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Essential oil composition and antioxidant activities of eight cultivars of Lavender and Lavandin from western Anatolia

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ABSTRACT

The aim of this study was to examine the essential oil compositions and antioxidant activities of six *L. angustifolia* cultivars and two *L. x intermedia* in Turkey. The chemical composition of essential oils obtained by steam-distillation from fresh flowers of *Lavandula* samples were analyzed using gas chromatography-mass spectrometry (GC/MS). Antioxidant activities of lavender and lavandin cultures were evaluated using β-Carotene Bleaching, DPPH· and ABTS·$^+$ assays. Sixty-six components were identified for all cultivars. The results indicate that there is a significant difference between *L. angustifolia* and *L. x intermedia* cultivars in terms of major components, linalool, linalyl acetate. Linalyl acetate (%46.887–%29.098) and linalool (%36.801–%28.102) were determined in six the cultivar of *L. angustifolia* and Super A cultivar of *L. x intermedia*. Linalool (%28.486) and eucalyptol (%15.650) were found as abundant in *L. x intermedia* Grey Hedge. Evaluation of antioxidant activity of the studied samples emphasizes that highest inhibition was observed in *L. angustifolia* Yubileina cultivar (23.67 ± 0.14 μg mL^{-1}). However, in the DPPH· assay, *L. x intermedia* Super A cultivars showed the highest inhibition activity (IC_{50}) 89.81 ± 0.17 μg mL^{-1}. In the ABTS·$^+$ assay, *L. angustifolia* Sevtopolis cultivar displayed highest radical scavenging activity with inhibition values of 61.23 ± 0.11 μg mL^{-1}. Essential oil composition of eight lavender and lavandin varieties, used in the industry, was analyzed. And there is a significant difference in terms of camphor composition. The obtained data have been inquired by principal components analysis (PCA), allowing differentiation of eight lavender and lavandin cultures by their variety origins. High levels of linalyl acetate and linalool, low level of camphor (< 0.5%) and high antioxidant activity shown that, these cultivars may be considered as a natural raw material source for pharmaceuticals and cosmetic products.

1. Introduction

Medicinal and aromatic plants have been used for variety of aims since ancient times and recently have a strong position as economical crops around the world for essential oil production (Bajalan et al., 2016). Nowadays, these plant essential oils have become commercially popular due to their impression as a “well-being” life style (Yang et al., 2010).

The industrial cultivation and production of *Lavandula angustifolia* Mill. and *Lavandula x intermedia* Emeric as medicinal and aromatic plants have been rapidly raised during the last years and the World’s interest for *Lavandula* essential oil is still increasing. Therefore, detailed analyses of produced essential oils to figure out their quality and quantity are highly important for the selection of industrial usage. The trade value of essential oil export in the world is approximately 1.90–2.00 billion dollars and about 50 million dollars of this currency belong to *Lavandula* essential oil (Gökdoğan, 2016).

Essential oils, obtained from medicinal and aromatic plants by using various methods such as steam distillation, hydro distillation, cold press or extraction, are mixtures of various chemical constituents including terpenes, alcohols, aldehydes, phenols and esters, which may produce significant fragrances (Grassmann and Elstner, 2003; Ali et al., 2015).

The genus *Lavandula* (*Lamiaceae* family) is one of the most well-known essential oil crop in the world with its 39 species, numerous hybrids and about 400 officially registered cultivars (Benabdelkader et al., 2011). The main producing regions are Europe, the Middle East, Asia and Northern Africa. France and Bulgaria. Those dominate the production but also Morocco, Yugoslavia, Hungary, Italy, Russia, Spain, Romania, Ukraine and Turkey have production in different amounts (Zheljazkov et al., 2013).

