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The investigation of total phenolic content and antioxidant properties of some essential oils in Turkey

Original Article

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Abstract:
This study targeted to determine the total phenolic content and antioxidant activity of essential oils *Cinnamomum zeylanicum*, *Vitis vinifera*, *Allium cepa* and *Sesamum indicum*. Total phenolic content was measured by Folin-Ciocalteu assays. The antioxidant potential of the tested extracts was evaluated using DPPH, CUPRAC and total antioxidant capacity (TAC) assays. The total phenolic compounds in essential oils varied widely, ranging from 80.26±0.007 µg GAE/mL to 268.20±0.008 µg GAE/mL. Cinnamon oil exhibited higher DPPH scavenging activity and CUPRAC activity. This study revealed that essential oils of *C. zeylanicum*, *V. vinifera*, *A. cepa* and *S. indicum* are good sources of natural antioxidants.

Key words:
essential oil, plant, antioxidant activity

Apstrakt:
Istraživanje ukupnog sadržaja fenola i antioksidativnih svojstava nekih etarskih ulja u Turskoj
Ova studija je imala za cilj da odredi ukupan sadržaj fenola i antioksidativnu aktivnost etarskih ulja vrsta *Cinnamomum zeylanicum*, *Vitis vinifera*, *Allium cepa* i *Sesamum indicum*. Ukupan sadržaj fenola je meren pomoću Folin-Ciocalteu testova. Za procenu antioksidativnog potencijala testiranih ekstrakata, korišćeni su DPPH, CUPRAC i TAC (Total Antioxidant Capacity) testovi. Ukupna količina fenolnih jedinjenja u etarskim uljima je varirala u širokom rasponu, od 80,26±0,007 µg GAE/mL do 268,20±0,008 µg GAE/mL. Ulje cimeta je pokazalo veću DPPH sposobnost vezivanja slobodnih radikala i CUPRAC aktivnost. Ova studija je pokazala da su etarska ulja *C. zeylanicum*, *V. vinifera*, *A. cepa* i *S. indicum* dobar izvor prirodnih antioksidanasa.

Ključne reči:
etarska ulja, biljka, antioksidativna aktivnost

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Introduction

Under stress, our bodies produce more reactive oxygen species than enzymatic antioxidants can counteract. This imbalance causes cell damage and health issues. A lack of antioxidants facilitates the development of degenerative diseases such as cardiovascular diseases, neurodegenerative disorders, Alzheimer's disease, inflammatory diseases, and cancer. One way to address this problem is to supplement the diet with antioxidant compounds found in natural plant sources (Krishnaiah et al., 2011). Plants are widely used as nutritional supplements because they are cheaper and safer, gaining attention among researchers as natural antioxidant sources (El-Miziani et al., 2015; Stanković et al., 2016).

Antioxidant components have the ability to scavenge free radicals and prolong shelf life by delaying the lipid peroxidation process, which leads to the deterioration of food and pharmaceutical products. Antioxidants play important roles in chain-breaking reactions, reducing the concentration of reactive oxygen species, and chelating transition metal catalysts (Erdoğan, 2022).

Essential oils are volatile, oily mixtures obtained from plants through hydrodistillation of water or water vapor, remaining liquid at room temperature. They are called "essential oils" or "etheric oils" because they can evaporate even at room temperature. Essential oils are secondary metabolites produced in plants (Erdoğan, 2022).

Cinnamomum zeylanicum, known as 'Ceylon cinnamon' or 'true cinnamon,' is an evergreen tree

that has been used as a medicinal plant. It is utilized in Ayurveda and folklore medicine as concoctions and decoctions. This plant has been used to treat inflammation of the eyes, dyspnea, leucorrhea, rheumatism, neuralgia, wounds, toothache, and diabetes (Weerasekera et al., 2021).

Vitis vinifera (grape) has been utilized for many years due to its nutritional and medicinal properties. The seeds and leaves of *V. vinifera* are used in herbal medicine, and its fruits are consumed as dietary supplements. Fresh grapes can be eaten or processed into wine and juice. Grape seeds are used in soaps and as a substitute for linseed. The sap of young branches is used as a remedy for skin diseases (Kanagarla et al., 2013).

Allium cepa, known as onion, is a perennial herb with an underground bulb stem. Onion peel can cure hypertrophic scars and prevent keloids. Studies also revealed that onion extract helps remove hypertrophic wounds. Regular consumption of onions lowers the risk of various cancers, including colorectal, lung, liver, brain, stomach, ovarian, prostate, and breast cancers (Chakraborty et al., 2022).

