

## Determination of certain biochemical properties of *Lavandula stoechas* L.

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**Abstract:** *Lavandula stoechas* L. (Lamiaceae) is an aromatic plant species recognized as a potential source of phenolic compounds and has been traditionally used in many countries for centuries. Essential oils derived from species of the *Lavandula* genus are widely utilized in perfumery, cosmetics, food processing, and aromatherapy due to their diverse biological properties. In this study, the aim was to determine the total phenolic content and antioxidant properties of the essential oil obtained from the dried aerial parts of *L. stoechas*. The total phenolic content of the essential oil was measured using the Folin–Ciocalteu method, while antioxidant activities were evaluated through CUPRAC and DPPH assays. According to the results, the total phenolic content was found to be 1.349 mg/g. The highest antioxidant capacity was observed with the CUPRAC method, yielding a value of 2.408 mg/g, whereas the mean DPPH value was determined to be 0.054 mg/g. These findings indicate that the essential oil of *L. stoechas* exhibits strong antioxidant potential, with the CUPRAC method reflecting a notably higher antioxidant activity.

**Keywords:** Phenolic, Antioxidants, *Lavandula stoechas*, CUPRAC, DPPH

## Karabaş (*Lavandula stoechas* L.)’ın bazı biyokimyasal özelliklerinin belirlenmesi

**Özet:** *Lavandula stoechas* L. (Lamiaceae) türü, fenolik bileşenler açısından potansiyel bir kaynak olarak değerlendirilen ve birçok ülkede uzun süredir geleneksel tıpta yaygın şekilde kullanılan aromatik bir bitkidir. *Lavandula* cinsine ait türlerden elde edilen uçucu yağlar, çeşitli biyolojik aktiviteleri nedeniyle parfümeri, kozmetik, gıda endüstrisi ve aromaterapi gibi alanlarda geniş uygulama alanı bulmaktadır. Bu çalışma kapsamında, *L. stoechas* bitkisinin kurutulmuş toprak üstü kısımlarından elde edilen uçucu yağın toplam fenolik içeriği ve antioksidan özellikleri belirlenmiştir. Uçucu yağların toplam fenolik içeriği Folin–Ciocalteu yöntemiyle, antioksidan aktiviteleri ise CUPRAC ve DPPH yöntemleriyle analiz edilmiştir. Analiz sonuçlarına göre, toplam fenolik içeriğin 1,349 mg/g olduğu saptanmıştır. Antioksidan kapasite açısından CUPRAC yöntemiyle ölçülen değer 2,408 mg/g ile en yüksek aktiviteyi göstermiştir. DPPH yöntemiyle belirlenen ortalama değer ise 0,054 mg/g olarak tespit edilmiştir. Elde edilen veriler, CUPRAC yöntemi ile belirlenen antioksidan kapasitenin daha yüksek olduğunu ve *L. stoechas* uçucu yağının güçlü bir antioksidan potansiyele sahip olduğunu göstermektedir.

**Anahtar kelimeler:** Fenolik, Antioksidan aktivite, *Lavandula stoechas*, CUPRAC, DPPH

### 1. Introduction

Traditionally, medicinal plants have been utilized for therapeutic purposes across the world since ancient times, offering natural remedies for a variety of diseases. These plants hold significant cultural value and are often associated with intergenerational knowledge regarding health preservation and healing practices (Safarzadeh et al. 2022; Alfuraydi et al. 2024). In contemporary times, traditional medicine continues to be a widely preferred healthcare approach for approximately two-thirds of the global population. This preference is largely driven by the increased awareness of the side effects associated with synthetic pharmaceuticals and the growing acceptance of herbal alternatives, especially in developed countries where sustainability initiatives encourage the use of locally available natural resources (Setacci et al. 2020). The rich biodiversity of the Mediterranean region plays a crucial role in the development of natural therapeutic methods, serving as a substantial reservoir of medicinal plants (Algieri et al. 2016; Gülmen 2018). Among the botanical families contributing to the expansion of the

