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Essential oil and extracts from *Lavandula angustifolia* Mill. cultivated in Bosnia and Herzegovina: Antioxidant activity and acetylcholinesterase inhibition

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ABSTRACT

Lavender (*Lavandula angustifolia* Mill.) is a perennial, aromatic, medicinal, and decorative plant widespread in the Mediterranean. Due to the high content of essential oil and numerous beneficial properties, it is an extremely valued plant species. In Bosnia and Herzegovina, lavender is cultivated mainly for the needs of the cosmetic and pharmaceutical industries. In this research, the antioxidant activity and acetylcholinesterase inhibition of essential oil (EO) and extracts isolated from lavender were evaluated. *L. angustifolia* EO was isolated using whole plant material in the flowering period by steam distillation and analyzed by gas chromatography (GC) with flame-ionization (FID) and mass spectrometric (MS) detection. Extracts were prepared by ultrasonic extraction in solvents of different polarities. Total phenolic content in extracts was determined using Folin–Ciocalteu reagent. The antioxidant activity of EO and extracts was examined by two methods, 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging activities and ferric reducing/antioxidant power (FRAP), while the acetylcholinesterase (AChE) inhibitory potential was determined using modified Ellman's method. The EO was high in content of linalool (27.72%) and linalyl acetate (22.82%), followed by α-pinene (9.82%), lavandulol acetate (7.32%), *trans*-caryophyllene (5.70%), and others. In total, 24 components were identified. Total phenol content was highest in water and ethanol extracts (45.3 and 14.40 mg gallic acid equivalent (GAE)/g dry extract). Polar extracts indicate good antioxidant power according to both methods, while EO can be considered as good inhibitor of AChE.

1. Introduction

Lavender, *Lavandula angustifolia* Mill. is an aromatic and medicinal perennial semi-shrub from the Lamiaceae family. The flowers are violet-blue, with an intense aromatic smell, and rich in essential oils (Costea et al., 2019). *L. angustifolia* is indigenous to the mountainous regions of the Mediterranean that are widely cultivated throughout the world. France and Bulgaria are the leading European countries in lavender production (Crișan et al., 2023; Giray, 2018). Since 2000, many lavender plantations have been established in Croatia, Italy, Greece, and the Mediterranean basin in general (Blažeković et al., 2010; Giannoulis et al., 2020; Pistelli et al., 2017). Due to the growing interest in the world market for this plant, plantation cultivation of *L. angustifolia* on small family farms in Bosnia and Herzegovina has become more common in recent years. Lavender is mostly cultivated for the production of essential oil.

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Commercial lavender essential oil is produced mainly by steam distillation and to a lesser extent by solvent extraction, microwave extraction, supercritical extraction, and hydro-diffusion methods (Kirimer et al., 2017). Lavender essential oil (EO) and extracts show outstanding aroma properties with a pleasant floral note (Guo & Wang, 2020). The lavender essential oil has a long tradition of application in various human activities, such as aromatherapy, traditional medicine, pharmacy, cosmetics, perfume industry, and insecticide industry (Wells et al., 2018). The composition and proportion of individual components in the essential oil significantly depend on the geographical origin, harvest time, and post-harvest processing of lavender. However, most authors listed linalool, linalyl acetate, caryophyllene, and lavandulyl acetate, as the main components of the essential oil (Da Porto et al., 2009; Kirimer et al., 2017; Smigielski et al., 2009; Verma et al., 2010).

L. angustifolia also accumulates phenolic compounds, which contribute to the bioactivity of lavender and its beneficial effect. Positive correlations were observed between the content of total phenols and antioxidant activity. The most present phenolic compounds reported in lavender include gallic acid, rosmarinic acid, caffeic acid, coumaric acid, and other polyphenols (Gallego et al., 2013; Radulescu et al., 2017; Spiridon et al., 2011).

Various studies reported that Lamiaceae species and their phytochemical substances have antioxidant and AChE inhibitory activity and thus could be beneficial in the prevention and treatment of health disorders such as Alzheimer's disease (Da Porto et al., 2009; Odak et al., 2015; Vladimir-Knežević et al., 2014). To the best of our knowledge, *L. angustifolia* phytochemical substances from mountain areas of Bosnia and Herzegovina have not been investigated yet. Hence, this study aimed to determine the chemical composition of the essential oil of *L. angustifolia* from Bosnia and Herzegovina, and examine the antioxidant properties of essential oil and extracts and their inhibitory effects on AChE.

2. Materials and methods

2.1. Chemicals

Acetone, ethyl acetate, ethanol, chloroform, *n*-hexane, acetylcholinesterase (AChE) from *Electrophorus electricus* (electric eel, Type VI-S, lyophilized powder, 200-1.000 units/mg protein), acetylthiocholine iodide (ATChI), galanthamine hydrobromide, 2.2'-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu reagent, gallic acid, TRIS-HCl buffer, trizma base, and *n*-pentane were purchased from Sigma-Aldrich (St. Louis, MO, USA). 5,5’-dithio-bis[2-nitrobenzoic acid] (DTNB) was purchased from Zwijndrecht (Belgium). Sodium acetate, acetic acid, hydrochloric acid, iron (III) chloride, and sodium sulfate were purchased from Merck (Germany). All chemicals were of analytical grade purity.