Sesamum indicum seeds have been used worldwide since prehistoric times. Sesame seeds are a rich source of protein and are used as ingredients in soap, cosmetics, lubricants, and medicines. Sesame seeds also contain two significant substances: sesamin and sesamol. These substances have cholesterol-lowering effects and can help prevent high blood pressure. Sesame oil acts as a laxative, emollient, and demulcent. It has been utilized in the treatment of several chronic diseases, including hepatitis, diabetes, and migraines, by Ayurvedic physicians in Holland (Anilakumar et al., 2010).

In the literature, many studies have been conducted on the antioxidant activity of essential oils from these plants, but location might affect the phytochemical composition of the plants and their essential oils. The current research was carried out to reveal the antioxidant activity and total phenolic content of essential oils of *C. zeylanicum* (cinnamon), *V. vinifera* (grape seed), *A. cepa* (onion), and *S. indicum* obtained from a herbal shop in Turkey.

Materials and Methods

Providing of the samples

Essential oils of cinnamon, grape seed, onion and sesamum were bought from herbal shop.

Total phenolic content

Total phenolic contents of the essential oils were determined in accordance with the method of Slinkard & Singleton (1977) utilizing gallic acid standard. Total phenolic content of the extracts was expressed as µg gallic acid equivalents (GAE)/mL by using the calibration curve. The tests were performed in triplicate.

Antioxidant Activity
DPPH radical scavenging activity

Appropriate dilution series (250-1000 µg/mL) of essential oils were prepared in DMSO. The absorbance of the solutions was measured using a spectrophotometer (Shimadzu UV mini-1240) at 517 nm (Brand-Williams et al., 1995). Rutin and Butylated hydroxytoluene (BHT) were used as standard antioxidants. The tests were performed in triplicate.

The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH Radical Scavenging Activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

A_0: Absorbance of control
A_1: Absorbance of extract or standard

Cupric reducing antioxidant capacity (CUPRAC)

Essential oils were prepared in 250-1000 µg/mL concentration. The absorbance was read by a spectrophotometer (Shimadzu UV mini-1240) at 450 nm. Butylated hydroxytoluene (BHT) was used as a standard antioxidant agent (Özyürek et al., 2009). The tests were performed in triplicate.

Total antioxidant capacity

The phosphomolybdenum method was used to determine the total antioxidant capacity of the extracts. Absorbance was measured using a spectrophotometer (Shimadzu UV mini-1240) at 695 nm. Ascorbic acid was used as the standard (Prieto et al., 1999). The total antioxidant capacity was expressed as µg ascorbic acid equivalent (AAE)/mL. The tests were performed in triplicate.

Results and discussion

Total Phenolic Content and Total Antioxidant Capacity

Phenolic substances are among the most crucial groups of natural antioxidants in plants (Öztürk et al., 2022). Phenolic compounds have redox properties responsible for their antioxidant capabilities and facilitating free radical scavenging. The phenolic content in each essential oil was determined using the Folin–Ciocalteu reagent (Aryal et al., 2019). The total phenolic compounds in the essential oils varied widely, ranging from 80.26±0.007 µg GAE/mL to 268.20±0.008 µg GAE/mL. The total phenolic contents of the essential oils increased in the following order: grape seed < sesame < onion <

Table 1. Total phenolic content and total antioxidant capacity of esssential oils

Essential oil	Total phenolic content (µg GAE/ml)	Total antioxidant capacity (µg AAE/ml)
Grape seed	80.26±0.007	56.03±0.002
Cinnamon	268.20±0.008	91.57±0.009
Onion	123.36±0.025	30.23±0.006
Sesamum	90.76±0.002	23.84±0.007

Data are presented as mean ± SD of triplicate determinations

cinnamon (**Tab. 1**).

In general, about 90% of the total amount of grape seed oil comprises mono- and polyunsaturated fatty acids, with linoleic acid (58–78%) being the most abundant, followed by oleic acid. The second largest group of lipophilic molecules in grape seeds are vitamins. Phytosterols, another important lipophilic molecule in grape seed oil, are biologically significant due to their antioxidant activity (Kapcsándi et al., 2021).

Sesame seed oil is among the most expensive and coveted edible oils globally. It is one of the healthiest oils, containing 38.84% oleic acid and 46.26% linoleic acids. Additionally, the high content of phytoestrogen and lignin in sesame seed oil can significantly impact oxidative stress and lipid profiles (Oboulbiga et al., 2023).

The major compounds present in onion essential oil are dipropyl disulfide and dipropyl trisulfide, which have been reported to possess antimicrobial and antioxidant activities (Mnayer et al., 2014). The essential oil of cinnamon is composed of cinnamaldehyde (56–78%), eugenol (4–10%), and other compounds such as pinene, linalool, epicatechin, and quercetin. Cinnamaldehyde, the main compound in cinnamon essential oil, has also been reported to have antioxidant activity (Unalan et al., 2021).