catalog of popular herbal medicines in the Mediterranean Basin, the Lamiaceae family is particularly notable for its abundance of medicinal and aromatic species (Algieri et al. 2016). This family, commonly known as the mint family, comprises 46 genera, with the genus *Lavandula* represented by six taxa in Türkiye. Globally, the genus *Lavandula*, which is native to the Mediterranean, consists of approximately 39 species and over 100 varieties (Carrasco et al. 2015). Among these species, *Lavandula stoechas* L. is native to the Mediterranean region of Türkiye. However, due to its adaptability to various climatic conditions and soil types, it is now distributed across many parts of the world (Gülmen 2018; Gallotte et al. 2020). Commonly known as “French lavender” or “topped lavender,” *L. stoechas*—locally referred to as “karabaş otu” or “karan”—holds a prominent commercial position among medicinal and aromatic plants, primarily due to its industrial uses (Davis 1982; Gallotte et al. 2020). It is a well-known species in traditional medicine, particularly for its essential oil content, and has been utilized for centuries in Türkiye for various therapeutic applications (Şahinler et al. 2022).

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Ethnobotanical and phytopharmacological studies report that in Morocco, *L. stoechas* is traditionally used for the treatment of rheumatic disorders and nephrotic syndromes, as an antispasmodic agent, and in alleviating pain and inflammatory conditions (El-Hilaly et al. 2003; Ez Zoubi et al. 2016). Moreover, it is commonly used in the preparation of traditional foods and herbal teas, as well as for cosmetic purposes (Msaada et al. 2011; Zuzarte et al. 2013; Montanari 2014). In the chemical composition of *L. stoechas*, the aerial parts have been found to contain flavonoids, catechin tannins, sterols, coumarins, leucoanthocyanins, and mucilages (Ez Zoubi et al. 2020). Previous studies on *Lavandula* taxa have extensively investigated their volatile constituents (Kırmızı Bekmez et al. 2009; Bella et al. 2015; Kıyma et al. 2017; Karaca et al. 2018; Küçük et al. 2018; Mokhtarzadeh 2019), genetic diversity (Kaya et al. 2012), anatomical, morphological, chorological, and biosystematic characteristics (Küçük et al. 2019), antioxidant activities (Uygun et al. 2017), pathogenic effects (Ökmen 2017), as well as their phytochemical and biochemical properties (Celep et al. 2018). Additionally, their cytotoxic (Çelik and Aslantürk 2007; Kalhan 2019; Abdel-Baki 2023), anti-inflammatory, antioxidant (Ceylan et al. 2015; Ayaz et al. 2020), antimicrobial (Sıcak et al. 2019), insecticidal, larvicidal, anticonvulsant, antispasmodic, sedative, hepatoprotective, nephroprotective, antidiabetic, and anticancer (Şahinler et al. 2022), antibacterial (Dadaloğlu and Evrendilek 2004), antifungal, and antiseptic activities (Öztürk et al. 2005) have also been well documented. Furthermore, *L. stoechas* has been traditionally used in folk medicine for wound healing, blood sugar regulation, headache relief, and treatment of vascular occlusions (Güner and Selvi 2016). The essential oil of *L. stoechas* has demonstrated antibacterial (Ez Zoubi et al. 2017 and 2020), insecticidal, antifungal (Bouyahya et al., 2017), antioxidant (Ez Zoubi et al. 2014; Messaoud et al. 2011), and anti-inflammatory properties (Benabdelkader et al.

2011; Ez Zoubi et al. 2014). Despite the extensive research on the chemical composition of *L. stoechas*, the majority of these studies have primarily focused on its essential oil constituents. However, investigations specifically addressing the total phenolic content, as a key biochemical property of its essential oil, remain limited. Therefore, the present study aims to determine the antioxidant activity of essential oils derived from *L. stoechas* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and the Cupric Ion Reducing Antioxidant Capacity (CUPRAC) assay, alongside quantification of total phenolic content.

