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Review Article

Chemical composition and comparative analysis of lavender essential oil samples from Bulgaria in relation to the pharmacological effects

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Abstract

Lavender essential oil is economically important and widely used in aromatherapy perfumery, food industry and pharmacy. Bulgaria is a global leader in lavender cultivation overtaking countries such as France, UK, China, India, and Spain during the last few years. The aim of this research is: 1) to characterize a lavender essential oil sample obtained from agricultural plantation near Pomorie, Bulgaria; 2) to perform descriptive statistical test based on a data set of 13 samples available in 4 publications, 3) to compare the varieties regarding the quantity of the most important components such as linalool and linalyl acetate 4) to summarize the pharmacological effects of the main components. As a result of GS/MS analysis of the essential oil sample obtained from agricultural plantation near Pomorie, we identified 44 compounds. The major constituents were linalyl acetate (27.5%) linalool (24.1%), *E*-β-ocimene (7.0%), terpinen-4-ol (5.1%) caryophyllene (4.5%), carvacrol (4.4%), lavandulyl acetate (3.5%), *Z*-β-farnesene (3.3%), and -*Z*-β-ocimene (3.2%). Linalool and linalyl acetate are the main ingredients based on which the quality of the essential oil is evaluated. In the studied samples they fluctuated between varieties depending on the year of extraction and the locations of origin in Bulgaria. Some varieties were characterized by a more stable ratio of linalool – linalyl acetate, compared to others. The main other components of our sample as well as the other examined Bulgarian samples fit the standards according to the requirements of ISO (2002) and the of European Pharmacopoeia (10th edn., Council of Europe 2020) with few exceptions. Lavender oil has numerous pharmacological applications based on its anxiolytic, sedative, antioxidant, anti-inflammatory, antitumor and antimicrobial activities. Although linalool and linalyl acetate largely contribute to these effects, the overall efficacy of lavender oil is proven to be due synergistic relationships between the components.

Keywords

Lavandula angustifolia Mill., linalool, linalyl acetate, synergy, anxiolytic, sedative, antioxidant, anti-inflammatory activity, antimicrobial

Introduction

Lavender oil is economically important and widely used in aromatherapy perfumery, food industry and pharmacy (Clarke 2002, 2009; Lis-Balchin 2002; Lawless 2013; Buckle 2014; Ramsey et al. 2020). Essential oil is produced industrially from lavender (*L. angustifolia*), spike lavender (*L. latifolia* L.) and lavandin – a sterile hybrid developed by crossing *L. angustifolia* × *L. latifolia*. However, the essential oil of *L. angustifolia* is more expensive and highly valued compared to that of the other *Lavandula* taxa (Lis-Balchin 2002; Lesage-Meessen et al. 2015; Giray 2018).

Lavender (*Lavandula angustifolia* Mill. subsp. *angustifolia* (*L. spica* L. var. *alpha*, *L. officinalis* Chaix, *L. fragrans* Jord., *L. vera* DC) is a woody shrub to 50 cm tall, with simple linear-lanceolate leaves, a compact spike with many flowers and broadly ovate-rhombic to obovate bracts (diagnostic feature). Native to SW and South-Central Europe (Italy, France and Spain) in mountainous areas usually over 1500 m. (Upson 2002).

