Turkish Journal of Forestry | Türkiye Ormancılık Dergisi
2024, 25(1): 113-117 | Research article (Araştırma makalesi)

Chemical composition of essential oil extracted from lavender growing in Kastamonu, Türkiye

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Abstract: Lavender and lavender oils have been used as traditional herbal medicines for centuries. Nowadays, lavender and its essential oil are valuable materials used in many industries, e.g., food, cosmetics, perfume, sanitary products, and pharmaceuticals. Since the components of valuable oil differ depending on the growing region, these components must be identified to ascertain the oil's quality and potential uses. The essential oil composition was obtained from the stemmed fresh flowers of Kastamonu, Türkiye cultivars of lavandin (*Lavandula x intermedia* var. Super) via a Clevenger-type hydrodistillation apparatus. Then, the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS), and 14 components were identified. Linalool and linalyl acetate were the main components, with 28.44% and 17.12%, respectively. In this study, we aimed to evaluate our results by comparing them with Pharmacopoeia Europaea and ISO standards to determine the quality of the essential oil and its possible areas of use. The findings of this study showed that lavandin essential oil is a high-quality essential oil as it contains high levels of linalool and is free of camphor. In addition, while the linalool, terpinen-4-ol, and camphor contents of the variety complied with the lavender oil standards set by ISO 3515:2002, the linalyl acetate content was found below this. Due to its high linalool content and lack of camphor, the extracted lavandin oil can be predicted to be suitable for use in the food, beverage, aroma, pharmaceutical, perfume and aromatherapy industries.
Keywords: Hydrodistillation, *Lavandula* sp., Linalyl acetate, Linalool

Kastamonu'da yetişen lavantadan elde edilen uçucu yağın kimyasal bileşimi

Özet: Lavanta ve lavanta yağları yüzyıllardır geleneksel bitkisel ilaç olarak kullanılmaktadır. Günümüzde lavanta ve esansiyel yağı gıda, kozmetik, parfüm, sıhhi ürünler ve ilaç gibi birçok endüstride kullanılan değerli malzemelerdir. Değerli yağın bileşenleri yetiştirme bölgesine bağlı olarak farklılık gösterdiğinden, yağın kalitesini ve potansiyel kullanım alanlarını belirlemek için bu bileşenlerin belirlenmesi gerekir. Esansiyel yağ bileşimi, Kastamonu, Türkiye lavanta çeşitlerinin saplı taze çiçeklerinden (*Lavandula x intermedia* var. Super) Clevenger tipi hidrodistilasyon cihazı kullanılarak elde edildi. Daha sonra uçucu yağın gaz kromatografisi-kütle spektrometresi (GC-MS) ile analizi sonucu 14 bileşen tanımlanmıştır. Linalool ve linalil asetat sırasıyla %28,44 ve %17,12 ile yağın ana bileşenleridir. Bu çalışmada elde edilen yağın kalitesini ve olası kullanım alanlarını belirlemek için sonuçlarımızı Avrupa Farmakopesi ve ISO standartları ile karşılaştırarak değerlendirmeyi amaçladık. Analiz sonuçları, lavandin esansiyel yağının yüksek düzeyde linalool içermesi ve kafur içermemesi nedeniyle yüksek kaliteli bir esansiyel yağ olduğunu göstermiştir. Ayrıca, linalool, terpinen-4-ol ve kafur içerikleri ISO 3515:2002'de belirtilen lavanta yağı standartlarına uygun iken, linalil asetat içeriği standard değerin altında bulunmuştur. Çalışmada ekstrakte edilen lavandin yağının yüksek linalool içeriği ve kafur içermemesi nedeniyle gıda, içecek, aroma, ilaç, parfüm ve aromaterapi endüstrilerinde kullanıma uygun olduğu öngörülebilir.
Anahtar kelimeler: Hidrodistilasyon, *Lavandula* sp., Linalil asetat, Linalool

1. Introduction

Türkiye has an important place in the world trade of medicinal and aromatic plants thanks to its geographical location. Medicinal and aromatic plants are used to prevent and treat disease and maintain health. The global use of aromatic plants and their essential oils (EOs) is increasing due to consumer demand for natural ingredients and concerns about synthetic additives. The World Health Organization (WHO) stated that more than 80% of the world's population uses herbal medicines. These plants and their EOs represent a diverse and unique source of natural products and are used in many industries, such as cosmetics, perfume, sanitary products, and pharmaceuticals. Additionally, EOs have potential applications in food products due to their strong antimicrobial and antioxidant activities. They are volatile and aromatic oils extracted from plants and named after their parent plants. The quality and quantity of EO components are affected by different factors, such as the genotype of the plant, the environmental conditions of the plant's growing and harvesting season, drying conditions, and the extraction method (Baydar, 2009; Avcı, 2010; Pinto et al., 2007).

