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Analysis of phenolic compounds in flowers and leaves of English Lavender (*Lavandula angustifolia* Mill.) using UPLC-ESI-MS/MS

İngiliz Lavantası (Lavandula angustifolia Mill.)'nın çiçek ve yapraklarının fenolik içeriklerinin UPLC ESI-MS/MS ile analizi

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Abstract
Lavender (*Lavandula angustifolia* Mill.) is used as raw material in various industries such as pharmaceuticals, cosmetics, etc. The aim of this study is to determine the phenolic compounds and ratios of these compounds medicinal lavender (*Lavandula angustifolia*) in its leaves and flowers. A total of 9 phenolic compounds were identified in the leaf and flower samples obtained from the lavenders grown in Ula (Mugla) during the flowering period. The high ratios of phenolic compounds detected in leaf and flower samples of Lavender (*Lavandula angustifolia* Mill.) were protocatechuic acid (189.38 µg/kg), 3,4-dihydroxybenzaldehyde (168.97 µg/kg), and 4-vanillic acid (77.54 µg/kg).

Özet
Lavender (*Lavandula angustifolia* Mill.), ilaç, kozmetik vb sanayi gibi farklı birçok endüstri kollarında hammadde olarak kullanılmaktadır. Bu çalışmanın amacı, tıbbi lavantanın (*Lavandula angustifolia*) yaprak ve çiçeklerindeki fenolik bileşikleri ve bu bileşiklerin oranlarını belirlemektir. Ula (Muğla) yetiştirilen çiçeklenme döneminde elde edilen yaprak ve çiçek örneklerinde toplam 9 fenolik bileşen belirlenmiştir. Lavender (*L. angustifolia* Mill.) yaprak ve çiçek örneklerinde; Protocatechuic acid 189,38 (µg/kg); 3-4-Dihydoxy benzaldehyde (168,97 µg/kg), 4- Vanilic acid 77,54 **(**µg/kg) yüksek oranlarda tespit edilen fenolik bileşenlerdir.

INTRODUCTION

Since ancient times, humankind has benefited from plants as both a source of basic nutrients and medicines. Edible parts of plants and their poisonous and healing properties have been discovered through trial and error methods, and medicinal plants have not only been collected, but also have been cultivated throughout history (Baydar 2005). The term "Medicinal Plants" refers to the plants utilized in both conventional and modern medical treatments as herbal remedies (Baydar 2007). Secondary metabolites, which are found in plants natural structure, and various combinations of these compounds are what give them their therapeutic effects. In addition to being used for therapeutic purposes, plants are used to produce economically important and irreplaceable natural products that are used in the fields of chemistry, food, cosmetics, and agricultural pest control, especially in the pharmaceutical industry (Sökmen and Gürel 2001). These products, which vary a lot in number and structure, can be grouped mainly terpenes, nitrogenous compounds and phenolic compounds. Of these compounds, especially phenolic compounds are polyphenolic compounds seen in all parts of plants and contain more than 8000 flavonoid groups, mostly in leaves, flowers, and roots in nature (Yıldız and Baysal 2003). Phenolic compounds are one of the most active natural antioxidants containing one or more hydroxyl groups bound to an aromatic ring and their antioxidant effects are realized by binding free radicals, forming chelates

with metals, and inhibiting the enzyme lipoxygenase (Güleşçi and Aygül 2016). The most common herbal phenolic antioxidants are flavonoids followed by cinnamic acid derivatives, coumarins, tocopherols, and phenolic acids especially rich in phenolics, are widely used in traditional medicine to treat many diseases, and extend the shelf life of foods (Aftab 2019, Harborne and Williams 2000, Silva et al. 2000, Merken et al. 2001).