## 2. Materials and methods

### 2.1. Plant material

A preliminary survey was initiated through a review of existing studies conducted in the research area and interviews with local residents. Field excursions were carried out to develop a work plan, with priority given to regions where the natural distribution of the species had been identified. The research material comprised the leafy shoots and flowers of *L. stoechas* L., commonly known as “French lavender,” collected in 2023 from the Karaağaç region of Köyceğiz, Muğla (Figure 1). The altitude and aspect data of the collection sites were recorded during field studies. The collected plant specimens were placed in labeled plastic bags, with details including collection date, location, and elevation written on each label. Samples were subsequently air-dried at room temperature (25°C) in a semi-shaded, well-ventilated environment to be used in further extraction analyses. Herbarium specimens were prepared using standard herbarium techniques (Figure 2), identified taxonomically, assigned specimen numbers, and deposited in the Herbarium of the Faculty of Forestry, Isparta University of Applied Sciences.

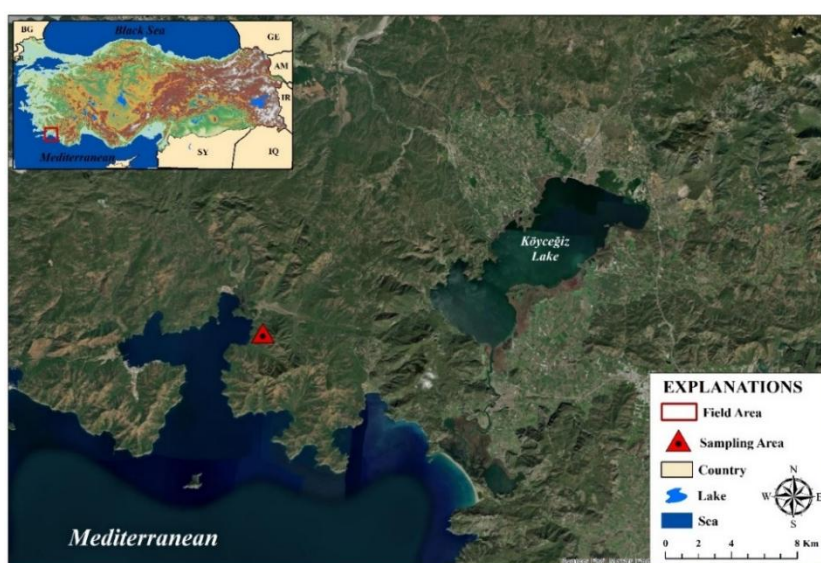


Figure 1. Study area (Karaağaç locality, Köyceğiz, Muğla)



Figure 2. General appearance of *L. Stoechas*

## 2.2. Methods

### 2.2.1. Determination of total phenolic content (TPC)

Determination of total phenolic and flavonoid contents  
The total phenolics of the samples, extracted with different solvents, were determined using the modified Folin-Ciocalteu method. The extract solution (0.1 mL) was mixed with 2.5 mL of deionized water and 0.1 mL of Folin-Ciocalteu reagent (Merck Company, Darmstadt, Germany), and the reaction was terminated using 0.5 mL of 20% sodium carbonate. The reaction mixture was incubated at RT for 30 min in the dark and the absorbance was measured at 760 nm with a UV-vis spectrophotometer (Shimadzu UV-1280, Kyoto, Japan). The standard curve was prepared using different concentrations of gallic acid (GA). Total flavonoids were measured by the aluminum chloride reaction (Sakanaka et al. 2005). The extract solution (0.25 mL) was mixed with 1.25 mL of deionized water, and 75  $\mu$ L of 5% sodium nitrate. After 6 min, 0.15 mL of 10% aluminum chloride was added; after 5 min, 0.5 mL of 1 M sodium hydroxide was added. The absorbance of all the sample solutions against a blank was measured at 510 nm and ( $\pm$ )-catechin concentrations were used to construct the standard curve.