Currently, Bulgaria is a global leader in lavender cultivation (Stanev et al. 2016; Giray 2018; Zagorcheva et al. 2020) with more than 11 145 ha of planted area and yield of 50126 tons for 2022 (Anonymous 2022). Lavender was introduced in Bulgaria for the first time in the early 20th century with plant material from France. The first large lavender plantations were established about 30 years later in the areas around Kazanlak and Karlovo using locally produced planting material and newly imported seeds from England and France. Later on, in the 1950's the breeders from the Institute of Roses and Aromatic Plants (IRAP), Kazanlak (Dimitrova 1959) expanded lavender clonal selection, intraspecific hybridization, gamma-irradiation, and chemical mutagenesis. This resulted in development of more than ten lavender varieties better adapted to the local environmental conditions, producing essential oil of high quantity (Dimitrova 1959; Topalov 1962, 1969; Raev and Boyadzhieva 1988; Staikov and Boyadzhieva 1989; Stanev 2007, 2010). The expansion of the total lavender areas during the last decade in Bulgaria is related with increasing cultivation outside the traditional areas in the country. Often the local soil characteristics and/or climate conditions in the new regions of lavender cultivation are not optimal for the currently available and cultivated varieties, which results in large variations of flower and essential oil yields, as well as lower lavender oil quality in some „bad" years. This requires additional parallel growing and testing of the existing pool of varieties and superior breeding lines and selection of the best performing for the new regions, or initiation of new lavender improvement program focused on specific regions. The testing and improvement work has to consider both adaptability of the tested varieties and breeding lines and stability of a number of growth parameters, flower and essential oil yields and essential oil quality (Stanev 2010). Currently, lavender cultivation in the country involves mainly seven varieties developed during the last century namely: 'Hemus', 'Druzhba', 'Karlovo', 'Sevtopolis', 'Jubileina', 'Raya' and 'Hebar'. The cultivation of selected lavender varieties, instead of population of seed derived lavender plants or propagated old lavender populations, resulted in an increase of both lavender flower and essential oil yields (Stanev et al. 2016). And yet there is a relatively high level of genetic diversity of Bulgarian industrially cultivated lavender essential oil varieties and breeding lines (Zagorcheva et al. 2020) The reason for this high genetic diversity is the increased seed propagation, which spoils the selected varieties during the last years. This genetic diversity together with the influence of many other factors such as climate specifics etc. suggests a big diversity in the composition of lavender essential oils (Zagorcheva et al. 2013, 2020; Giray 2018).

The International Organization for Standardization or ISO, defines Oil of lavender (*Lavandula angustifolia* Mill.) regarding the chemical components as follows: ester value: 108–165 (38–58% ester content), camphor content: minimum 0.5% (m/m), as well as typical chromatograms and proportions of the characteristic constituents are given (ISO 2002). The established chromatographic according to the requirements of the of European Pharmacopoeia (10th edn., Council of Europe 2020) are slightly different concerning some components.

The aim of this research is: 1) to characterize a lavender essential oil sample obtained from agricultural plantation near Pomorie, Bulgaria; 2) to perform comparative descriptive statistical tests based on a data set of 13 samples available in 4 publications, 3) to compare the varieties regarding the quantity of most important components such as linalool and linalyl acetate 4) to summarize the pharmacological effects of main components.

Materials and methods

Essential oil extraction and analysis

Lavandula angustifolia inflorescences (Fig. 1a) material was collected from agricultural planation near the town of Pomorie, Black Sea coast, Bulgaria (Fig. 1b) in third decade of June 2022. The plantation owner stated it was 'Sevtopolis' variety. Molecular markers test was not performed to confirm this. Steam extraction of the essential oil was performed using Clevenger apparatus for 4 h. GS/MS analysis of the composition of the essential oil was performed. Gas chromatography-mass spectroscopy (GC/MS) analysis was conducted according to European Pharmacopoeia (10th edn.). The GC-MS analysis of diluted (1:1000) *L. angustifolia* essential oils was performed on Exactive Orbitrap GC-MS system (ThermoFisher Scientific) operating at 70 eV, ion source temperature 230 °C, interface temperature 280 °C with split injection (1 μL, ratio 20:1) at 270 °C injector temperature. A fused silica capillary column, 5% phenyl/95% methyl polysiloxane (TG5SILMS 30 m × 0.25 mm × 0.25 μm, Thermo) was used.

