular: EYK ve ÜÇE'nin iki farklı *in vitro* yönteme göre belirlenen antioksidan aktiviteleri DPPH için sırasıyla % 79.0 ve % 74.7, TEAC için sırasıyla 276.51 µM/100g ve 83.0 µM/100g'dır. EYK ve ÜÇE'nin toplam fenolik madde içerikleri sırasıyla 437.84 mg GAE/g ve 175.50 mg GAE/g'dır. EYK'nın başlıca fenolik bileşenleri karvakrol (% 42.08), timol (% 4.17), ojenol (% 22.38), kuminaldehit (% 5.04) ve safranal (% 2.69)'dir. ÜÇE'nin yapısında kondanse tanen konsantrasyonu 45.88 g/100g'dır.

Sonuç: Bu çalışmadan elde edilen bulgulara göre EYK ve ÜÇE antioksidan potansiyele sahiptir. Ancak EYK'nın toplam fenolik madde içeriği ve iki yöntemle (DPPH radikal süpürme aktivitesi ve troloks eşdeğeri antioksidan kapasitesi) belirlenen antioksidan aktivitesi ÜÇE'ye göre daha yüksektir. Her iki yöntemle elde edilen sonuçlar birbirleri ile uyumlu ve oldukça benzerdir. Bu *in vitro* sonuçlar *in vivo* çalışmalarla desteklenmelidir.

INTRODUCTION

Herbs and spices, as well as products derived thereof, are mainly comprised of essential oils and extracts, which are the most important targets to search for produce functional food with specific health effects and improve the quality and nutritional value of food (Kahkönen et al. 1999). Also, they have been receiving a lot of attention as feed additives in animal nutrition due to decrease the detrimental effects of oxidative stress, and to decrease oxidative deterioration in animal products such as egg and meat (Basmacıoğlu-Malayoğlu et al. 2011a).

The essential oils from a number of herbs and spices have been confirmed to possess diverse biological properties including antioxidant activity (Bozin et al. 2007; Tepe et al. 2004; Wei and Shibamoto, 2007). The antioxidant activities of essential oils from oregano (Han et al. 2017), laurel, rosemary, sage, coriander (Baratta et al. 1998; Yashin et al. 2017) anise, clove (Basmacıoğlu-Malayoğlu et al. 2011a; Yashin et al. 2017), cumin (Thippeswamy and Akhilender Naidu, 2005) are well documented. Essential oils exhibited potent antioxidant activities (Peschel et al. 2006) due to their redox properties by acting as reducing agents, hydrogen donators, singlet oxygen quenchers (Brannan and Mah, 2007) and binding metal chelation. However, there is limited research on antioxidant activity of the blend of essential oils.

In recent years, there is a growing interest in agro-industrial by products due to their antioxidant potential (Aktaş et al. 2013). Grape seed extract is a by-product derived from the grape seeds that obtained from wine and grape juice processing. The proanthocyanidins (condensed tannins) and oligomers of flavan-3-ol units, especially catechin and epicatechin present in grape seed extract (Lau and King, 2003).

The aim of the present study was to determine the total phenolic contents and main phenolic compounds from EOB and GSE and to evaluate the antioxidant activities by two common methods (DPPH and TEAC) for utilization as natural antioxidants in feed, food and pharmaceutical industries. This *in vitro* study has also planned to shed light on our *in vivo* further study in broiler.

MATERIAL and METHOD

Plant Material

The plant material consisted of leaves, flower buds and fruits (seed). The EOB was composed of 56.25 % oregano (*Origanum onites*), 28.75 % clove (*Syzygium aromaticum*) and 15 % cumin (*Cuminum cyminum*) oils, which were obtained by using steam distillation method and manufactured by commercial firms. This mixture was chosen according to results of the *in vitro* study (Basmacıoğlu-Malayoğlu et al. 2011b). The essential oils were mixed together by homogeniser in the laboratory and stored at 4 ºC in airtight containers. Grape (*Vitis vinifera* L.) specimens named as Antep Karası collected from Gaziantep location. Ethanol extract of grape seed was obtained from commercial firm (Edremit-Balıkesir, Turkey).

Method

Determination of Main Phenolic Compounds of EOB and GSE

EOB compounds were calculated from the individual essential oils compounds analyzed by GC/MS (HP 6890GC/5973 MSD) system (Basmacıoğlu-Malayoğlu et al. 2011b).