Among the plants containing essential oils, lavender (*Lavandula* sp.), which belongs to the Lamiaceae family, is a

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✓ **Received** (Geliş tarihi): 27.10.2023, **Accepted** (Kabul tarihi): 21.12.2023

Citation (Atıf)**:** Tahmas-Kahyaoğlu, D., Özen, M.B., Yıldız, H., 2024. Chemical composition of essential oil extracted from lavender growing in Kastamonu, Türkiye. Turkish Journal of Forestry, 25(1): 113-117.
DOI: [10.18182/tjf.1382213](https://doi.org/10.18182/tjf.1382213)

very valuable and profitable plant because it contains high-quality EOs. The Food and Drug Administration (FDA) recognizes it as safe for use in food (Bethesda, 2006). Thus, lavender essential oil (LEO) has become a popular ingredient in the food industry due to its beneficial properties and pleasant fragrance. While its antimicrobial activity is well known, including against Candida albicans, Escherichia coli, and Staphylococcus aureus, its antioxidant properties have captured the attention of many researchers (Hammer et al., 1999; Moon et al., 2004; Gülcin et al., 2004; Topal et al., 2008; Hui et al., 2010; Danh et al., 2013). Therefore, it is mainly used in food, perfume, sanitary products, cosmetics, and medicine. Lavenders consist of 25–30 species of flowering plants (Hui et al., 2010). The most commonly used lavender species for oil extraction are lavender (*Lavandula angustifolia* Mill.) and lavandin (*Lavandula intermedia* Emeric ex Loisel.). Although lavender has the most beautiful scent and high quality, its oil production is much lower than that of high-camphor lavandin (Adam, 2006). For this reason, oils derived from lavandin are often blended with lavender oil or other commercially available EOs to create a pleasant aroma. Lavender oil exhibits analgesic, sedative, disinfectant, bactericidal, fungicidal, and antidepressant activities as well as alleviates depression and anxiety, improves mood, and induces sleep (Smigielski et al., 2011). The main components in LEO are linalyl acetate (20-60%) and linalool (20-35%). On the one hand, while linalool is commonly used in food, beverages, flavorings, and pharmaceuticals, linalyl acetate is found to be a valuable component used in cosmetics and perfumes. On the other hand, LEOs rich in camphor and 1,8-cineole are used in the pharmaceutical and insecticide industries (Katar et al., 2020). Lavender flowers can contain as much as 3% EO, which varies by genotype, climate, soil structure, extraction method, etc. (Adaszyńska-Skwirzyńska and Szczerbińska, 2019).

Oil production from lavender is commonly carried out using various methods such as hydrodistillation, Soxhlet extraction, and supercritical carbon dioxide extraction. Hydrodistillation is a traditional technique where plant material is boiled in water with direct contact between the two. Depending on the region of cultivation, it is necessary to identify the valuable components of the extracted lavender oil to assess the oil's quality and potential applications.The most important components determining the quality of LEO are linalool and linalyl acetate (Kara and Baydar, 2013). However, as the camphor content increases, the smell of the EO deteriorates (Adam, 2006). On the one hand, camphor-rich lavender oils are valuable to the pharmaceutical and pesticide industries (Baydar, 2013). On the other hand, the absence or scarcity of camphor in EO indicates high quality and makes it ideal for perfume and aromatherapy (Adam, 2006; Kara and Baydar, 2013).

Gas chromatography-mass spectrometry (GC/MS) is an effective method for identifying the volatile composition of distilled EOs due to its fast and comprehensive separation and detection capabilities. Additionally, commercially available MS libraries identify these compounds' mass spectra.

This study aimed to obtain EO from a lavandin variety grown in Kastamonu by the hydrodistillation method and to understand the quality and usage areas of the oil by determining the volatile components of this oil by GC-MS.