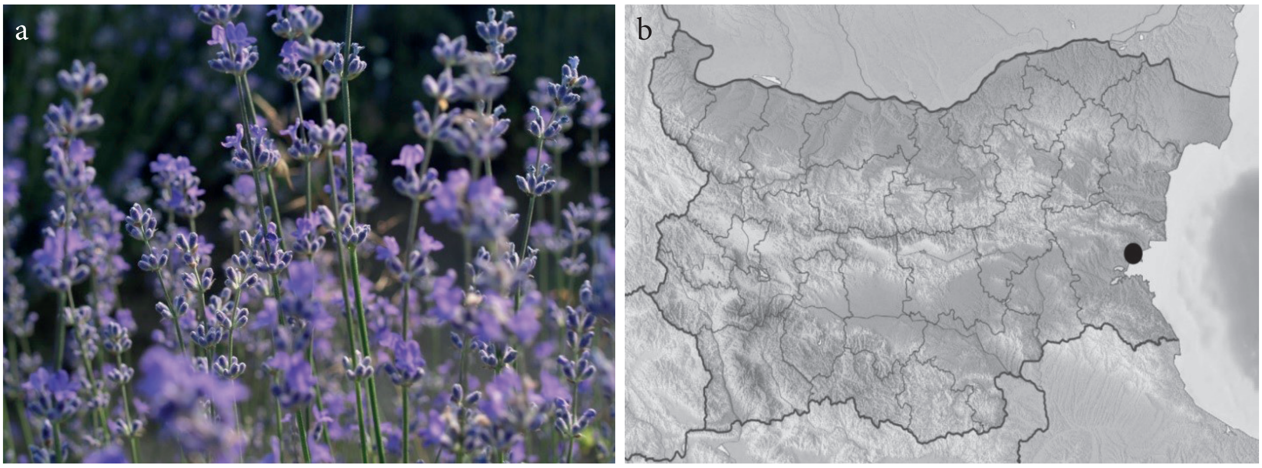

Figure 1. a. Agricultural planation – *Lavandula angustifolia* inflorescences; **b.** planation location.

Data set and analyses preparation

We accessed the Scholar Google, Web of Science, and PubMed to identify publications for the period 1900–2023 with the search string: "*Lavandula angustifolia* + essential oil", "linalool", "linalyl acetate", "Bulgaria", "pharmacological effect/s", etc. We selected 4 publications (Schmidt 2003; Prusinowska and Śmigielski 2014; Konaktchiev 2015; Zagorcheva et al. 2016) with totally 13 samples (Suppl. material 1) in which presented the full components' lists in result of GC or GC-MS analyses of lavender (*L. angustifolia*) essential oil obtained by hydrodistillation form various varieties, under different cultivation regimes and extraction protocols (e.g. 40 min to 4 hours). We checked for synonyms in the compound names and eliminated the duplications (compounds were alphabetically sorted). Also, the records of isomers and coeluates were combined and generalized.

Descriptive statistics

Linalool and linalyl acetate of the sample from Pomorie, which we characterized were compared to data set from the published research We used the data of linalool and linalyl acetate content from SE Bulgaria for a period of 5 years (Stanev and Angelova 2022). Average, Minimum and Maximum percent of the main components were calculated for Bulgarian samples and compared to the standards.

Results and discussion

Composition of the lavender essential oil – sample from Pomorie

The yield of the lavender from Pomorie was 1.15 ml/100 g. We identified 44 components (Fig. 2, Table 1) with major constituents: linalyl acetate (27.52%), linalool (24.11%), *E*-β-ocimene (7.01%), terpinen-4-ol (5.11%), caryophyllene (4.46%), carvacrol (4.42%), lavandulyl acetate (3.52%), *Z*-β-farnesene (3.33%), *Z*-β-ocimene (3.15%).

Descriptive statistics and comparative analyses

Linalool and linalyl acetate are the main ingredients based on which is evaluated the quality of the lavender essen-

