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Bazı Bitki Türlerinden Elde Edilen Uçucu Yağların Toplam Fenol İçerikleri ve Antioksidan Aktiviteleri

Total phenolic contents and antioxidant activities of the essential oils from some plant species

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Anahtar Sözcükler:

Uçucu yağlar, antioksidan aktivite, DPPH, TEAC

ÖZET

Bu çalışmada, anason *(Pimpinella anisum),* biberiye (*Rosmarinus officinalis),* defne (*Laurus nobilis),* karanfil *(Syzygium aromaticum),* kekik (*Oreganum onites* spp.) ve kimyon (*Cuminum cyminum)* bitkilerinden buhar distilasyonu yöntemi ile elde edilen uçucu yağların radikal süpürme kapasite ve antioksidan aktivitelerinin araştırılması amaçlanmıştır. Uçucu yağların radikal süpürme kapasiteleri ile antioksidan aktiviteleri toplam fenol (TPA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical süpürme aktivitesi ve Troloks eşdeğeri antioksidan kapasitesi (TEAC) gibi *in vitro* yöntemlerle belirlenmiştir. Elde edilen bulgulara göre karanfil ve kekik uçucu yağları yüksek eugenol ve karvakrol içeriklerine bağlı olarak en yüksek radikal süpürme aktivite (%98.32, 70.67) ve antioksidan kapasite (421, 225 µM troloks/100g kuru örnek) göstermiştir. Bununla birlikte, toplam fenol ve DPPH (r = 0.97), toplam fenol ve TEAC (r = 0.99) değerleri arasında yüksek korelasyon saptanmıştır. Sonuç olarak, bu çalışma karanfil>kekik>defne>biberiye>kimyon>anosan etkililik sırasında uçucu yağların antioksidan potansiyellerini göstermiştir. Özellikle, karanfil ve kekik uçucu yağları yüksek antioksidan aktivitelerinden dolayı ilaç, gıda sanayisi ve hayvan beslemede doğal antioksidan ajanlar olarak kullanılabilir.

Key Words:

Essential oils, antioxidant activity, DPPH, TEAC

ABSTRACT

This study was designed to investigate the radical scavenging and antioxidant activities of the essential oils obtained by the steam distillation process from anise (*Pimpinella anisum),* rosemary (*Rosmarinus officinalis),* laurel (*Laurus nobilis),* clove *(Syzygium aromaticum),* oregano (*Origanum onites* spp.) and cumin (*Cuminum cyminum*). The radical scavenging capacities and antioxidant activities of essential oils were determined by *in vitro* assays such as total phenol assay (TPA), 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity and Trolox equivalent antioxidant capacity (TEAC). According to results obtained, clove essential oil and oregano essential oil exhibited the highest radical scavenging activity (98.32, 70.67%) and the antioxidant capacity (421, 225 µM trolox/100g dry sample) due to the high content of eugenol and carvacrol, respectively. Additionally, obtained data revealed excellent correlations between the total phenol and DPPH (r = 0.97), total phenol and TEAC values (r = 0.99). In conclusion, clove and oregano essential oils can be used as natural antioxidant agents due to their high antioxidant activities in pharmaceutical, food industries and animal nutrition.

INTRODUCTION

There is a growing interest in natural additives because of the world-wide trend towards the usage in production of food, cosmetics and pharmaceuticals. Herbs and spices, as well as products derived thereof, are mainly comprised of extracts and essential oils, which are the most important targets to search for natural antioxidant and antimicrobials. Also, these phytogenic products from natural sources due to their antimicrobial and antioxidant properties have been receiving a lot of attention as feed additives in animal nutrition (Basmacıoğlu et al., 2004; Hernández et al., 2004). The aims of using these natural feed additives as antioxidants are to minimize or decrease the detrimental effects of oxidative stress, to produce antioxidant rich functional foods, to decrease oxidative deterioration in animal products such as egg and meat.

Lipid deterioration, oxidation and spoilage by microorganisms, are major causes of food spoilage. It is responsible for undesirable off-flavors, deterioration of the color, texture, with decrease in nutritional quality and safety by formation of toxic products.

These phytogenic additives that are rich in antioxidant compounds are attractive to the food, serving as an alternative to the synthetic antioxidants, such as traditionally applied buthylated hydroxyanisole (BHA) and hydroxytoluene (BHT), which might exhibit carcinogenic effects in living organisms. Furthermore, animal nutritionists investigate new phytogenic compounds as alternatives to α-tocopheryl acetate commonly added to diet in poultry nutrition.