Many *Lavandula* species have essential oils with aromatic and medicinal properties that able use in the cosmetic, pharmaceutical and food industries (Torras-Claveria et al., 2007) but specially three of these species are important with their high commercial value: Lavender

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(*Lavandula angustifolia* Mill. syn. *L. officinalis* Chaix ex Vill syn. *L. vera* DC syn. *L. spica* (true lavender, fine lavender or English lavender), Lavandin (*Lavandula intermedia* Emeric ex Loisel syn. *L. hybrid,* a hybrid of *L. angustifolia* and *L. latifolia*), and Spike lavender (*Lavandula latifolia* Medicus) (Lesage-Meessen et al., 2015). The world production of lavender oil is approximately 200 tons per year. Bulgaria, UK and France are dominating the lavender essential oil production. The world production of lavandin oil is about 1200 tons per year with a rate of 90% representing by France (Karapandzova et al., 2012).

The essential oils of the *Lavandula* species have the same chemical composition but these components are present in different proportions. Oil composition and oil yield of *Lavandula* differentiate each other. Common criteria for the determination of oil quality are camphor, linalool and linalyl acetate levels of essential oil (Baydar and Kineci, 2009). According to the ISO 3515:2002 standard, lavender essential oil contains linalool (25–38%), linalyl acetate (25–45%) and camphor (0.5–1.0%), lavandin essential oil contains linalool (24–35%), linalyl acetate (28–38%) and camphor (6–8%) according to the ISO 8902:2009.

Due to these specifications, *lavandula* essential oil is used in food manufacturing industry as flavoring agent, preservative additives for cosmetics and fragrance industry including soaps, colognes, perfumes, skin lotions (Da Porto et al., 2009; Muyima et al., 2002; Kunicka-Styczyńska et al., 2009; Fakhari et al., 2005). Paricularly, while Lavender essential oil is used in industrial areas such as perfumery, pharmaceuticals and cosmetic due to its high linalool and linalyl acetate content, lavandin essential oil is commonly used in hygiene products, industrial and household cleaner products, detergents and insecticides due to high levels of camphor (Cavanagh and Wilkinson, 2002; Lesage-Meessen et al., 2015). Additionally, altough lavandin essential oil is produced in higher yields than lavender essential oil (120 kg/ha, 40 kg/ha, respectively) (Carrasco et al., 2016a; Kara and Baydar 2013), lavender essential oil quality and price (85–150€/kg) is higher than lavandin essential oil (19€/kg) (Lesage-Meessen et al., 2015).

Lavandula essential oil, with the main active constituents linalool, linalyl acetate, 1,8-cineole, *cis* and *trans*-ocimene, terpinen-4-ol and camphor, has been reported to have antimicrobial, anticholinesterase and antioxidant activities (Costa et al., 2012; Hanamanthagouda et al., 2010; Cavanagh and Wilkinson, 2002; Gonçalves and Romano, 2013). Thus, *Lavandula* oil promotes healing symptoms for stress, exhaustion, migraines, anxiety, insomnia and depression (Rafie et al., 2016; Danh et al., 2013; Fismer and Pilkington, 2012; Koulivand et al., 2013).

When we consider all the pharmacological properties and the rich chemical content, *lavandula* essential oil is a significant product and encourage the cultivation of this plant as an industrial crop for essential oil production (Stanev et al., 2016; Adal et al., 2015).

In the literature, there is a lot of research dedicated to essential oil compositions and antioxidant activities of *L. angustifolia* species (Hanamanthagouda et al., 2010; Costa et al., 2012; Kiran Babu et al., 2016; Fakhari et al., 2005), its Bulgarian cultivars (Zagorcheva et al., 2013; Baser et al., 2005; Stanev et al., 2016) and *L. x intermedia* (Bajalan et al., 2016; Blazekovic et al., 2010; Carrasco et al., 2016b; Erbaş and Baydar, 2008) but there is no comperative study on chemical compositon and antioxidant activites of *L. angustifolia* Mill. ("Sevtopolis", "Yubileina", "Druzhba", "Raya", "Hebar", "Hemus") and *L. x intermedia* ("Super A", "Grey Hedge") essential oils in Turkey.