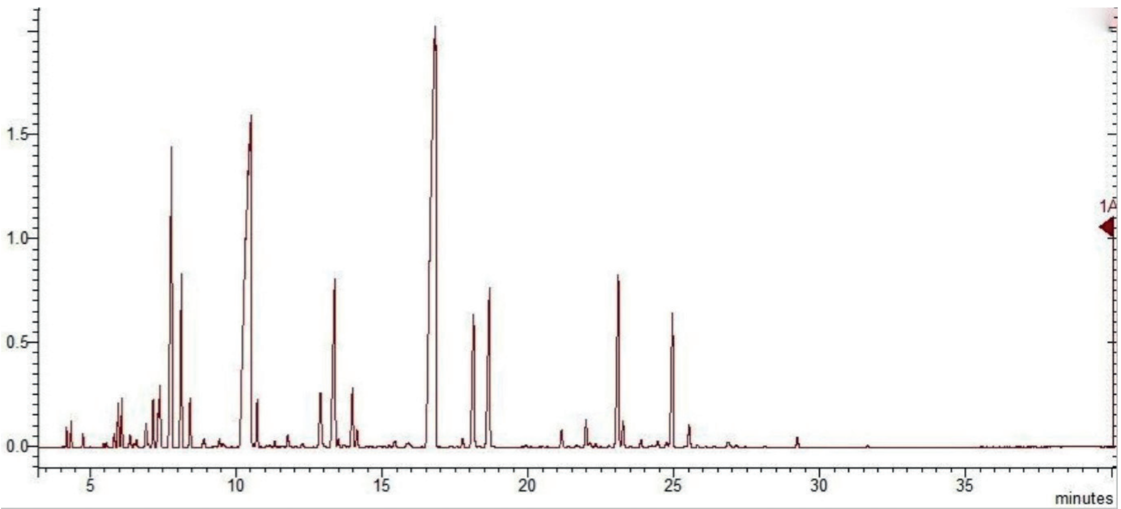

Figure 2. GS/MS analysis of the composition of *L. angustifolia* essential oil – Origin Pomorie.

tial oil. Our sample contained 26.84% linalool and 30.64% linalyl acetate (Table 1). We compared the contents of these two components to several samples from the 7 varieties from different origin and during different years (Fig. 3).

The content of linalool and linalyl acetate vary as between varieties so within the years and the locations of origin in Bulgaria. As is shown in previous research 'Hemus' and 'Hebar' varieties are characterized by a more stable ratio of linalool – linalyl acetate, compared to 'Karlovo', 'Drujba', 'Raya' and 'Sevtopolis' and the most stable one is 'Jubileina' (Stanev and Angelova 2022). The results of our descriptive statistics confirm the previously reported findings for the lavender essential oil samples from Bulgaria from various origins (Fig. 3), which fits the standards of ISO and/or European Pharmacopoeia (10th edn., Table 2).

The main other components of our sample as well as the other examined Bulgarian samples fit the standards according to the requirements of ISO (2002) and the of European Pharmacopoeia (10th edn., Council of Europe 2020) with few exceptions (Table 2).

Pharmacological effects of lavender essential oil

Insomnia, anxiety and pain

In aromatherapy, lavender oil is used for its sedative activity. This is due to its main components, the monoterpenes linalool and linalyl acetate, which possess spasmolytic, sedative and tranquilizing effect (Buchbauer et al. 1991).

Table 1. Components detected in the *L. angustifolia* essential oil – Origin Pomorie.

Compound	% of total	Compound	% of total	Compound	% of total
tricyclene	0.02	*E*-β-ocimene	7.01	linalyl acetate	27.53
β-thujene	0.23	*Z*-β-ocimene	3.15	bornyl acetate	0.18
α-pinene	0.32	γ-terpinene	0.82	lavandulyl acetate	3.52
camphene	0.17	terpinolene	0.13	carvacrol	4.42
β-terpinene	0.04	linalool	24.11	nerol acetate	0.36
β-pinene	0.06	1-octen-3-yl-acetate	0.78	geranyl acetate	0.62
1-octen-3-ol 0.222 3-octanone	0.67	3-octanol,acetate 0.05 allo-ocimene	0.12	caryophyllene	4.46
β-myrcene	0.69	caren-4-ol	0.01	α-santalene	0.53
3-octanol	0.24	camphor	0.26	*E*-α-bergamotene	0.16
α-phellandrene	0.06	lavandulol	1.47	humulene	0.14
3-carene	0.11	terpinen-4-ol	5.11	β-sesquiphellandrene	0.12
acetic acid, hexyl ester	0.51	crypton	0.13	*Z*-β-farnesene	3.33
o-cymene	0.84	α-terpineol	1.36	germacrene D	0.52
limonene	0.53	butanoic acid,hexyl ester	0.36	caryophyllene oxide	0.24
eucalyptol	1.09	*Z*-geraniol	0.16		