Total phenolic contents of the tested essential oils have been studied by other researchers. For instance, Aktaş et al. (2018) found the total phenolic content of grape seed essential oil to be 437.84 mg GAE/g. Behbahani et al. (2020) reported the total phenolic content of cinnamon oil as 106.19±0.63 mg GAE/g. Bopitiya & Madhujith (2013) concluded that the total phenolic content of sesame oil collected from Sri Lanka was 26.00±0.14 mg GAE/g.

The total antioxidant capacity (TAC) was determined by the reduction of Mo(VI) to Mo(V) by the essential oils and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH (Aliyu et al., 2013). The TAC of the extracts was determined using the phosphomolybdenum method as described by Prieto et al. (1999). Cinnamon oil exhibited the highest total antioxidant capacity (91.57±0.009 µg AAE/mL), while sesame oil had the lowest (23.84±0.007 µg AAE/mL) (**Tab. 1**).

CUPRAC and DPPH Radical Scavenging Activity

Antioxidants also have the ability to reduce higher valence elements such as copper and iron. The redox potential of an antioxidant is a significant indicator of its activity. The CUPRAC assay is a redox potential-based method in which the copper(II)-neocuproine complex is reduced to copper(I)-neocuproine chelate by antioxidants (Arıtuluk et al., 2016). In the CUPRAC method, cinnamon oil showed a remarkable reducing capacity, even better than BHT, which is used as a standard antioxidant agent. The reducing capacities decreased in the order: cinnamon oil > BHT > grape seed oil > sesame oil > onion oil at 1000 µg/mL concentrations (**Tab. 2**).

The hydrogen atom donating capacity of the essential oils was revealed by the decolorization of 2,2-diphenyl-1-picrylhydrazyl (DPPH). In a methanol solution, DPPH produces a violet/purple color that fades to yellow in the presence of an antioxidant in the essential oil (Rahman et al., 2015). Among the oils, cinnamon oil possessed the highest activity. At a concentration of 1000 µg/mL, the scavenging activity of grape seed oil, cinnamon oil, *Allium cepa* oil, and sesame oil was 30.38±0.031, 93.27±0.005, 29.65±0.013, and 16.09±0.011, respectively. Cinnamon oil exhibited higher activity than BHT and rutin, which are standard antioxidant agents. The other essential oils showed lower effects than BHT and rutin. The DPPH scavenging activities of the essential oils and standards were dose-dependent and increased with increasing concentrations (**Tab. 2**).

There are many studies in the literature about the DPPH scavenging activity of essential oils from grape seed, cinnamon, onion, and sesame. Aktaş et al. (2018) reported the DPPH scavenging activity of grape seed oil to be 79%. Behbahani et al. (2020) reported the DPPH scavenging activity of cinnamon essential oil to be 71.12±0.77%. Shalaby et al. (2011) studied the DPPH activity of onion essential

Table 2. CUPRAC and DPPH radical scavenging activity of essential oils

Essential oil	Concentration (µg/ml)	CUPRAC Activity (nm)	DPPH Radical Scavenging Activity (%)
Grape seed	250	0.1597±0.007	22.75±0.017
	500	0.2309±0.025	24.74±0.001
	750	0.3440±0.004	26.19±0.007
	1000	0.3810±0.014	30.38±0.031
Cinnamon	250	1.1459±0.1424	82.01±0.006
	500	1.9497±0.022	85.74±0.002
	750	2.2245±0.025	91.65±0.005
	1000	2.2462±0.008	93.27±0.005
Onion	250	0.1179±0.011	14.69±0.007
	500	0.1353±0.032	17.74±0.002
	750	0.1791±0.013	21.53±0.013
	1000	0.2490±0.012	29.65±0.013
Sesamum	250	0.1368±0.013	6.25±0.021
	500	0.1842±0.006	6.76±0.020
	750	0.2554±0.034	10.96±0.063
	1000	0.2861±0.025	16.09±0.011
BHT	250	0.6529±0.003	84.85±0.022
	500	0.7567±0.0025	88.69±0.013
	750	0.8909±0.005	90.56±0.017
	1000	1.0978±0.006	92.46±0.010
Rutin	250	-	83.37±0.008
	500	-	86.20±0.016
	750	-	90.88±0.009
	1000	-	91.31±0.005

Data are presented as mean ± SD of triplicate determinations

oil collected from Egypt and found an IC_{50} value of 80 µg/mL. Bopitiya and Madhujith (2013) found the DPPH scavenging activity (IC_{50}) of sesame oil to be 0.026 mg/mL.

Conclusion

In the current study, we investigated and reported the total phenolic content and antioxidant properties of oils from grape seed, cinnamon, onion, and sesame. This research serves as a pioneering detailed study in describing and evaluating the chemical properties of the tested essential oils. The obtained data also show that these essential oils can be used as natural antioxidants. Hence, further investigations and tests are needed to characterize and isolate the compounds with antioxidative properties in these essential oils.

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