Turkey is one of the most prominent gene centers for the Lamiaceae family. Turkey is represented by 45 genera in this family, 565 species, and a total of 735 taxa (Güner et al. 2012). Since most members of the Lamiaceae family are rich in essential oils, aromatic oils, and similar secondary metabolites, they are of great importance in medicine, pharmacy, food, cosmetics, and perfumery fields (Baytop 1984). There are 39 species, several hybrids, and over 400 officially registered varieties of the genus *Lavandula* (Lamiaceae family) (Benabdelkader et al. 2011). The name lavender comes from lavo, lavare verbs, which means to wash or clean in Latin. Lavender is also mentioned in Dioscorides' work "De Materia Medica", in which he praised its medicinal properties, and has been known since ancient times (Góra and Lis 2005).

L. angustifolia Mill. is a medicinal aromatic plant, in the *Lamiaceae* family, with strong biological properties and multiple-use and its effectiveness has been proven against many diseases (Zhao et al. 2015). It has traditionally been used for numerous disorders such as restlessness, insomnia, spasms, migraines, dizziness, hysteria, high fever, infectious diseases, loss of appetite, neural disorders, gastrointestinal complaints, bladder inflammation, rheumatism, Metrorrhagia (uterine hemorrhage), leucorrhoea (vaginal discharge), intestinal parasites, upper respiratory diseases (Valnet 2001). *Lavandula angustifolia* (formerly *Lavandula officinalis* chaix or *Lavandula vera*), known by names such as genuine lavender and medicinal lavender, is a multi-year medicinal, aromatic and ornamental, half-bushy plant, 20-60 in length, with flowers in lilac or grayish-blue color, which is naturally spreading in the central highlands of the northern Mediterranean (600-1500 m) from Spain to Greece, and farmed all over the world (Ceylan 1996, Baytop 1999, Lis-Balchin 2002, Prusinowska and Śmigielski 2014, Zhao et al. 2015, Donadu et al. 2017).

Although there are different species of the genus *Lavandula* in the flora of Turkey, this species does not spread naturally (Davis 1982, Baytop 1999). However, this species is found as an ornamental plant in parks and gardens, and its agriculture is performed in well-drained, fertile and calcified soils in Turkey (Zeybek and Zeybek 1994, Góra et al. 2005). In addition to being a valuable ornamental plant, with its fragrant and decorative flowers, Lavender (*Lavandula angustifolia*) is widely used in the cosmetic, perfume, food, and aromatherapy industries, thanks to its essential oils, which have a wide range of uses (Ceylan et al. 1988, Ceylan et al. 1996). *L. angustifolia* contains large amounts of essential (e.g. aromatic compounds) and non-essential phenolics, anthocyanidins, flavones (luteolin and apigenin, kaempferol glycosides, rutin, quercitrin, hesperidin), phenolcarboxylic acids (rosmarinic acid, caffeic acid), phytochemicals (H´eral et al. 2020, Zhao et al. 2015, Zheng et al. 2019, Rădulescu et al. 2017) and is rich in mineral elements (calcium, magnesium, zinc, manganese) (Imelouane et al. 2011, Prusinowska et al. 2014). The two most important components in lavender oil are linalyl acetate (20-60%) and linalool (20-35%). The best quality lavender oil is obtained from the *L. angustifolia* species (Baydar 2007). Various biological and pharmacological properties of the lavender genus, such as antitumor, antimicrobial, and antioxidant properties, have long attracted the attention of researchers. Although there are many studies on reported biological activities and their essential compounds, there is a limited number of studies on phenolic compounds of lavender plants. For this reason, this study aims to reveal the phenolic compounds of Lavender (*L. angustifolia*), which have been utilized for many years in numerous industrial areas, such as cosmetics and body care, as well as in folk medicine today.

MATERIAL and METHODS

Plant Material

The plant materials *Lavandula angustifolia* Mill. which has 1+0 age flowers and leaf samples were obtained from Muğla-Gökova planted in trial fields in 2021. *L. angustifolia* were chosen since *L. angustifolia* was widely planted in this area. The leafy stalks and flowers were gathered for use in extraction analyses. The samples were collected and placed in plastic bags, with each bag being named. Each plastic bag's label was labeled with information on the collecting time, location, and elevation. These samples were then dried in an airy place and semi-shadowy at room temperature (at 25°C) before the extraction analyses. These plants samples were kept and identified at Muğla Sıtkı Koçman University Research Center Laboratory.