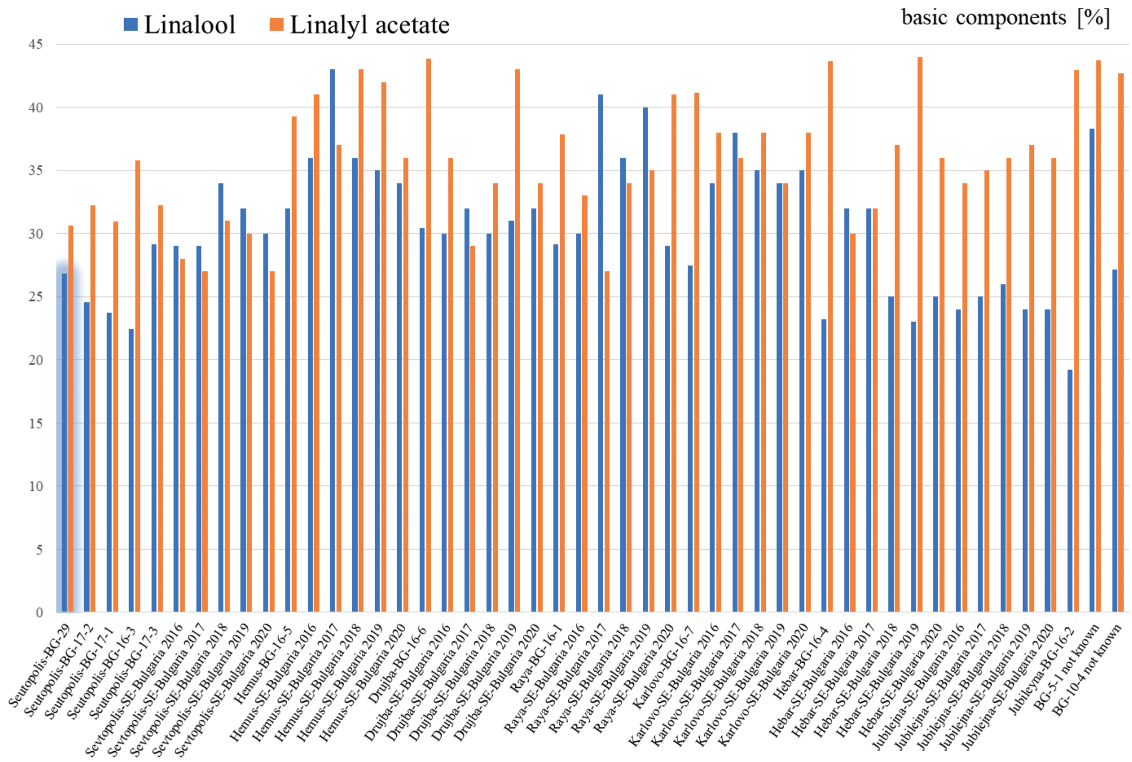

Figure 3. Linalool and linalyl acetate in our sample from Pomorie (Sevtopolis-BG-29) and 'Sevtopolis', 'Hemus', 'Druzhba', 'Raya', 'Karlovo', 'Hebar', and 'Jubileina', from different origin and during different years.

Table 2. Content of the main lavender essential oil components in the sample from Pomorie, Average, Minimum and Maximum values calculated for 13 samples from Bulgarian and compared to the standards.

Components	Sample from Pomorie	Samples from Bulgaria (n = 13)			ISO and/or European Pharmacopoeia (10th edn.)	
		Average [%]	Min [%]	Max [%]	Min [%]	Max [%]
Camphor	0.26	0.12	0.00	0.29	–	1.20 or 1.50
1,8-Cineole	1.21	0.35	0.00	3.06	–	2.50 or 3.00
1,8-Cineole + Phellandrene	–	0.65	0.00	2.21	–	–
Phellandrene	0.06	–	–	–	–	1.00
D-Limonene	0.53	0.38	0.00	0.80	–	1.00
Z-β-Ocimene	3.15	5.18	0.00	9.04	1.00	10.00
E-β-Ocimene	7.01	2.78	0.90	8.71	0.50	6.00
Lavandulol	1.47	0.82	0.00	1.64	0.1	3.00
Lavandulyl acetate	3.52	3.78	0.00	5.83	0.20	8.00
Linalool	24.11	27.20	19.22	38.32	20.00	45.00
Linalyl acetate	27.53	38.24	30.64	43.84	25.00	47.00
3-Octanone	0.74	0.96	0.00	2.61	0.10	5.00
Terpinen-4-ol	1.51	2.58	0.00	6.84	0.10	8.00
α-Terpineol	1.36	0.68	0.00	1.51	–	2.00

Lavender fragrance show a beneficial effect on insomnia and depression on a clinical study of women college students (Lee et al. 2006). Furthermore, the use of lavender oil is effective in reducing challenging behaviours in individuals with dementia (van der Ploeg et al. 2010). Iinhalation of lavender essential oil is experimentally proven to have beneficial effects on PVC pain, anxiety, and satisfaction level of patients undergoing surgery (Karaman et al. 2016). It has ability to decrease primary dysmenorrhea (painful menses with cramping sensation in the lower abdomen with normal ovulatory cycles, which has no pelvic pathology) (Zayeri et al. 2019). A study by Sanna et al. (2019) showed that orally administered lavender oil, besides having antidepressant-like and anxiolytic-like activity, could reduce spared nerve injury (SNI)-induced neuropathic pain symptoms in mice.