Butanol-HCl method was used for determination of condensed tannin of GSE (Makkar, 1995). 0.01 gram of samples was weighed in tubes and 6 ml butanol-HCl reagent (95 ml butanol + 5 ml HCl + 1 g Fe_2SO_4) was added. The tubes were then placed into boiling water bath and heated 100 ºC for an hour, then cooled. The tubes were centrifuged at 3000 X g for 100 minutes. The absorbance was read at 550 nm by using spectrophotometer (Amersdam 2100 UV, UK).

Determination of Total Phenolic Contents and Antioxidant Activities of EOB and GSE

Total phenolic contents of EOB and GSE were determined by Folin-Ciocalteu method described by Dorman et al. (2003) with some modifications. Briefly, 10 µl aliquot of oil or extract sample was added in a tube containing Milli-Q water (final volume 10 ml). Then 500 µl of Folin-Ciocalteu's reagent was added. Finally, 1.5 ml of saturated sodium carbonate solution was added, mixed and left to stand at room temperature for an hour. The absorbance solution was read at 760 nm by using a spectrophotometer (Amersdam 2100 UV, UK). A standard curve was prepared by gallic acid and the results were given as mg GAE per gram of essential oil or extract.

The antioxidant activities of EOB and GSE were evaluated by DPPH and TEAC methods. DPPH method was carried out as described by Amarowicz et al. (2004) with minor modifications. EOB and GSE were dissolved in 4 ml of methanol and then added to 1 mM methanolic solution of DPPH• (final volume 4.5 ml). The contents were mixed for 15 seconds and then left to stand at room temperature for 30 min. The absorbance of the solution was read against methanol at 517 nm by using a spectrophotometer (Amersdam 2100 UV, UK). The radical scavenging activity (RSA) calculated according to the equation below;

$$\% RSA = 100 \times (1 - A_E/A_D)$$

A_E: the absorbance of the solution containing antioxidant essential oil or extract.

A_D: the absorbance of the DPPH• solution.

TEAC method was carried out as described by Re et al. (1999) with slight modifications. ABTS [2', 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium] is dissolved in water to a concentration of 7 mM and reacted with 2.45 mM potassium persulfate at a molar ratio of 2:1 to form the ABTS• radical, left in the dark room overnight for 16 hours. Stock solutions of extract, essential oil blend, and trolox were prepared in ethanol. The ABTS• solution was diluted with ethanol until an absorbance of 0.70 ± 0.02 AU at 734 nm was reached. After addition of 1.9 ml of diluted ABTS• solution to 10 µl GSE, EOB or trolox standards (final concentration 5-25 µM) in ethanol the absorbance reading was taken at 1 min after initial mixing and up to 6 min. The results are expressed as µM Trolox per 100 g of sample.

Stastical Analysis

Statistical analysis of the data was determined by Student's t-test. A probability value of $P<0.05$ was considered to denote a statistically significant difference. Data were given as the mean ± standard deviation (SD).

RESULTS

The EOB including carvacrol (42.08 %), thymol (4.17 %), eugenol (22.38 %), cuminaldhyde (5.04 %) and safranal (2.69 %) as active compounds were composed of three totally different essential oils (oregano oil, clove oil and cumin oil). The condensed tannin in GSE was determined as 45.88 g/100g (Table 1).

The total phenolic contents and antioxidant activities of EOB and GSE for each method are shown in Table 2. EOB and GSE total phenolic contents determined by the Folin-Ciocalteu method to be 437.84 mg GAE/g and 175.50 mg GAE/g, respectively. According to TEAC method, EOB (276.51 µM trolox/100g) exhibited highest antioxidant activity than GSE (83.0 µM trolox/100g). According to DPPH method, EOB and GSE DPPH radical scavenging activity ranged from 79.0 % to 74.7 %, respectively (Figure 1).

Table 1. Main phenolic compound/compounds of EOB and GSE

Çizelge 1. *EYK ve ÜÇE'nin başlıca fenolik bileşen/bileşenleri*

	Phenolic compounds (%)					
EOB	**Eugenol**	**Carvacrol**	**Thymol**	**Cuminaldhyde**	**Safranal**	**Others**
	22.38	42.08	4.17	5.04	2.69	23.64
GSE	**Condensed tannin (g/100g)**					
	45.88					

Table 2. Total phenolic contents and antioxidant activities of EOB and GSE[1]

Çizelge 2. *EYK ve ÜÇE'nin toplam fenolik madde içerikleri ve antioksidan aktiviteleri*[1]

	Total phenolic content[2]	**DPPH Radical scavenging activity**[3]	**TEAC**[4]
EOB	437.84 ± 2.50	79.00 ± 1.45	276.51 ± 1.58
GSE	175.5 ± 2.36	74.7 ± 1.08	83.0 ± 1.56

[1] Each value corresponds to the mean and standard deviation (n=3).
[2] Data of total phenolic contents are expressed as milligrams of GAE per gram essential oil or extract.
[3] Data of DPPH Radical scavenging activity are expressed as %.
[4] TEAC are expressed as micromoles of Trolox equivalents per 100 gram essential oil or extract.