Extraction of Samples

Dry flowers separated from leafy stalks and flowers were extracted for analysis of their phenolic content. 320-g-sample was dried, extracted with 400 mL of 96% ethanol for were mixed using an orbital shaker for 30 minutes. Then extraction was continued until obtain raw extracts.

Standards and Reagents

HPLC grade acetonitrile, hexane, and methanol for phenolic component extraction and chromatographic separation were acquired from Merck (Darmstadt, Germany). The standards used for quantification and identification of phenolic compounds were: p-coumaric acid, gentisic acid, pyrogallol, 3,4-dihydroxybenzoic acid, 4-hydroxy benzoic acid, vanillic acid, pyrocatechol, 3,4-dihydroxybenzaldehyde, juteolin, galantamine, vanillin, epicatechin, myricetin, syringic acid, ferulic acid, homogentisic acid, catechin gallate, rutin, trans2-hydroxy cinnamic acid, transcinnamic acid, resveratrol, caffeic acid, catechin hydrate, apigenin, naringenin, genistein, quercetin, chlorogenic acid chrysin, hesperetin, kaempferol were supplied from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). HPLC grade water was 18.2 MΩ.

Ultrasonic-assisted Extraction of Phenolic Compounds From Plant Samples

Ultrasonic-assisted extraction of phenolic compounds from *L. angustifolia* samples was utilized a liquid-liquid extraction process which made minor adjustments (Kıvrak et al. (2018). In a centrifuge tube, 2.0 g of Lavandula angustifolia samples were weighed, and 30 mL acetonitrile and 15 mL n-hexane were added. The mixture is stirred for 2 minutes before being extracted in an ultrasonic bath for 10 minutes and centrifuged for 5 minutes at 4000 rpm. The acetonitrile layer was separated from the rest of the mixture. Those processes were repeated two more times. The acetonitrile extracts were mixed and rinsed via petroleum ether before being dried with a nitrogen evaporator. The sediment was solved in methanol:water mixture (40:60, v/v), and then leached with syringe filter (pore size: 0.20 µm and PTFE membrane) and 2 µL injected into UPLC-ESI-MS/MS instrument. The C18 column was used to analyze phenolic compounds in oil samples, and gradient elution was used to separate the compounds. (S1) 0.5% acetic acid in water and (S2) 0.5% acetic acid in acetonitrile were used as mobile phases. In a column oven at 40°C, elution was performed with eluents (S1) and (S2) at a flow rate of 0.650 mL/min (S2). The elution sequence included a linear gradient mode for 1 minute at 99% of (S1), a 10-minute transition from 99% to 70% of (S1), a 2-minute transition from 70% to 99% of (S1), and a 3-minute plateau at 99% of (S1) (S1). The last plateau resulted in the column's re-equilibration. Ion mode (ESI), source temperature 150°C, desolvation temperature 500°C and nebulizer 7.0 bar were the conditions for tandem mass spectrometry. Table 1 explains the confirmation/quantification of mass transitions (m/z) and their collision energies.

UPLC-MS/MS Analysis of Individual Phenolic Compounds

Individual phenolic compounds were examined in samples of *L. angustifolia* were detected via an UPLC–ESI–MS/MS instrument. The mass spectrometry parameters, confirmation and quantification mass transition (*m/z*), and their collision energies are articulated with other study (Kıvrak et al. 2019). Separation processes were conducted with a C18 column.

RESULTS and DISCUSSION

In this study, the phenolic composition of *L. angustifolia* leafy stalks and flower samples was analyzed and identifies by UPLC-ESI-MS/MS, and a total of 9 phenolic compounds were specified. Among them, the major compounds were protocatechuic acid (189.38µg/kg), 3,4-dihydroxybenzaldehyde (168.97µg/kg), and vanillic acid (77.54µg/kg). Other compounds identified in the study at higher rates were ferulic acid (57.53 mg/kg), and caffeic acid (51.37 µg/kg) (Table 1).