Anti-allergic properties Lavender oil possess anti-allergic properties, namely it inhibits immediate-type allergic reactions by inhibition of mast cell degranulation in vivo and in vitro (Kim et al. 1999). At the same time oxidation products of linalool may be potential allergens (Karlberg 2003, Woronuk et al. 2011) as well as oxidation products of linalyl acetate (Sköld et al. 2008).

Anti-inflammatory properties Lavender oil has anti-inflammatory properties and is used as massage oil as wound healing agent. The proposed mechanism of the anti-inflammatory properties of lavender essential oil involves the participation of prostanoids, NO, proinflammatory cytokines, and histamine (Cardia et al. 2018). Moreover, in a recent study lavender oil and its main components linalool and linalyl acetate show effectiveness against Imiquimod induced psoriasis in mice (Rai et al. 2020).

Antimicrobial activity Lodhia et al. (2009) studied the antibacterial properties of essential oils from various plant species. Lavender essential oil showed potent effect on gram-negative organisms. It could be used as an antibacterial agent against pathogenic bacteria isolated from pet turtle (Hossain et al. 2017).

Pest control and insect repellent Lavender essential oil is well known insect repellent (Erland and Mahmoud 2016). It is an ingredient of commensal sprays against mosquitoes, ticks, flees etc. Moreover, lavender essential oil could be used against *Tyrophagus longior*, a mite (Perrucci 1995) and *Sitophilus oryzae*, a beetle (Al-Harbi et al. 2021) that are pests in stored food.

Pharmacological effects of main components

Namely linalool and linalyl acetate are the precious constituents of lavender essential oil that possess anti-inflammatory activity (Peana et al. 2002) and sedative effect (Woronuk et al. 2011).

Linalool is a monoterpene compound, which can be found in the essential oil of different aromatic plant species. Naturally occurring is its (–)-enantiomer, or (–)-linalool. Inhalation of linalool has been shown to impart positive psychopharmacological effects in humans (Woronuk et al. 2011). Linalool is the major pharmacologically active constituent involved in the anti-anxiety effect of lavender oil (Umezu et al. 2006). Linalool acts on the somatic sensory system with local anesthetic properties, since it blocks the action potential by acting on voltage-dependent Na+ channels (Leal-Cardoso et al. 2010). A study of Paena et al. (2003) show that (–)-linalool inhibits the inflammatory pain in mice, as determined by a significant reduction in acetic acid-induced abdominal constrictions, whereas higher dose is effective in the hot plate test, which is a model of supraspinal analgesia. Later Paena et al. (2006) explain possible mechanism of the antinociceptive effect as reduction of nitric oxide (NO) production/release, where cholinergic and glutamatergic systems are involved. *In vitro* studies of linalool show dose-dependent sedative effects at the central nervous system, including hypnotic, anticonvulsant and hypothermic properties. Re et al. (2000) report inhibitory effect of linalool on the acetylcholine (ACh) release and on the channel open time in the mouse neuromuscular junction. Camargo et al. (2016) suggest that linalool possesses antihypertensive potential. Their study show that linalool is able to reduce cardiac hypertrophy, decrease vascular reactivity to a vasoconstrictor agent phenylephrine and to increase pharmacological potency to the vasodilator agent sodium nitroprusside, which is an example of its synergetic properties (Camargo et al. 2016). Dias et al. (2017) analyze the antifungal activity of linalool against *Candida* species. The result show that the best antifungal activity of linalool is on *Candida tropicalis* (MIC = 500 mg/mL), followed by *Candida albicans* (MIC = 1.000 mg/mL), and *Candida krusei* (MIC = 2.000 mg/mL). Linalool can also be a potential antifungal agent against *Microsporum canis* and *Microsporum gypseum* (Silva et al. 2017). The influence of (R)-(–)-linalool on airborne microbes when vaporized with an air washer has been reported and the average reduction in germ count was above 40% (Sato et al. 2007). Linalool has high antioxidant properties that can be used in treating different diseases, including cancer (Jabir et al. 2018). Iwasaki et al. (2016) show that linalool induces apoptosis of human colon cancer cells via cancer-specific oxidative stress. In prostate cancer cells, linalool treatment result in the appearance of cell shrinkage and membrane blebbing, which are characteristic features of cell apoptosis (Sun et al. 2015). Repellent activity of linalool was evaluated by Müller et al. (2009). Linalool diffusers provided a 93% repellency rate indoors and a 58% repellency rate outdoors, which shows that linalool can be used as an alternative to toxic insecticides. (Müller et al. 2009). Linalool is rated as only a mild irritant to man and *in vivo* studies on mousse and rats, show low acute toxicity, orally and through the skin (Kamatou and Viljoen 2008).