The aim of this study to investigate the essential oil composition and antioxidant activity of *L. angustifolia* cultivars ("Sevtopolis", "Yubileina", "Druzhba", "Raya", "Hebar", "Hemus") and *L. x intermedia* cultivars ("Super A", "Grey Hedge") in Turkey and encourage the breeder to growth of this industrial crops by showing their quality specifications and possible commercial value.

2. Materials and methods

2.1. Plant material

Whole plant material of six *Lavandula angustifolia* Mill. cultivars including "Sevtopolis", "Yubileina", "Druzhba", "Raya", "Hebar", "Hemus" were collected from Fethiye, Kabaağaç (36°33′45.80" N–29°17′21.07" E), *Lavandula x intermedia* var. Grey Hedge from Fethiye, Göcek, Gökçeovacık (36°47′11.67" N–28°58′51.17" E), and *Lavandula x intermedia* var. Super A were collected from Burdur, Akçaköy (37°42′28.14" N–29°52′37.94" E) Turkey in June 2016 when the crop was full of blossom. The spices were identified from Muğla Sıtkı Koçman University, Faculty of Science, Department of Molecular Biology and Genetics. The plant materials were studied fresh.

2.2. Isolation of the essential oil

Aerial parts of freshly harvested plants were immediately subjected to steam distillation for 2 h to extract the essential oil. The resulting oil was dried with anhydrous sodium sulphate and stored in an amber bottle at +4 °C in a refrigerator until time of analysis.

2.3. GC/MS analysis

GC/MS analyses were carried out using an Agilent 6890N Gas Chromatograph equipped with a split/splitless injector (200 °C), a DB-1MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm) and coupled with an Agilent 5975C MS Detector, operating in the electron impact (EI) mode at 70 eV. Transfer line temperature was set at 250 °C. The carrier gas was He (2.6 mL min^{-1}), and the oven temperature was programmed from 60 °C to 280 °C at a rate of 3 °C/min. The injected volume was 1 μL and the split ratio 50:1.

The identification of the compounds was based on the comparison of their retention times (RT) and mass spectra with those from the NIST and Wiley 2008 libraries. Relative percentages of compounds were calculated based on the peak areas from their GC–MS chromatograms.

2.4. Antioxidant activity

The in-vitro antioxidant activity of Lavander and Lavandin essential oils were examined by three complementary methods, inhibition of β-carotene bleaching assay, DPPH radical scavenging activity and ABTS cation radical decolorization assay and results calculated with the same equations given by Kıvrak and Kıvrak (2014). α-Tocopherol were used as standard and all tests were done in triplicate.

2.4.1. Inhibition of β-carotene bleaching assay

The total antioxidant activity was determined using β-carotene-linoleic acid test method (Miller, 1971) based on the detection of inhibition of conjugated dien hydroperoxides because of oxidation of linoleic acid with slight modifications described by Kıvrak (2015). β-Carotene (0.5 mg), dissolved in 1 mL of chloroform, was mixed with linoleic acid (25 μL) and Tween 40 emulsifier (200 mg). Chloroform was evaporated under vacuum, 50 mL of distilled water saturated with oxygen was added by vigorous shaking. Aliquots (160 μL) of this emulsion were added to 40 μL of the extract solutions at different concentrations. As soon as the emulsion was added to each tube, the zero time absorbance was initially measured at 470 nm, and then the absorbance measurements were done for every 30 min until 120 min. The results were given as 50% inhibition concentration (IC_{50}). The sample concentration inhibiting 50% antioxidant activity (IC_{50}) was calculated from the graph of activity percentage against sample concentration. The antioxidant activity was calculated in terms of percent inhibition relative to the control, using eq. (1)

$$\text{Antioxidant activity (\%)} = (A_{Control} - A_{Sample})/A_{Control} \times 100 \quad (1)$$