### 2.2.2. Antioxidant analyses of *L. stoechas*

Antioxidant activity of *L. stoechas* was evaluated using two distinct methods: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the Cupric Reducing Antioxidant Capacity (CUPRAC) assay.

### 2.2.3. DPPH radical scavenging method

The antiradical activity was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method as described by Shimata et al. (1992). A 0.25 mL aliquot of the sample (at a concentration of 250 ppm) was mixed thoroughly with 1 mL of 0.2 mM DPPH solution and vortexed. The mixture

was incubated in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm. The radical scavenging activity (SRS) was calculated using the following equation (Equation 1):

$$SRS \text{ (mmol TR g}^{-1} \text{ ekstrakt)} = \frac{\Delta A}{\varepsilon_{TR}} \times \frac{V_m}{V_s} \times Sf \times \frac{V_E}{m} \quad (1)$$

Where  $\varepsilon_{TR}$ : CUPRAC molar absorptivity of Trolox ( $1.67 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ );  $V_s$ : volume of the sample;  $V_m$ : total reaction volume (4 mL);  $Sf$ : dilution factor (if applicable);  $V_E$ : extract volume ve  $m$ : mass of the extract.

### 2.2.5. CUPRAC (Cupric Reducing Antioxidant Capacity) method

The CUPRAC assay was conducted based on the method developed by Apak et al. (2004). A 100 ppm extract solution was mixed with 0.01 M  $\text{CuCl}_2$ , 7.5 mM neocuproine, 1 M ammonium acetate buffer (pH 7.0), and 1 mL of distilled water. Ethanol was used as a blank control. The mixture was incubated in the dark for 1 hour. Absorbance was measured at 450 nm. Antioxidant capacity was expressed in terms of Trolox equivalents (TE) per gram of dry weight (Equation 2):

$$CUPRAC \text{ (}\mu\text{mol TE g}^{-1}\text{)} = \frac{A}{\varepsilon_{TR}} \times \frac{V_m}{V_s} \times D_f \times \frac{V_E}{m} \times 1000 \quad (2)$$

Where  $A$ : absorbance at 450 nm;  $\varepsilon_{TR}$ : CUPRAC molar absorptivity of Trolox ( $1.67 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ );  $V_m$ : CUPRAC total reaction volume;  $V_s$ : sample volume (mL);  $D_f$ : dilution factor;  $V_E$ : extract volume (mL); ve  $m$ : mass of dry herb (g).



### 3. Results and discussion

Plant chemical constituents are generally classified into primary and secondary metabolites based on their biosynthetic pathways and biological functions (Topcu and Çölgeçen 2019; Ülger and Ayhan 2020). Among these, secondary metabolites are not directly involved in vital physiological processes but play significant ecological and functional roles. One of the most important classes of secondary metabolites is phenolic compounds, characterized by one or more hydroxyl groups (-OH) attached to aromatic benzene rings (Vermerris and Nicholson 2007; Carochio and Ferreira 2013; Stewart and Stewart 2008).

Numerous studies have demonstrated that essential oils (EOs) derived from various plant species exhibit a range of beneficial properties including antimicrobial, antioxidant, anti-inflammatory, and even anticancer activities. These bioactivities are largely attributed to the chemical constituents of the essential oils, which vary depending on plant species, drying methods, durations, and extraction techniques. Djeridane et al. (2006) reported a positive correlation between antioxidant capacity and total phenolic content. Similarly, Ennajar et al. (2009) observed that an increase in phenolic content is associated with enhanced antioxidant activity, suggesting that phenolic compounds are key contributors to the antioxidant potential of essential oils.

Phenolic compounds are well known for their ability to neutralize free radicals, which underpins their health-promoting effects in various plant extracts and essential oils. Multiple studies have confirmed the radical-scavenging capabilities of phenolics and flavonoids, highlighting their potential as natural antioxidants. The total phenolic content (TPC) and antioxidant activity of plant extracts are critical indicators of their ability to mitigate oxidative stress by neutralizing free radicals.