2.2. Plant material

The *L. angustifolia* was harvested in the flowering period at a local plantation (M.T., Goranci, Mostar, Bosnia and Herzegovina; altitude 700 m, 43°25'48.4"N, 17°42'43.2"E) in June 2020. The plant material was identified by a professor of Botany Ph.D. Anđelka Lasić (Department of Biology, Faculty of Science and Education, University of Mostar) and duplicate samples were preserved in the herbarium of the department with number FPMOZ-SB-4-2020.

L. angustifolia essential oil was extracted by steam distillation of whole plant material, for 2 h in a distillery plant for medicinal herbs.

L. angustifolia extracts were prepared using ultrasonic extraction. Dry ground plant material (5 g) and solvent (100 ml) were mixed and submitted to ultrasonic extraction, 35 kHz at 30 °C for 60 min. Solvents of different polarities (water, acetone, ethyl acetate, ethanol, chloroform, and *n*-hexane) were applied. The extracts were filtered and evaporated under reduced pressure to dryness. The experiments were performed in triplicate. All dry extracts were dissolved in TRIS buffer (1; 2.5; 5; 7 and 10 mg/ml) for subsequent analysis.

2.3. Determination of total polyphenols

The total phenolic content in *L. angustifolia* extracts was determined by the conventional spectrophotometric method with the Folin-Ciocalteu reagent (Hernandez et al., 2010). The procedure was described in our previous study (Talić et al., 2019). Data are presented as the average of triplicate analyses for all six different extracts.

2.4. Gas chromatography-mass spectrometry (GC-FID/MS) analysis

The GC-FID/MS analysis of the *L. angustifolia* essential oil was performed on a Shimadzu GC-2010 Plus and a Shimadzu GCMS-QP2010, equipped with an AOC-20i autosampler. Fused silica capillary column Inert Cap (5% diphenyl-95% dimethylpolysiloxane, 30 m × 0.25 mm internal diameter, film thickness 0.25 µm) was used. The solution of essential oil in pentane (1:500 v/v) (1.0 µl) was injected in splitless mode with helium as carrier gas. The operating conditions were as follows: injection temperature 260 °C; helium flow rate, 1.11 ml/min; oven temperature program: 50 °C (2 min), 50-120 °C (2 °C/min), 120-220 °C (5 °C/min). FID conditions: 260 °C, air/H_2 flow: 400/50 ml/min. MS (EI) conditions: ion source temperature: 200 °C, interface temperature: 280 °C, ionization voltage: 70 eV, mass range: m/z 40-400 u; scan time: 0.5 sec. The quantification (%) of each detected component of *L. angustifolia* essential oil was determined by normalizing the peak area in FID analysis. The components in the essential oil were identified via comparison of their retention index on the non-polar column with the *n*-alkane series (C_8-C_{40}). Their mass spectra were compared with the Wiley 7 and NIST spectrum library. The analyses were done in triplicate and the results were represented as mean values ± standard deviation.

2.5. DPPH radical scavenging assay

The antioxidant activity of six different extracts and the essential oil was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH (Brand-Williams et al., 1995). Extracts prepared in TRIS buffer (1-20 g/l) and EO prepared in 86% ethanol (75-300 g/l) were tested. The 50 µl of the sample was added in 1 ml of 96% ethanol solution of DPPH (6 × 10^{-5} M). Post incubation of 30 min the absorbance was measured at 517 nm. The BHT was used as a positive control (1-20 mg/ml). All measurements were performed in triplicate. Inhibition of DPPH was calculated according to the formula below.

$$Inhibition\ (\%) = \frac{A_{C(0)} - A_{A(t)}}{A_{C(0)}} x\ 100$$

where $A_{C(0)}$ is the absorbance of the control at t = 0 min, and $A_{A(t)}$ is the absorbance of the antioxidant at t = 30 min. The results were classified as low: 5-25%; moderate: 25-50%; or good: 50-100%.

2.6. Ferric reducing/antioxidant power (FRAP) assay

The method is based on the ferric-reducing ability of plasma as a measure of antioxidant power (Benzie & Strain, 1996). In this assay, the antioxidant activity of the samples tested was calculated with reference to the reaction signal given by a Fe^{2+} solution of known concentration, representing a one-electron exchange reaction. The preparation of the FRAP reagent and the procedure of measurement was described in a previous study (Talić et al., 2019). The corresponding regression calibration equation for $FeSO^4 \times 7\ H_2O$ (1.0-10.0 mg/ml) was A = 0.1463c + 0.2315 (R^2 = 0.9973). Plant extracts solutions (1.0-10.0 mg/ml, prepared in TRIS buffer) and EO solutions (1.0-10.0 mg/ml, prepared in 86% ethanol) were tested. The BHT was used as the positive control (1.0-10.0 mg/ml). All measurements were performed in triplicate.