Essential oils are obtained from plants or from part thereof by steam or hydro-distillation and organic-solvent extraction methods. Most essential oils consist variable mixtures of terpenoids (monoterpenes [C10], sesquiterpenes [C15] and diterpenes [C20]), low molecular weight aliphatic hydrocarbons, alcohols, aldehydes, acyclic esters or lactones and exceptionally nitrogen- and sulphur-containing compounds, coumarins and homologues phenylpropanoids (Bozin et al., 2006). The antioxidant activity of essential oils is assigned to terpenes and phenolic compounds (Ruberto and Baratta, 2000). This activity is mainly due to their redox potantial, which can play an important role in absorbing and neutralizing free radicals, quenching reactive oxygen species, and chelating metal, especially iron and copper cations (Balasundram et al., 2006).

The essential oils from a number of herbs and spices have been confirmed to possess antimicrobial (Balasundram et al., 2006; Bozin et al., 2006; Dorman and Deans, 2000; Ruberto and Baratta, 2000) and antioxidant activities (Baratta et al., 1998; Bozin et al., 2007; Tepe et al., 2004; Wei and Shibamoto, 2010) in literature. It should be noted that the extract and the essential oil of a particular plant might exhibit totally different biological activities; for instance the extract might have high antioxidant activity but a very poor antimicrobial activity or the essential oil might show a high antimicrobial activity but a very low antioxidant activity. Therefore, it is of prime importance to obtain bio-additives from plant materials exhibiting multi-bioactivities (potential multi-purpose functional use) so that when these additives are incorporated to the final product, the functionality will be enhanced while providing economically sound solutions. In addition, the evaluation of total antioxidant capacity of these bio-additives cannot be performed accurately by any single method due to the complex nature of phytochemicals. For this purpose, multiple-tests must be taken into account whenever assays of essential oils are performed to allow functional properties. Many methods have been proposed to evaluate the antioxidant potential of natural sources of antioxidants. Among methods, Trolox equivalent antioxidant capacity (TEAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) are useful for determining the activity of both lipophilic and hydrophilic species. Taking this into account, the in vitro antioxidant activity of 6 essential oils tested was determined by three different methods, namely, DPPH, TEAC and total phenol assays.

The aim of this study were to: (1) determine the radical scavenging capacity and antioxidant activities of anise, rosemary, laurel, clove, oregano and cumin essential oils by three common antioxidant activity methods; (2) investigate the relationship between antioxidant activity and phenolic compounds or antimicrobial activity of essential oils, which were determined in one of our previous studies (Basmacıoğlu-Malayoğlu et al., 2011), and relationship between antioxidant activity methods and total phenol content.

MATERIALS AND METHODS

Essential oils

Cumin and clove essential oils were obtained from Özdrog Company (Hatay, Turkey), laurel and anise essential oils from İnan Agriculture Company (Antalya, Turkey), oregano essential oil from Türer Agriculture Company (İzmir, Turkey), rosemary essential oil from Set Agriculture Company (Aydın, Turkey). The herbs used for steam-distilled essential oils were characterizated in Table 1.

Table 1. Characterization of the herbs used for steam-distilled essential oils .

Herbs	Family	Botanical name	Plant part	Collection site
Anise	Umbelliferae	*Pimpinella anisum*	Fruits (Seeds)	Burdur
Rosemary	Labiatae	*Rosmarinus officinalis*	Leaves	Mersin
Laurel	Lauraceae	*Laurus nobilis*	Leaves	Hatay
Clove	Myrtaceae	*Syzygium aromaticum*	Flower bud	Madagascar
Oregano	Labiatae	*Origanum onites spp.*	Leaves	İzmir
Cumin	Umbelliferae	*Cuminum cyminum*	Fruits (Seeds)	Sultandağı-Akşehir

The essential oils of anise (*P. anisum)*, rosemary (*R. officinalis),* laurel (*L. nobilis),* clove *(S. aromaticum)*, oregano (*O. onites* ssp.) and cumin (*C. cyminum)* were screened for radical scavenging and antioxidant activities by various *in vitro* assays.