In previous studies, the effects of ferulic acid, caffeic acid, p-coumaric acid, which are among the phenolic compounds identified in the leafy stalks and flowers of *L. angustifolia*, have been investigated in different types of cancer (Rusak et al. 2010, Janicke et al. 2011, Hudson et al. 2000). Moreover, Kampa et al. (2004) have investigated the antiproliferative effect of ferulic acid, 3,4-dihydroxybenzoic acid, syringic acid and caffeic acid on human breast cancer cells (Moon et al. 2006). Therefore, this situation shows the importance of phenolic compound content in terms of human health.

There are many studies in the literature investigating the chemical composition and antioxidant properties of the lavender plant. Adaszyńska-Skwirzyńska and Dzięcioł (2017) were used HPLC to analyze the phenolic acid content in the methanol extracts of the two different

Table 1. Phenolic compounds of L. angustifolia (µg/kg) and method parameters for the phenolic compounds analysis via UPLC-ESI-MS/MS

COMPOUNDS	*L. angustifolia*	Quantification>confirmatory transition (m/z)	[c]RT(Min)
4-Hydroxy benzoic acid	32.54±0.212132	136.98 > 93.03. 65.10	2.75
3-4-Dihydoxy benzaldehyde	168.97±1.400071	137.00 > 91.93, 107.94, 136.00	2.76
trans-cinnamic acid	ND		
Vanillin	12.55 ±0.014142	150.95 > 135.94, 91.90, 107.97	2.74
Gentisic acid	26.92±0.021213	153.05 > 109.04, 108.03, 81.00	1.85
Protocatechuic acid	189.38±0.007071	153.06 > 108.00, 81.01, 91.01	1.85
p-coumaric acid	1.32±0.015556	163.01 > 119.04, 93.00, 117.01	4.65
Vanilic acid	77.54±0.009192	166.98 > 151.97, 108.03, 123.03	3.61
Caffeic acid	51.37±0.015556	179.10 > 135.14, 107.10, 133.9	3.65
Ferulic acid	57.53±0.009192	193.03 > 134.06, 178.00, 149.02	5.36
Kaempferol	ND		
Myricetin	ND		

ND: not determined

cultivars of *L. angustifolia* (cultivars of *L. angustifolia*: 'Ellagance Purple' and 'Blue River', containing leafy stalks and flowers). According to chromatographic analyses, it was determined that the tested varieties and different morphological parts of *L. angustifolia* varied in phenolic acid content rosmarinic acid (1.505-5.569 mg g^{-1} d.m.) was determined at relatively high rates, in all samples. In addition, each sample was also found to contain caffeic acid, but the amounts were at low quantities (0.080-0.120 mg g^{-1} d.m.), and the ferulic acid compound was found only in leafy stalks (2.281-2.687 mg g^{-1} d.m.).