2. Material and method

2.1. Plant material

The stemmed fresh lavandin (*Lavandula x intermedia* var. Super) flowers were collected from Kastamonu province, Türkiye in May 2022 and used to acquire EO.

2.2. Essential oil isolation

A Clevenger-type hydrodistillation apparatus was used to obtain EO from the stemmed, fresh lavandin flowers. However, sample preparation is required before hydrodistillation. For this purpose, a 500 g lavandin sample and 500 mL of water were blended via a blender. While the blending process continued, another 2500 mL of water was slowly added to this mixture to obtain a 6:1 ratio of water to the lavender sample (Sintim et al., 2015). Then, the mixture was taken into the hydrodistillation flask and steam-distilled for 3 hours (h) for the extraction of the EO. Lastly, the isolated EO was stored in amber-colored, airtight, sealed vials at 4 °C before analysis.

2.3. Essential oil component determination

The gas chromatography-mass spectrometer (GC-MS) technique (GCMS-QP 2010 Ultra, Shimadzu, Japan) was used to identify LEO components. A modified version of the method used by Kammoun et al. (2021) was employed as the analysis protocol. Principally, the EO samples must be prepared for GC-MS analysis. Therefore, the LEO sample was diluted at 1:100 in hexane (Sigma-Aldrich, Canada). All components of the EO were separated on an Rtx-5MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm) utilizing helium as a carrier gas at a flow rate of 3 mL/min, split ratio of injection 1/25 with a 250 °C injector, and detector temperature. Oven program: 40 °C for 3 min; from 40 °C to 240 °C at 4 °C/min. The mass selective detector was operated with a set mass scan for the acquisition from 40 to 450 (m/z) to obtain total ion chromatograms. Individual chemical compounds in the analyzed samples were determined using the commercial mass spectra library (W9N11) by comparing the achieved mass spectra of each volatile compound. The quantification of the relative abundance of all volatile compounds in the sample was done based on the relative area of the total ion chromatogram peaks.

3. Results

According to the 3 h hydrodistillation results, 1% EO was extracted from the lavandin used in the study. It has been reported that lavender flowers can contain up to 3% EO, which varies by genotype, climate, soil structure, extraction method, etc. (Adaszyńska-Skwirzyńska and Szczerbińska, 2019). Some researchers claimed that the lavandin plant contains from 1% to 1.5% of its total weight in EO in fresh stemless flowers and from 5.0% to 6.0% in dried stemless flowers (Kara and Baydar, 2013; Kara and Baydar, 2011; Baydar, 2009). In terms of the EO from fresh stem flowers, our results are consistent with previous findings.

Volatile components of the LEO were revealed via the GC-MS technique. In Table 1, the percentage composition of a total of 14 individual components found in LEO can be seen. The main ones are linalool (28.44%), linalyl acetate

(17.12%), trans-β-ocimene (8.33%), lavandulyl acetate (6.11%), α-terpineol (5.94%), geranyl acetate (5.66%), terpinen-4-ol (5.12%), and caryophyllene (3.07%) as seen in Table 1.