2.7. Acetylcholinesterase inhibition assay

The acetylcholinesterase (AChE) inhibitory potentials of *L. angustifolia* extracts and EO were measured by modified Ellman's method (Ellman et al., 1961) as described by Wszelaki et al. (2010). Briefly, 180 µl of TrisHCl buffer (50 mM, pH 8.0), 10 µl of AChE (0.055 U/ml, prepared in 20 mM TrisHCl buffer, pH 7.5), and 10 µl of tested solution (1.0-10.0 mg/ml of plant extracts, 1.0-10.0 mg/ml of EO solutions) were mixed and incubated for 20 minutes (4 °C). The reaction was initiated with the addition of 10 µl of DTNB (0.6 mM) and 10 µl of ATChI (10 mM). Galantamine was used as a positive control (1.0-10.0 mg/ml). Inhibition of AChE was measured using a 96-well microplate reader (IRE 96, SFRI Medical Diagnostics) at 405 nm over a period of 30 minutes at 25 °C. The experiment was run in triplicate. The percentage of enzyme inhibition was calculated according to the formula below.

$$AChE\ inhibition\ (\%) = \frac{A_C - A_T}{A_C} x\ 100$$

where A_C is the activity of the enzyme without the test sample and A_T is the activity of the enzyme with the test sample.

2.8. Statistical analysis

The SPSS (SPSS Inc., Chicago, IL, USA) statistical analysis was applied. All data are expressed as mean ± standard deviation (SD). One Way Analysis of Variance (ANOVA) coupled with Tukey's posthoc tests was used to compare mean values between different extracts, positive control samples, and essential oil. '*p*' value for non-statistical differences was presented. The $p < 0.050$ was defined as statistically significant.

3. Results and discussion

As part of our ongoing research on the properties and possible applications of aromatic herbs from Bosnia and Herzegovina, the extracts at different polarities and essential oil from *L. angustifolia* collected in these regions were studied.

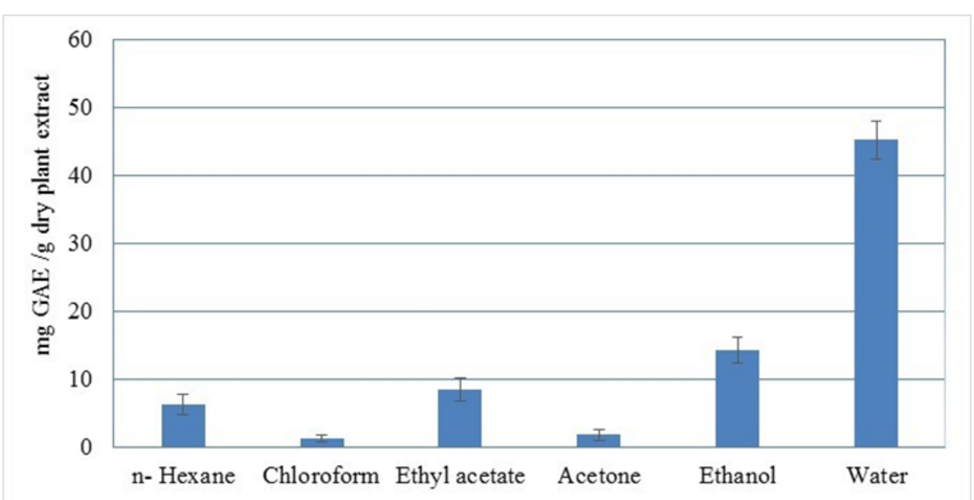

Figure 1. Total polyphenols content in *L. angustifolia* extracts

3.1. Total polyphenols in L. angustifolia extracts

Polyphenols were found in aromatic and medicinal plants, fruits, vegetables, and seeds. In the last few decades, it has been confirmed that polyphenols have several beneficial effects on health, especially due to their antioxidant properties (Hano & Tungmunnithum, 2020). Their role as natural antioxidants is important in the prevention and treatment of inflammatory, cardiovascular, and neurodegenerative diseases and cancer (Quideau et al., 2011). Furthermore, they have a wide range of applications as food supplements, pharmaceutical, and cosmetic additives (Tungmunnithum et al., 2018).