In the present study, the total phenolic content of *L. stoechas* essential oil was determined to be 1.349 mg g<sup>-1</sup>, while its antioxidant activities were measured as 0.054 mg g<sup>-1</sup> for DPPH radical scavenging and 2.408 mg g<sup>-1</sup> for CUPRAC, as shown in Table 1.

Studies conducted with *Lavandula* species from various geographic regions have demonstrated that total phenolic content (TPC) and antioxidant capacity show considerable variability. Karabagias et al. (2019) reported that the aqueous extract obtained from the flowers of *L. stoechas* had a significantly higher TPC (approximately 4289 mg L<sup>-1</sup>) compared to its methanolic extract. This result suggests that the higher polarity of the aqueous extract allows for more efficient release and isolation of

phytochemicals, particularly phenolic acids, flavonoids, and reducing agents such as ascorbic acid.

In a study by Messaoud et al. (2013), essential oils obtained from three different *Lavandula* species had TPC values of 31.3 mg GAE g<sup>-1</sup> for *L. coronopifolia*, 30.8 mg GAE g<sup>-1</sup> for *L. multifida*, and 25.2 mg GAE g<sup>-1</sup> for *L. stoechas*. Similarly, Deligiannidou et al. (2018) noted that beverages prepared by infusion or decoction from *L. angustifolia* flowers exhibited higher phenolic content when water, a polar solvent, was used for extraction. In another study by Spiridon et al. (2011), the total phenolic content of alcoholic and aqueous extracts (1:1, v/v) of *L. angustifolia* from Romania was found to be 50.6 ± 3.2 mg GAE g<sup>-1</sup>, a significantly lower value compared to the findings of the present study. These results highlight the importance of the solvent used in extraction, indicating that polar solvents such as water are more effective in extracting phenolic compounds.

Antioxidant compounds play a key role in preventing oxidative damage in biological systems by limiting radical formation, terminating radical reactions, and neutralizing existing radicals (Atak et al. 2002; Uysal 2015). These compounds act at different stages of the oxidative chain reaction by reducing oxygen concentration, chelating metal ions that catalyze reactive oxygen species production, and functioning as chain-breaking agents. Analytical methods and testing conditions significantly influence antioxidant capacity measurements, even within the same food type (Ghiselli et al. 2000; Büyüktuncel 2013). In recent years, various methods based on free radical scavenging activity have been developed to assess antioxidant potential, among which the CUPRAC method stands out as a simple and adaptable technique for evaluating both natural and synthetic antioxidants.

In this context, the antioxidant activities of *L. stoechas* samples in our study were determined using the DPPH and CUPRAC methods (Table 1). The findings indicate that *L. stoechas* possesses high antioxidant activity, although the values vary depending on the method used.

Numerous studies in the literature have reported that essential oils derived from various *Lavandula* species exhibit a wide range of therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, and even anticancer effects. Sebai et al. (2015) demonstrated that essential oils extracted from the aerial parts of *L. stoechas* significantly increased DPPH radical scavenging activity (RSA) in a dose-dependent manner when compared to ascorbic acid. However, while these essential oils showed considerable RSA (EC<sub>50</sub> = 221.43 µg/mL), the activity was lower than that of ascorbic acid (EC<sub>50</sub> = 87.57 µg/mL).