In the flowers of *L. angustifolia*, which was assessed by Arceusz and Wesołowski (2011), ferulic acid (0.0196 mg g^{-1} dm) and caffeic acid (0.0589 mg g^{-1} dm) were the most common, whereas there was no gallic acid detected. However, Komes et al. (2011) have characterized these compounds by an increased concentration in the flowers of *L. angustifolia* in the following order: ferulic acid > rosmarinic acid > caffeic acid. In other studies, methanolic extracts of *L. angustifolia* were found to contain a large variety of phenolic acids (vanillic acid, chlorogenic acid, syringic acid, o-coumaric acid, methoxy-cinnamic acid, ferulic acid, 4-hydroxybenzoic, p-coumaric acid and sinapic acid) (Blažeković et al. 2010, Spiridon et al. 2011, Sytar et al. 2016). In their study, Alasalvar and Yildirim (2021) identified gallic acid (1.05 ± 0.61 mg/g), ferulic acid (0.48 ± 0.27 mg/g), caffeic acid (0.19 ± 0.02 mg/g), rosmarinic acid (5.49 ± 0.96 mg/g), and rutin (11.13 ± 0.97 mg/g) using HPLC-DAD in *L. angustifolia* samples obtained under optimized conditions. In another study, Radulescu et al. (2017) analyzed the extracts from flowers of *L. angustifolia* and identified primary phenolics gallic acid, isoquercitroside, umbelliferone, vitexin, luteolin 7-O-glucoside, and chlorogenic acid. Spiridon et al. (2011) identified 16 compounds in the ethyl acetate extracts of *L. angustifolia* leaves and flowers of which ferulic acid (17.29%) and kaemferol malonyl glucoside (15.22%) had the highest rates, but apigenin rhamnosyl glucoside, rosmarinic acid, caffeic acid, chlorogenic acid, ferulic acid-4-O-glucoside, kaemferol-3-coumaryl glucoside, ursolic acid, i-vitexin were found at much lower concentrations. In the same study, although rosmarinic acid (9.53%) was detected at a lower concentration, the phenolic acid compound was determined at high rates and trace amounts of caffeic acid (0.23%) were detected. In *L. viridis* methanol extracts, rosmarinic acid and luteolin-7-O-glucoside compounds were determined at high rates (Costa et al. 2011). Adaszyńska-Skwirzyńska and Dzięcioł (2017) The rosmarinic acid compound determined as 1.505-5.569 mg g^{-1} d.m. in their study but the compound were not determined in our samples of *L. angustifolia* investigation. In this study, while protocatechuic acid (189.38µg/kg);3,4-dihydroxybenzoic acid (168.97 µg/kg) and vanillic acid (77.54 µg/kg) identified at the high rates, and ferulic acid (57.53 µg/kg), caffeic acid (51.37µg/kg) as the other high rates compounds were identified in leafy stalks and flower samples of *L. angustifolia.* When compare these results with the literature may be seen that the phenolic compounds concentrations some differed from in other studies but ferulic acid and caffeic acid determined high concentrations phenolic compounds were similarities the literature.

CONCLUSION

Due to its geographical location, Turkey is a country with the most favorable climatic and soil conditions for growing medicinal aromatic plants in the world. It should be encouraged to establish small-scale industrial facilities, through cooperation in rural areas particularly, in order to produce high value-added products by growing lavender plant, which has a large market, in medium-sized areas in certain regions, and to process the products provided from lavender farming areas. It is seen that the production areas of aromatic plants have increased with the <growth in the natural products market in the world and in Turkey in recent years. Some important requirements must be fulfilled in order to provide products to target markets with desired standards and efficiency and to follow developments by taking the right steps in lavender agriculture and industry in global market. Between $1.8 billion and $2 billion of essential oil are exported worldwide each year, of which about $50 million is lavender oil (Baydar 2007, Boelens 1995). Turkey's lavender oil imports, however, are increasing every year.

Phenolic compounds form a class of bioactive metabolites that are important for extraction from plants, characterization, and medicinal applications. Phenolic compounds continue to attract the increasing attention of scientists as they form a rich and abundant phytochemical class with potential benefits to human health (Araujo et al. 2015, Yassine et al. 2021, Dinu et al. 2016). Further characterization of the phenolic composition is also needed to take advantage of natural sources of antioxidants (Kahkönen et al. 1999). In this study, the major compounds detected were protocatechuic acid (189.38µg/kg), 3.4 dihydroxybenzaldehyde (168.97µg/kg), and vanillic acid (77.54µg/kg). According to the results obtained, the values of phenolic compounds in *L. angustifolia*, which have economic significance for Turkey and the world, show similarities compared to the values published in the literature, despite some differences in the values. Chemically, these differences in contents can be explained by geographical origins, different environmental conditions, parts of the plant extracted, and extraction methods.

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