In this study, the total polyphenol content in *L. angustifolia* extracts, prepared in solvents of different polarities, was measured. The total phenolic content, expressed as mg GAE/g dry extract (d.e.) is shown in Figure 1. Water extract was highest in total phenol content (45.30 mg GAE/g d.e.), followed by ethanol (14.40 mg GAE/g d.e.), ethyl acetate (8.50 mg GAE/g d.e.), *n*-hexane (6.40 mg GAE/g d.e.), acetone (1.80 mg GAE/g d.e.) and chloroform (1.20 mg GAE/g d.e.). In general, there is a difference in extracts obtained by polar solvents compared to nonpolar ($p < 0.050$). Pairs of extracts *n*-hexane/ethyl acetate ($p = 0.679$), *n*-hexane/acetone ($p = 0.060$), and chloroform/acetone ($p = 0.998$) displayed no significant differences.

This study confirmed that water as a polar solvent under the given conditions of ultrasonic extraction (35 kHz, 60 min, 30 °C) isolated the highest amount of polyphenolic compounds from *L. angustifolia* extracts. Other similar studies have confirmed that the concentration of total phenols is highly dependent on the solvent and extraction conditions (Radulescu et al., 2017), plant harvesting time (Duda et al., 2015), cultivation conditions, and the parts of a

plant (Adaszyńska-Skwirzyńska & Dzięcioł, 2017; Blažeković et al., 2010). Radulescu et al. (2017) have determined that the use of aqueous alcohol extraction isolated more phenolics compounds, where the main phenolics were gallic acid, umbelliferone, chlorogenic acid, luteolin 7-*O*-glucoside, vitexin, and isoquercitroside. Adaszyńska-Skwirzyńska and Dzięcioł (2017) reported that total phenolics in *L. angustifolia* flowers (1.13-1.14 mg/g dry material) were lower than total phenolics in leafy stalks (3.71-4.06 mg/g dry material). The phenolic content of the whole plant harvested in Romania had 12.44-18.16 mg GAE/g dry plant (Duda et al., 2015). Some studies have confirmed that a higher amount is obtained at the beginning of flowering, than in full bloom, due to the fact that metabolic pathways in the blooming period are activated to produce volatile compounds (Hassiotis et al., 2014).

Table 1. Chemical compositions of *L. angustifolia* essential oil

No	Name of the constituents	RI	Area (%)	Standard deviation (±)
1	β-Myrcene	987	2.15	0.26
2	4-Carene	1009	2.50	0.17
3	*α*-Pinene	1032	9.82	1.13
4	*trans*-Ocimene	1043	1.40	0.77
5	Linalool	1100	27.72	0.46
6	Alloocimene	1126	1.17	0.12
7	Camphor	1146	0.90	0.03
8	Lavandulol	1161	1.24	0.05
9	Borneol	1171	4.20	0.15
10	Terpinen-4-ol	1179	5.20	0.26
11	Cryptone	1185	0.44	0.02
12	Hexyl butanoate	1190	0.26	0.04
13	*α*-Terpineol	1193	1.09	0.05
14	*p*-Cumic aldehyde	1240	0.26	0.05
15	Linalyl acetate	1250	22.82	0.33
16	Lavandulol acetate	1281	7.32	0.17
17	Neryl acetate	1355	0.60	0.05
18	Geranyl acetate	1375	1.32	0.12
19	*trans*-Caryophyllene	1418	5.70	0.35
20	β-Farnesene	1449	1.80	0.25
21	Germacrene-D	1478	0.34	0.03
22	*γ*-Cadinene	1510	0.38	0.03
23	Caryophyllene oxide	1579	1.04	0.12
24	Δ-Cadinene	1638	0.30	0.11
	Total		99.97	

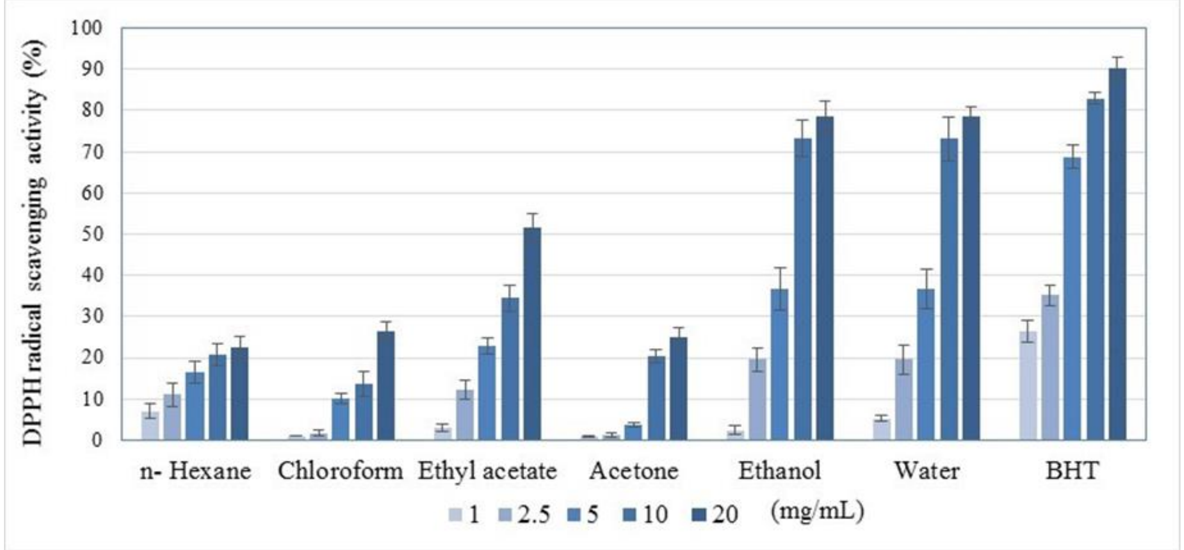

Figure 2. DPPH free radical scavenging activity of BHT and different *L. angustifolia* extracts