**Table 1.** Total phenolic content and antioxidant activity of *L. stoechas*

	Total Fenolik Mean (mg g <sup>-1</sup> )	Dpph Mean (mg g <sup>-1</sup> )	Cuprac Mean (mg g <sup>-1</sup> )
<i>L. stoechas</i> extracts	1.349	0.054	2.408

Ceylan et al. (2015) evaluated the antioxidant activity of *L. stoechas* extract using the DPPH method and found that it inhibited the DPPH radical by  $69.31 \pm 1.24\%$  at a concentration of 0.5 mg/mL. In comparison, the synthetic antioxidants BHT and BHA exhibited inhibition percentages of  $89.16 \pm 1.83\%$  and  $80.01 \pm 1.78\%$ , respectively. The IC<sub>50</sub> value of the methanolic extract from *L. stoechas* leaves was measured at  $0.300 \pm 0.010$  mg/mL, whereas BHT and BHA had IC<sub>50</sub> values of  $0.020 \pm 0.001$  mg/mL and  $0.035 \pm 0.007$  mg/mL, respectively. These results indicate that although the extract of *L. stoechas* is a potent natural antioxidant, its efficacy is lower than that of synthetic antioxidants. Nevertheless, plant extracts are considered valuable sources of antioxidants.

Sariri et al. (2009) investigated the antioxidant potential of water extracts from fresh leaves and flower buds of four *Lavandula* species from northern Iran. The total antioxidant activity values were reported as 9.2 µg/mL for *L. angustifolia*, 12.5 µg/mL for *L. stoechas*, 38.7 µg/mL for *L. dentata*, and 65.1 µg/mL for *L. latifolia*. Similarly, Sebai et al. (2013) found that the essential oils from dried aerial parts of *L. stoechas* showed high antioxidant capacity with an IC<sub>50</sub> value of 221.43 µg/mL, although this was still lower than the IC<sub>50</sub> value of ascorbic acid (87.57 µg/mL).

Messaoud et al. (2013) reported IC<sub>50</sub> values of 15.8 mg/mL for *L. coronopifolia* and 34.2 mg/mL for *L. stoechas* essential oils. Furthermore, the methanolic extracts of *L. coronopifolia* (IC<sub>50</sub> = 15.8 mg/mL) and *L. multifida* (IC<sub>50</sub> = 19.3 mg/mL) exhibited stronger antioxidant activities than the synthetic antioxidant BHT (IC<sub>50</sub> = 26.5 mg/mL), though they were less effective than Trolox (IC<sub>50</sub> = 12.8 mg/mL). Essential oils also demonstrated radical scavenging capacity, albeit lower than that of methanolic extracts and synthetic antioxidants, with IC<sub>50</sub> values of 162.2 mg/mL for *L. coronopifolia*, 201.6 mg/mL for *L. multifida*, and 2321.7 mg/mL for *L. stoechas*.

Finally, Barkat and Laib (2012) reported an IC<sub>50</sub> value of  $584 \pm 0.58$  µg/mL for essential oils extracted from dried *L. stoechas* flowers collected in Algeria.

In conclusion, numerous studies on plant extracts have shown that phenolic compounds and flavonoids are potent antioxidants due to their free radical scavenging capacity. The findings indicate that *L. stoechas* possesses notable antioxidant properties, and phenolic compounds are the primary contributors to this activity. These results underscore the potential of *L. stoechas* as a valuable natural source of antioxidants with possible therapeutic applications.

#### 4. Conclusion

Many researchers have reported that essential oils (EO) derived from various plant sources possess a wide range of beneficial properties, particularly antimicrobial, antioxidant, anti-inflammatory, and even anticancerous effects. These pharmacological activities are largely attributed to the chemical compounds found in the

essential oils, which can vary depending on the plant species, extraction methods, drying techniques, drying durations, and the environmental conditions under which the plant material is cultivated.

*Lavandula* species have long been used in traditional medicine to treat a variety of ailments in different forms. Despite numerous studies on the chemical composition of *L. stoechas*, the majority of these investigations have focused on the essential oil components of the plant. In this study, the total phenolic content of *L. stoechas* flowers, roots, and leaves was determined, with a focus on exploring its antioxidant potential. While the biosynthesis of secondary metabolites in medicinal and aromatic plants is genetically controlled, it is also strongly influenced by environmental factors. Consequently, this study highlights the high total phenolic content and significant antioxidant potential of the essential oils derived from *L. stoechas* samples grown in the Muğla region.

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