Lavender essential oil is characterized by a high level of linalool and linalyl acetate in its chemical composition (Kara and Baydar, 2013; Pokajewicz et al., 2021). It also contains moderate amounts of terpinen-4-ol, lavandulyl acetate, and lavandulol (Kara and Baydar, 2013; Pokajewicz et al., 2021). Additionally, there are varying levels of eucalyptol (1,8-cineol) and camphor (Kara and Baydar, 2013; Pokajewicz et al., 2021). These are just a few of the many other components found in oil that contribute to its physical, chemical, and biological properties. The essential oil of *L. angustifolia* can have a highly varied chemical composition, which is mainly determined by the plant's genotype. However, environmental and ontogenetic factors, as well as the region, conditions of cultivation, post-harvest processing procedures, and even the part of the plant used, can also influence the chemical composition of the essential oil. The quality of lavender oil can vary depending on its intended use and has been classified by mainly the International Organization for Standardization (ISO 3515:2002) and the European Pharmacopoeia (Ph. Eur.). While the Ph. Eur. has set maximum limits for the EO components for pharmaceutical and perfume use, the ISO has defined specific characteristics of LEOs from different origins to simplify the assessment of their quality (ISO 3515:2002; Pokajewicz et al., 2021). The quality of lavender oil can be determined by two main factors: the pleasant aroma, which is a subjective characteristic, and the desired combination of ingredients (Pokajewicz et al., 2021). In this study, we aimed to evaluate our results by comparing them with standards to determine the quality of the essential oil and its possible areas of use. On the one hand, according to the ISO 3515:2002 standard, the percentages of linalool, linalyl acetate, cymene, terpinen-4-ol, and camphor should be between 25.0-38.0%, 25.0-45.0%, 4.0-10.0%, 2.0-6.0%, and 0-0.5%, respectively (ISO 3515:2002). On the other hand, the Ph. Eur. 10th edition defines the limits of linalool, linalyl acetate, cymene, terpinen-4-ol, and camphor as 20.0-45.0%, 25-47%, no limits, 0.1-8.0%, and maximum 1.2%, respectively (Katar et al., 2020; Pokajewicz et al., 2021). In this study, the linalool, terpinen-4-ol, and camphor contents of the cultivar complied with the LEO standards set by ISO 3515:2002 and the Ph. Eur. 10th edition (ISO 3515:2002). However, the linalyl acetate content was below these standards. By the way, it is also worth noting that the values specified in these standards are based on GC analysis with a flame ionization detector (FID) (Pokajewicz et al., 2021). However, currently, the most commonly used method for quantitative sample analysis in scientific studies is GC-MS, with its greatly improved detection of small chemical components in EOs (Pokajewicz et al., 2021; Aparicio-Ruiz et al., 2018; Shellie et al., 2002). Generally, GC-MS is useful for qualitative analysis; GC-FID, on the other hand, provides a more accurate quantitative measurement of many components (Pokajewicz et al., 2021; Aparicio-Ruiz et al., 2018). Therefore, based on the GC-MS results obtained, we can conclude that the amount of linalyl acetate, the main component of lavender oil, is not within the range of the specified standards.

Table 1. Determined volatile compositions of LEO via GC-MS

Chemical name	Content (%)
Linalool	28.44
Linalyl acetate	17.12
Trans-β-ocimene	8.33
Lavandulyl acetate	6.11
α-Terpineol	5.94
Geranyl acetate	5.66
Terpinen-4-ol	5.12
Caryophyllene	3.07
3-Octyl acetate	2.79
β-Farnesene	2.56
3-Octanone	1.49
Myrcene	1.48
Caryophyllene oxide	1.12
Nerol	0.92
Total content	90.15

The table does not include compounds whose concentrations are below the detection limit.

There have been other studies on the LEO from different regions in Türkiye. For instance, Katar et al. (2020) reported in their study that the samples of Afyon and Isparta locations had linalool contents of 51.10% and 48.84%, respectively. They also found that at 53.10%, the Eskişehir location had the highest linalool content, while the Uşak location had the lowest at 41.34%. The group of Katar (2020) determined the rate of linalyl acetate at 23.54% for the Isparta location, and the lowest value was obtained at 1.83% for the Eskişehir location. Additionally, they also examined the linalyl acetate ratio, which was 21.58% for the Uşak location and 3.14% for the Afyon location. In this study, while the linalool content of LEO obtained from the Kastamonu location was lower than the study results of Katar et al. (2020) it was determined that the linalyl acetate content was almost five times higher than the value obtained from the Uşak region. Many researchers have stated that the EO composition of lavender and lavandin varies based on genotype, harvest time, and extraction method (Pinto et al., 2007; Baydar, 2009; Avcı, 2010; Kara and Baydar, 2013).

β-ocimene is a common volatile substance released in significant amounts from the leaves and flowers of many plants. This component may play a role in some biological functions in plants (influencing flower visitors, defense response). This component, which is abundant in most plants, serves to attract pollinators to flowers. It has two stereoisomers: cis- and trans-β-ocimene. The trans isomer is more common and more abundant in floral fragrances than the cis isomer (Farré-Armengol et al., 2017). In our study, the trans-β-ocimene amount was determined to be 8.33% (Table 1). The amount of β-ocimene in lavender essential oil grown in Afyon, Eskişehir, Isparta, and Uşak regions was determined to be 0.51%, 0.53%, 2.44%, and 1.21%, respectively (Katar et al., 2020). In the study conducted to develop a new approach to determining the quality of lavender essential oil, the amount of trans-β-ocimene was determined to be 5.18% (Marincaş and Feher, 2018). In a study examining Polish and Bulgarian lavender, trans-β-ocimene was detected only in Polish lavender, and its amount was 0.26%. (Dębczak et al., 2022).