3.2. Chemical composition of L. angustifolia essential oil

The *L. angustifolia* EO was obtained by steam distillation and analyzed by GC-FID/MS. The detected compounds of EO are presented in Table 1. In total, 24 compounds were identified in the essential oil, representing 99.97 % of the oil composition. The compounds are presented in order of elution from the nonpolar column. The main detected compounds of *L. angustifolia* EO cultivated in Bosnia and Herzegovina were linalool (27.72%) and linalyl acetate (22.82%), both of which accounted for close to half of the content. The following compounds were α-pinene (9.82%), lavandulol acetate (7.32%), *trans*-caryophyllene (5.70 %), terpinen-4-ol (5.20%), borneol (4.20%), 4-carene (2.50%), and β-myrcene (2.15%).

The biological activity and use of *L. angustifolia* EOs depend on their chemical composition. *L. angustifolia* EO is beneficial for nervousness, insomnia, depression, migraine headaches, sprains, nerve pain, sores, and other diseases (Woronuk et al., 2011). In addition, *L. angustifolia* EO is a medicine used for the treatment of

Alzheimer's disease (Xu et al., 2017). EO rich in linalool and linalyl acetate can be considered as high in quality due to the many beneficial effects of these compounds, such as anti-inflammatory (Peana et al., 2002), anti-bacterial, anti-microbial (Białoń et al., 2019) and different therapeutic properties (Woronuk et al., 2011). According to the literature data, *L. angustifolia* EO has variable chemical composition. *L. angustifolia* cultivated in Poland as the major constituents had linalool (30.6%), linalyl acetate (14.2%), geraniol (5.3%), β-caryophyllene (4.7%), and lavandulyl acetate (4.4%) (Smigielski et al., 2018). *L. angustifolia* cultivated in the mid hills of Uttarakhand (India) had linalyl acetate (47.6%), linalool (28.1%), lavandulyl acetate (4.3%), and α-terpineol (3.7%) as the main compounds (Verma et al., 2010). Linalool and linalyl acetate were also found as the main compounds in *L. angustifolia* EO from Crimean (34.1-52.7%, 23.3-36.6%) (Białoń et al., 2019), and Australia (23.0-57.5%, 4.0-35.4%) (Shellie et al., 2002). However, a completely different composition was found in *L. angustifolia* EO from Iran. The main compounds in this essential oil were carvacrol (26.2%), limonene (19.6%), 1,8-cineole (11.8%), terpinen-4-ol (7.6%), spathulenol (4.9%), α-pinene (4.2%) and *p*-cymene(4.2%) (Bakhsha et al., 2014). Da Porto et al. (2009) investigated the chemical composition of Italian *L. angustifolia* EO, and the main compounds were linalool, linalyl acetate, 1,8-cineole, camphor, and β-caryophyllene. A higher proportion of linalool (54.0%) and a lower proportion of linalyl acetate (11.6%) were found in the essential oil from Croatia (Blažeković et al., 2018) compared to oil from Bosnia and Herzegovina. Both essential oils were characterized by a low amount of camphor (≤ 1%). All these reports suggest that the environmental conditions strongly affect the chemical profile of essential oil either in a quantitative or qualitative extent, which in turn determines their biological activity and application. EO with a high content of linalool and linalyl acetate and a low content of camphor, such as our investigated oil, are among the finest and most desirable lavender oils in the cosmetics and aromatherapy industries (Woronuk et al., 2011).

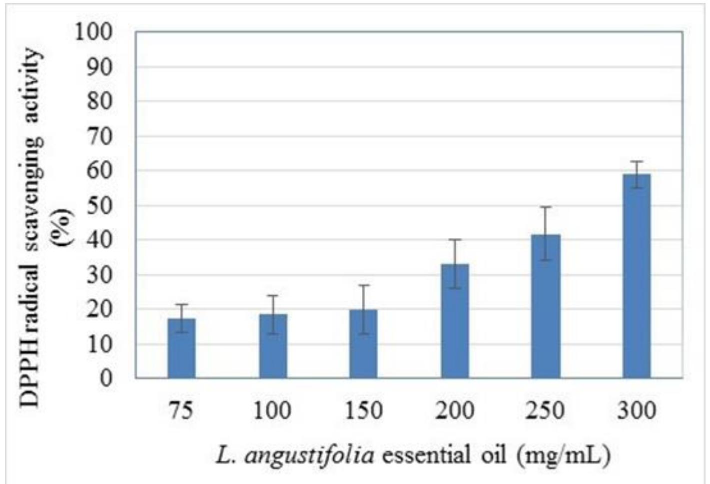

Figure 3. DPPH free radical scavenging activity by *L. angustifolia* essential oil