Antioxidant Assays

Total Phenol Assay (TPA)

The total phenols in the essential oils were determined by Folin-Ciocalteu method described by Dorman et al. (2003) with some modifications. Briefly, 10 µl aliquot of oil samples was added into a tube containing Milli-Q water (final volume 10 ml). Then 500 µl of Folin-Ciocalteu's reagent (Merck, UN3264)) was added and the solution is stirred vigorously by vortex and left to stand for 5 minutes. Finally, 1.5 ml of saturated sodium carbonate (Carlo Erba, CE367 707) solution is added, stirred vigorously for the last time and left to stand at room temperature for an hour. Absorbance was determined spectrophotometrically (Amersdam 2100 UV spectrophotometer, UK) at 760 nm. A standard curve was prepared by gallic acid which was further used to in quantifying the concentration of total phenols in the samples. Determination of total phenols was carried out in duplicate, the results are mean values and given as gallic acid equivalent (GAE) per gram of sample.

DPPH radical scavenging activity (RSA) assay

DPPH (2,2-diphenyl-1- picrylhydrazyl assay was carried out as described by Amarowicz et al. (2004) with minor modifications. This radical serves as the oxidizing radical to be reduced by the antioxidant (AH) and as the indicator for the reaction $DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet}$ (Re et al., 1999). Essential oils were dissolved in 4 ml of methanol and then added to 1 mM methanolic solution of $DPPH^{\bullet}$ (Sigma-Aldrich, D9132) (final volume 4.5 ml). The contents were stirred vigorously for 15 sc. and then left to stand at room temperature for 30 min. Decrease in colorization was measured spectrophotometrically (Amersdam 2100 UV spectrophotometer, UK) at 517 nm. The radical scavenging activity (RSA) was carried out in duplicate and calculated using the equation below;

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

A_E is the absorbance of the solution containing antioxidant extract whereas A_D is the absorbance of the $DPPH^{\bullet}$ solution.

Trolox equivalent antioxidant capacity (TEAC) Assay

TEAC assay was carried out as described in a protocol by Re et al. (1999) with slight modifications. This method is based on the reaction between ABTS [2', 2'- azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium] and potassium persulfate giving blue/green ABTS radical ($ABTS^{\bullet}$). With the addition of the antioxidants, decolorization is attained and measured spectrophotometrically at 734 nm. The results are expressed as µM Trolox per 100 g of sample. ABTS (Sigma, A1888) is dissolved in water to a concentration of 7 mM and reacted with 2.45 mM potassium persulfate (Fluka, 60490) at a molar ratio of 2:1 to form the $ABTS^{\bullet}$ radical, left in the dark room overnight for 16 hours. Stock solutions of essential oils and trolox were prepared in ethanol. Ten µl aliquot of both the essential oils and trolox were pipetted into tubes, then the $ABTS^{\bullet}$ solution was added which had been diluted with PBS (pH 7.4) to an absorbance of 0.70 ± 0.02 AU at 734 nm, stirred vigorously and the absorbance was measured in time which was carried out in duplicate.

Statistics

Statistical analyses of the data were performed by Student's *t*-test. A probability value of $P \leq 0.05$ was considered to denote a statistically significant difference, and $P \leq 0.01$ was also used to show the power of the significance. Data are presented as mean values ± S.E.M. (standard error of the mean). Additionally, the data sets of three antioxidant assays were correlated in order to figure out if certain correlations exist.

RESULTS AND DISCUSSION

Variation of total phenol content

The total phenols determined by the Folin-Ciocalteu method varied from 12.28 to 794.28 mg

GAE/g sample. Essential oils (EOs) of clove and oregano had the highest total phenol content (794.28, 306.66 mgGAE/g, respectively), whereas laurel EO possessed the lowest total phenols (Table 2). Basmacıoğlu-Malayoğlu et al. (2011) determined the chemical composition of the essential oils used in this study by GC/MS. Based on their findings, the major components of clove EO possessing the highest total phenols are eugenol (77.85%) and β-caryophyllen (6.98%), whereas the major components of oregano EO are carvacrol (74.01%) and thymol (7.33%) comprising more than 80% of the EOs. Suresh et al. (1992) suggested that the profound antimicrobial activity of clove EO was due to the high content of eugenol. The high total phenol value of clove EO can be due to the high content of eugenol in the oil sample. In regards to the oregano EO, carvacrol was the dominating compounds which is in accordance with the study of Oflaz et al. (2002) reporting 59-82% of carvacrol for EOs of various oregano samples. Therefore, the high total phenol value could be associated with the high content of carvacrol and thymol.