Linalyl acetate is the acetate ester of linalool, and because of that, the two often occur in conjunction. The two compounds possess similar pharmacological activity. Linalyl acetate, as well as linalool play a major role in the anti-inflammatory activity displayed by the essential oils containing them. This statement is evaluated by reduction of the edema in Carrageenin-induced edema in rats (Peana et al. 2002). Moon et al. (2018) suggests that linalyl acetate would be beneficial to treatment of atopic and inflammatory diseases by reducing Thymic stromal lymphopoietin (Moon et al. 2018). A study by Seo et al. (2021) suggests that lavender oil and in particular, linalyl acetate may have preventive role in rheumatoid arthritis against muscle wasting in rats, which are chronically exposed to nicotine. Linalyl acetate prevents olmesartan-induced intestinal hypermotility mediated by interference of the sympathetic inhibitory pathway in hypertensive rats (Kwon et al. 2018). Also, linalyl acetate acts as a muscle relaxant on the vascular smooth muscle tissue in rabbits (Koto et al. 2006). Linalyl acetate can suppress the production of melanin and thus exhibits anti-hyperpigmentation properties (Peng et al. 2014). |It has anti-hypertensive properties that can prevent hypertension-related ischemic injury (Hsieh et al. 2018). Linalyl acetate can prevent the development of Type 2 diabetes mellitus by reducing insulin resistance and stress levels and by improving mitochondrial dysfunction in male rats exposed to high fat diet and chronic stress (Shin et al. 2020).

Synergism

Although particular components have certain pharmacological effects the essential oil as a whole often possess higher efficiency due to the synergism of the components. There are many studies, confirming the synergistic effect of lavender essential oil in the antimicrobial therapy. The combination of lavender oil with piperacillin significantly alters the outer membrane permeabilization of *Escherichia coli* compared to a control (Yap et al. 2014). The combination of essential oil and conventional antibiotics could reduce the beta-lactam antibiotic resistance (Yap et al. 2013). The combination of gentamicin and linalool towards Methicillin-resistant *Staphylococcus aureus* shows a strong bactericidal action (Adaszyńska-Skwirzyńska et al. 2019). Lavender oil can be combined not only with antibiotics, but also with other essential oils. A synergistic action between lavender and tea tree essential oil results in enhanced antifungal activity (Cassella et al. 2002). The combination of *Artemisia herba-alba*, *Lavandula angustifolia* and *Rosmarinus officinalis* essential oils showes synergism at very low concentrations compared to MICs (Moussii et al. 2020).

Conclusion

Linalool and linalyl acetate which are the main ingredients of lavender essential oil characterizing its quality vary. The samples from Bulgaria regarded in this study meet the standards with only few exceptions. The main pharmacological effects of lavender essential oil are related to insomnia, anxiety and pain relief, anti-inflammatory and anti-allergic properties and anti-microbial activity, insect repellent and acaricidal. Linalool in particular is responsible for the sedative and painkiller effect as well as anti-inflammatory activity. It has pronounced antioxidant and antitumor effect as well as antimicrobial activity. Linalyl acetate has pronounced ani-inflammatory effect can prevent hypertension-related ischemic injury and development of Type 2 diabetes mellitus. Although particular components have certain pharmacological effects the essential oil as a whole often possess higher efficiency due to the synergism of the components.

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Supplementary material 1

Lavender essential oil from Bulgaria and its pharmacological effects

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Data type: constituents of essential oil samples (PDF file)

Explanation note: Main components detected in the 13 samples of *Lavandula angustifolia* essential oil [%].

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