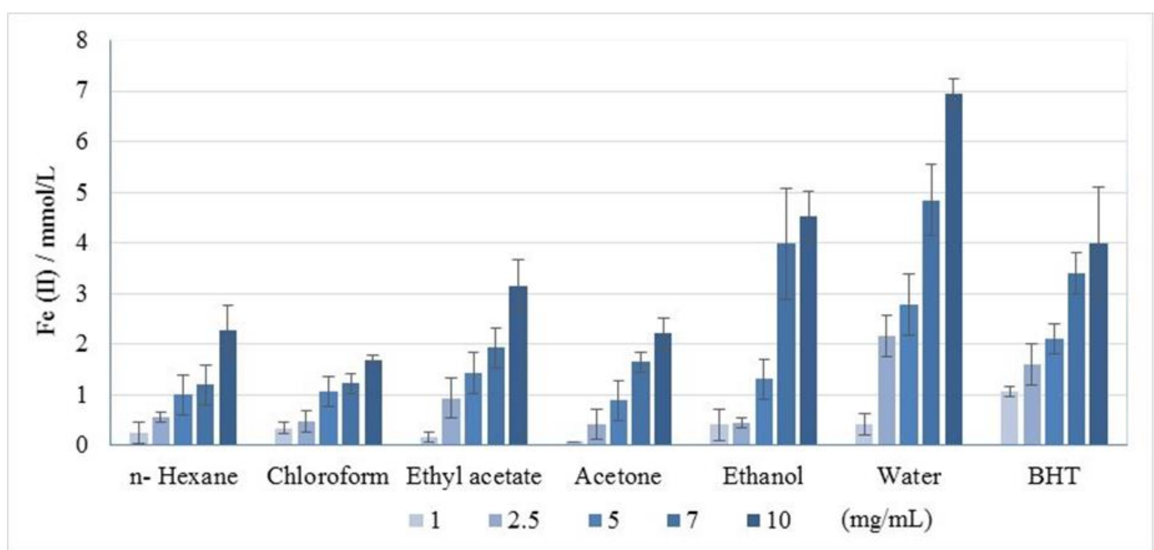

Figure 4. The antioxidant capacity of BHT and different *L. angustifolia* exstracts by FRAP method

3.3. Antioxidant activity

The antioxidant activity of *L. angustifolia* extracts and EO were determined by two methods, DPPH and FRAP. The extracts were prepared in nonpolar and polar solvents.

DPPH radical scavenging activity of *L. angustifolia* extracts is shown in Figure 2. Extracts prepared in *n*-hexane, acetone, and chloroform showed low radical scavenging activity (5-25%). The ethyl acetate extract induced 50% radical scavenging activity at the highest concentration (20 g/l), being considered moderate (25-50%).

However, water and ethanol extracts showed good antioxidant power and reached 73.1-78.5% radical scavenging activity at 10 and 20 mg/ml, respectively (Figure 2). DPPH free radical scavenging activity of BHT compared to each *L. angustifolia* extract was elevated ($p < 0.050$ for all extracts). The difference was also observed in extracts made by polar solvents compared to nonpolar ($p < 0.001$). Pairs of extracts *n*-hexane/chloroform ($p = 0.671$), *n*-hexane/acetone ($p = 0.946$), chloroform/acetone ($p = 0.995$), water/ethanol ($p = 1.00$) displayed no statistically significant difference.

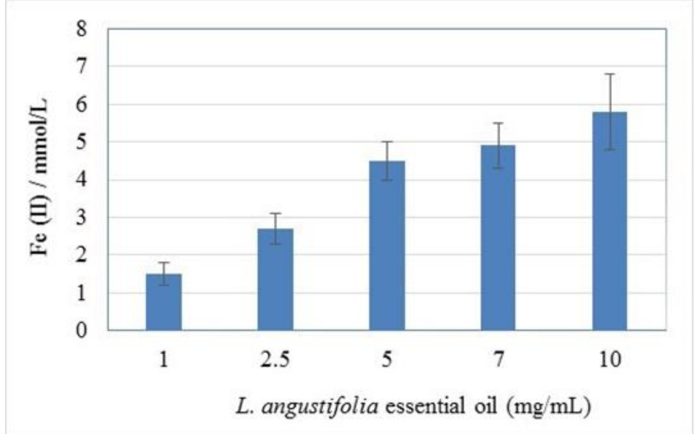

Figure 5. The antioxidant capacity of *L. angustifolia* essential oil by FRAP method