Lavandulyl acetate has generally been noted to be active against Gram-positive bacteria and yeasts and may have a bactericidal or bacteriostatic effect depending on the type of bacteria. It is also in high demand in cosmetics and perfumery (Dębczak et al., 2022). In our study, the amount of lavandulyl acetate was determined to be 6.11% (Table 1). In another study, Polish and Bulgarian lavenders were examined, and the amounts of lavandulyl acetate were determined to be 5.17% and 3.02%, respectively (Dębczak et al., 2022). In a study comparing two Czech lavender varieties, the average amount of lavandulyl acetate was found to be 8.62% in Krajová' lavender and 8.88% in Beta lavender (Dušková et al., 2016). In lavenders in the Afyon, Eskişehir, Isparta, and Uşak regions, lavandulyl acetate was detected only in the Uşak region, and its amount was 2.47% (Katar et al., 2020). In another study, the amount of lavandulyl acetate was found to be 0.07% (Marincaş and Feher, 2018).

Terpineols are monocyclic monoterpenoid tertiary alcohols. There are four most commonly known isomers of terpineols: α-, β-, γ-, and δ-terpineol. α-terpineol is widely found in nature and has been detected in more than 150 essential oils. Other isomers are not commonly found in nature. Studies have shown that α-terpineol, in addition to its traditional use, has many biological properties such as antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic (Sales et al., 2020). In our study, the amount of α-terpineol was determined to be 5.94% (Table 1). The amount of α-terpineol in lavender essential oil grown in Afyon, Eskişehir, Isparta, and Uşak regions was determined to be 0.48%, 0.45%, 2.96%, and 3.30%, respectively (Katar et al., 2020). In a study examining Polish and Bulgarian lavender, α-terpineol was detected only in Polish lavender, and its amount was 0.31% (Dębczak et al., 2022). In another study, the amount of α-terpineol was found to be 1.03% (Marincaş and Feher, 2018).

Geranyl acetate, an ester of geraniol, represents a flavor with different odor characteristics. It is also the main component of many essential oils (such as lime oil) that have high economic value. Geranyl acetate is considered the most valuable geranyl ester for its color, flavor, and aroma properties, and it has been used as a flavoring agent in food, cosmetics, and fragrance production (Zeferino et al., 2021). In our study, the geranyl acetate amount was determined to be 5.66% (Table 1). The amount of geranyl acetate in lavender essential oil grown in Afyon, Eskişehir, Isparta, and Uşak regions was determined as 0.49%, -, 1.25%, and 1.28%, respectively (Katar et al., 2020).

It is stated that linalyl acetate and linalool, the main components of LEO, have a calming effect, while camphor has a stimulating effect on humans (Tomi et al., 2018). Due to the stimulant effect of camphor on sympathetic nerves, the higher amount of camphor in lavandin oil may counteract the relaxing effects of linalool and linalyl acetate in true lavender (Tomi et al., 2018). Additionally, as the camphor content increases, the LEOs scent deteriorates, so it is not preferred in the perfume industry (Kara and Baydar, 2013).

In the Katar group's study (2020), the camphor content in EO samples varied from 4.48% to 7.60% in Afyon, Isparta, Eskişehir, and Uşak locations. According to the values obtained from this study, camphor wasn't determined from the LEO used. The fact that LEO obtained from a lavandin variety grown in Kastamonu does not contain camphor shows high quality. This EO is therefore suitable for use in the perfume and aromatherapy industries. Additionally, as stated in the Ph. Eur., the camphor content is required to be at most 1.2% for being used in the perfume and pharmaceutical industries (Katar et al., 2020; Pokajewicz et al., 2021).

4. Conclusion

Plant-derived EOs have various applications in the food, pharmaceutical, and cosmetics industries. Different factors, such as the environmental conditions, the plant's growing season, and the extraction method of its oil, affect the quality and quantity of EO components. Essential oils must be characterized by reliable techniques to ensure quality in pharmaceutical, cosmetic, and food products. This study revealed the volatile composition of LEO via the GC-MS technique. The key constituents of the LEO were identified as linalool (28.44%) and linalyl acetate (17.12%). In addition, the high values of linalool and the absence of camphor in the LEO used in this study indicate its high quality and make it suitable for use in the food, beverage, flavorings, pharmaceutical, perfume, and aromatherapy industries.

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