Table 2. Radical scavenging capacities and antioxidant activities of essential oils

Essential oils	Total phenol assay (mg GAE/ g sample)	DPPH Radical scavenging activity (%)	TEAC value µM trolox/100g dry sample
Anise	15.30 ± 1.09	25.68 ± 2.31	2.1 ± 0.18
Rosemary	23.32 ± 1.99	32.00 ± 1.98	2.2 ± 0.05
Laurel	12.28 ± 1.05	39.70 ± 0.67	3.1 ± 0.11
Clove	794.28 ± 1.56	98.32 ± 0.81	421.0 ± 0.57
Oregano	306.66 ± 1.78	70.67 ± 1.16	225.0 ± 0.66
Cumin	20.16 ± 2.09	27.50 ± 0.68	8.3 ± 0.12

Variation of antioxidant efficiency

The RSA values varied from 25.68 % to 98.32 % showing a similar pattern. EOs of clove and oregano showed the highest RSA values (98.32, 70.67 %, respectively), whereas anise EO showed the lowest value, 25.68 %. RSA values of the essential oils from high to low can be expressed as clove > oregano > laurel > rosemary > cumin > anise. It is worth to mention that the RSA values of essential oils were comparatively lower than the RSA values of extracts particularly from rosemary and laurel. Dang et al. (2001) noted that the phenolic compounds induced the antioxidant activity of rosemary extract are non-volatile and are not present in the essential oil from rosemary, which explains its weaker antioxidant properties.

In the other studies, effectiveness in the *β*-carotene assay was: black pepper > monarda > oregano > geranium > clove > nutmeg (Dorman, 1999), free radical-scavenger effectiveness in the DPPH test was in the following descending order: clove> cinnamon > nutmeg > basil > oregano > thyme (Tomaino et al., 2005). TEAC values ranged between 2.1 to 421.0 µM Trolox/100 g sample. Among those, EOs of clove and oregano had the highest values (421.0, 225.0 µM Trolox/100 g sample), whereas TEAC values of anise, rosemary and laurel were very similar, just cumin showing a comparatively higher TEAC value (8.3 µM Trolox/ 100 g sample) than those.

It can be concluded that the major components of essential oils of anise, rosemary, laurel and cumin, being trans anethol (95.40%), α-pinene (%24.50), 1,8 cineol (%53.74) and cuminal, (33.57%) (Basmacıoğlu-Malayoğlu et al., 2011) in combination with the rest of the compounds possess lower antioxidant capacity.

Relation between different antioxidant assays

With a holistic view to the results of different assays, it can be concluded that the results of total phenol content and DPPH (r=0.97), DPPH and TEAC (r=0.98) and also total phenol content and TEAC results (r=0.99) revealed very good correlations, indicating a similar trend for the findings (Figure 1). Generally, a positive correlation between total phenolic constituents and antioxidant capacity is reported (Tepe et al., 2004; Tomaino et al., 2005).

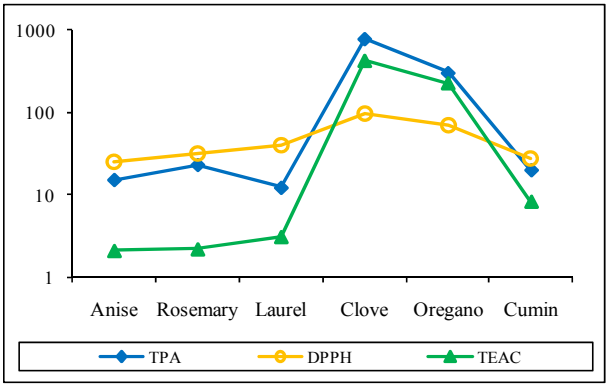

Figure 1 Aggregated graphs presenting correlations of the results of *in vitro* antioxidant assays.

CONCLUSION

The utilization of essential oils as phytochemicals in food, cosmetic, medicine industries and animal nutrition is gaining more attention due to growing consumer requirements towards natural products and also increasing concern about potentially harmful synthetic additives. The importance of research studies in regards to essential oils lie in identification of chemical compositions, highlighting various

biological activities and possibility of linking the chemical contents with functional properties. Collaboration of food manufacturers, animal nutritionists and life science engineers with the ultimate aim of combining *in vivo* studies with *in vitro* results would present valuable insights in order to transfer the know-how to the industry, thereby as novel products to the consumers.

The findings obtained from this study confirm that clove and oregano samples showed high capacity in terms of neutralizing free radicals (DPPH· and ABTS). Taking into consideration the high antimicrobial activities of clove and oregano essential oils (Basmacıoğlu-Malayoğlu et al., 2011) besides their significantly high antioxidant activities, these essential oils can be utilized as phytochemicals in food industry and animal nutrition owing to the possibility of sustaining both antimicrobial and antioxidant properties with one single ingredient.

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