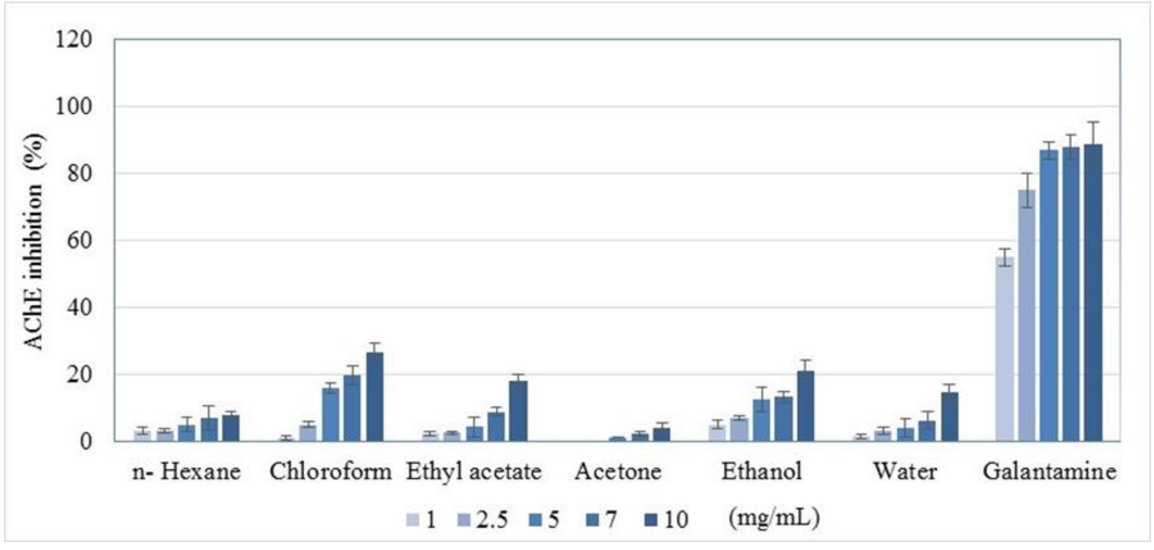

Figure 6. Acetylcholinesterase inhibition by galantamine and different extracts of *L. angustifolia*

DPPH free radical scavenging activity of *L. angustifolia* EO is presented in Figure 3. The EO did not show significant antioxidant activity as only the concentration of 300 mg/ml reached more than 50% free radical scavenging activity. *L. angustifolia* EO also showed weak antioxidant capacity in our previous study for Lamiaceae species (13.3% at 20 mg /ml) according to this method (Odak et al., 2015).

FRAP is the second used method for measuring the antioxidant power of extracts and EO from *L. angustifolia*. All samples used in this assay reduced ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The antioxidant property of *L. angustifolia* extracts by the FRAP method is presented in Figure 4. The BHT was used as a positive control (10 mg/ml is eq. to 4.0 mM Fe^{2+}).

There is a difference in the antioxidant power of extracts obtained using polar solvents compared to nonpolar ones ($p < 0.050$). Acetone, chloroform, and *n*-hexane extracts showed lower antioxidant properties than extracts prepared in ethanol and water. However, there was no difference found in antioxidant capacity between ethyl acetate extract and ethanol extract ($p = 0.274$). Water and ethanol extracts showed the greatest reducing power at 10 mg/ml (4.5 and 6.9 mM Fe^{2+}). Based on both methods used, these two extracts displayed better antioxidant activity than the others. Pairs of extracts *n*-hexane/chloroform ($p = 0.937$), *n*-hexane/ethyl acetate ($p = 0.681$), *n*-hexane/acetone ($p = 1.00$), chloroform/ethyl acetate ($p = 0.149$), chloroform/acetone ($p = 0.975$) and ethyl acetate/acetone ($p = 0.569$) displayed no statistically significant differences. Elevated polyphenols content in aqueous and ethanol extracts of *L. angustifolia* is considered to be responsible for good antioxidant effects. Previous studies confirmed that *L. angustifolia* contains rosmarinic acid, chlorogenic acid, and caffeic acid, which are known as powerful antioxidants (Blažeković et al., 2010). Duda et al. (2015) also reported that ethanolic extracts of *L. angustifolia* possess antioxidant power (2.5 to 8.6 mM Fe^{2+}/100 g dry sample) and correlate well with the total polyphenol content.

Unlike the DPPH method, *L. angustifolia* essential oil showed antioxidant power according to the FRAP method. Reduction to ferrous iron (Fe^{2+}) was dependent on the concentration of essential oils (1-10 mg/ml) and ranged from 1.5 to 5.8 mM Fe^{2+} (Figure 5). In comparison of the antioxidant activity of the essential oil and the extract of similar concentrations, stronger antioxidant activity of the essential oil compared to the extracts was observed ($p < 0.050$), except for ethanol ($p = 0.274$) and water ($p = 0.459$) extracts, and BHT ($p = 0.054$).