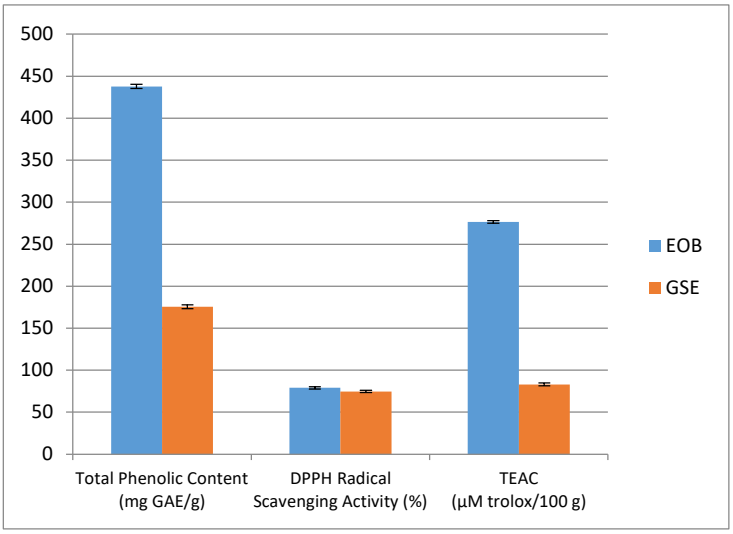

Figure 1. Bar graph illustrating total phenolic contents and antioxidant activities (Mean±SD)

Şekil 1. *Toplam fenolik madde içerikleri ve antioksidan aktiviteleri gösteren sütun grafiği (Ortalama±SD)*

DISCUSSION and CONCLUSION

In this study, the total phenolic content of EOB was determined 437.84 mg GAE/g. According to Rocha-Guzman et al. (2006), total phenolic content of oregano essential oil was 151 mg GAE/ml. Zheng and Wang (2001) studied with various herbal extracts. They found that Greek mountain oregano and Mexican oregano had higher total phenolic content 11.8 mg GAE/g and 17.51 mg GAE/g, respectively. Shan et al (2005) investigated the total phenolic content and total antioxidant capacity of 26 spices from 12 botanical families as determined by Folin-Ciocalteu method and TEAC. They found that the cloves, cinnamon and oregano were the three spices with the highest values. It is clear that oregano, clove and cumin oils contain phenolic compounds such as thymol, carvacrol, eugenol and cuminaldhyde and hence their antioxidant activity could be due to these compounds. In our previous *in vitro* study (Basmacıoğlu-Malayoğlu et al. 2011a), clove essential oil alone exhibited highest TEAC and DPPH value 421 µM trolox/100g and 98.32 %, respectively. Oregano essential oil alone showed TEAC activity 225 µM trolox/100g and DPPH radical scavenging activity 70.67 %. Cumin essential oil alone exhibited lowest antioxidant activity for TEAC (8.3 µM trolox/100g) and DPPH (27.50 %). According to these results, EOB presented a lower TEAC (276.51 µM trolox/100g) and DPPH value (79 %) than clove essential oil alone. However, EOB exhibited highest TEAC and DPPH values than oregano and cumin essential oils alone. The results suggested that the antioxidant activity is depend on the clove essential oil content. The findings obtained this study were in agreement with the findings of the study (Baj et al. 2018) who determined the antioxidant activity of basil, marjoram and rosemary essential oils and their blends. They suggested that basil, marjoram and rosemary blend of essential oils antioxidant activity is depend on the marjoram essential oil content which exhibited the highest antioxidant activity as 87.9 % according to DPPH method.

In this study, the total phenolic content of GSE (Antep Karası) was found 175.50 mg GAE/g. However, Göktürk-Baydar et al. (2006) reported that the total phenolic content of grape seed extracts collected from different location of Turkey were 589.09 mg GAE/g (Hasandede), 506.60 mg GAE/g (Emir) and 549.54 mg GAE/g (Kalecik Karası). The significant differences between the results were likely due to genotypic and environmental differences within species, choice of parts tested, time of taking samples and determination methods (Yesil-Celiktas et al. 2007).

The results from this study showed that EOB and GSE have potent antioxidant activity. However, EOB has higher total phenolic content and antioxidant activity determined by two methods (DPPH and TEAC) than GSE. The results obtained by both methods are compatible and quite similar. It is necessary to support these in vitro results with in vivo studies.

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