3.4. Inhibition of acetylcholinesterase (AChE) activity

AChE inhibitory activity of the EO and extracts of *L. angustifolia* is shown in Figures 6 and 7. Galantamine was used as the reference AChE inhibitor. All the extracts induced low AChE inhibitory activity compared to galantamine ($p < 0.001$). Among them, chloroform extract achieved a slightly greater inhibitory effect of 25% (26.5% at 10 mg/ml). Extracts in polar solvents were compared to nonpolar; water/*n*-hexane ($p = 0.225$), water/ethyl acetate ($p = 0.863$), ethanol/chloroform ($p = 0.456$), ethanol/ethyl acetate ($p = 0.941$) displayed no significant differences. Pairs of extracts *n*-hexane/acetone ($p = 0.790$), and chloroform/ethyl acetate ($p = 0.078$) also displayed no statistically significant AChE inhibitory activity differences.

Similar studies of *L. angustifolia* ethanol extracts confirm a weak to moderate AChE inhibitory effect (Ferreira et al., 2006; Vladimir-Knežević et al., 2014). Research on animal models of Alzheimer's disease showed that the aqueous extract of *L. angustifolia* improves memory (Soheili et al., 2012).

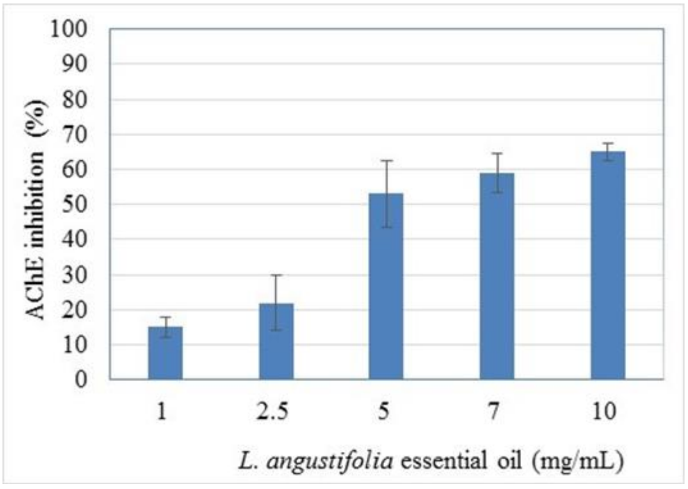

Figure 7. Acetylcholinesterase inhibition by *L. angustifolia* essential oil

L. angustifolia EO showed AChE inhibitory activity as a function of concentration. The results indicated that concentrations 5, 7, and 10 mg/ml achieved over 50% inhibition of AChE activity. Therefore, according to this research, EO can be considered as good inhibitor of AChE at a concentration of 10 g/l. AChE inhibition by EO is elevated compared to all extracts used ($p < 0.001$). Ferreira et al. (2006) also reported that lavender essential oils are good inhibitors (39.5% at 1mg/ml) of AChE.

Cholinesterase inhibition has become the most widely employed clinical approach to treating neurodegenerative diseases. EO and its major component, linalool, have shown multiple bioactivities, especially for Alzheimer's disease (Hancianu et al., 2013; Hritcu et al., 2012; Xu et al., 2017). Numerous studies have confirmed that the accumulation of amyloid beta (Aβ) plaques lead to decreased cognitive function and neurodegenerative disorders such as Alzheimer's disease (Hampel et al., 2010; Querfurth & LaFerla, 2010; Soheili et al., 2012). Aβ plaques are formed due to hyperactivity of AChE and reduction of choline. One possible reason for the accumulation of Aβ in Alzheimer's disease is oxidative stress, mediated by the production of reactive oxygen species (Zuo et al., 2015). Consequently, the antioxidant capacity of essential oils and tested extracts of *L. angustifolia* could contribute to the prevention of plaque formation. Linalool reverses the histopathological hallmarks of AD at mice and restores cognitive and emotional functions via an anti-inflammatory effect (Sabogal-Guáqueta et al., 2016). The chemical composition and biological activity of *L. angustifolia* extracts prepared in water and ethanol should be the subject of future research.

4. Conclusions

Ethanol and water extracts of L. angustifolia are found to contain a high proportion of total polyphenols and have an impressive antioxidant capacity. The main components of the essential oil were linalool, linalyl acetate, α-pinene, and lavandulol acetate. Due to the high content of beneficial health compounds, linalool and linalyl acetate (together over 50%), this oil can be considered as a high-quality oil. In addition, EO showed good inhibitory activity for AChE. It was confirmed that *L. angustifolia* could be used as a source of biologically active compounds and as a valuable raw material for the pharmaceutical and cosmetic industries. The results obtained contribute to the better evaluation and sustainable use of *L. angustifolia* grown in Bosnia and Herzegovina.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

In this study, no method requiring the permission of the "Ethics Committee" was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Supplementary File

None.

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