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ORIGINAL ARTICLE

Evaluation of antimicrobial activity and activity on the autonomic nervous system of the lavender essential oils from Montenegro

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Summary. This study investigates chemical composition and antimicrobial activity of essential oil of *Lavandula officinalis* from Montenegro, Mediterranean, and effects on autonomic nervous system. The oil was analysed by GC-MS technique in order to determine the majority compounds while dilution method was used to determine minimal inhibitory concentration. The present study assessed autonomic parameters such as blood pressure, heart rate, and skin temperature to determine the arousal level of the autonomic nervous system. In addition, subjects were asked to estimate their mood responses such as feeling pleasant or unpleasant, uncomfortable, sensuality, relaxation, or refreshing in order to assess subjective behavioural arousal. Chemical analysis by GC-MS identified 31 compounds in lavender oil representing 96.88% of the total oil. Linalool (24.84%), was a major component, together with linalyl acetate (22.39%), 1,8 cineole (18.13%) and camphor (12.88%). The investigated lavender oil consisted mostly of oxygenated monoterpenes (87.95%) and monoterpene hydrocarbons (7%). The lavender oil exhibited antimicrobial activity against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19111), *Proteus mirabilis* (ATCC 25933) and *Candida albicans* (ATCC 10231) with an MIC of 1.4µl/ml. The results revealed that lavender oil caused significant decreases of blood pressure and heart rate, which indicated a decrease of autonomic arousal.

Key words: antimicrobial activity, lavender essential oils, trans-dermal application

Introduction

Medicinal and aromatic plants have the ability to inhibit the growth of a wide range of pathogenic microorganisms due to presence of essential oils. Essential oils, their chemical composition, antioxidant and antibacterial activity affect numerous physiological processes, in both plants and humans, and thus protect against free radicals and growth of pathogenic microorganisms (1). Resistance of some bacteria and fungi to commercial antimicrobial drugs and possible negative effects of synthetic antioxidants have justifiably increased the interest for essential oil research (2, 3). Also, essential oils are used in aromatherapy due to the effects of their constituents in the treatment and prevention of certain diseases related to the Central Nervous System (4).

Lamiaceae is a large and well-defined family with about 252 genera and approx. 700 species (5). Lavandulla is one of the most important genera for medical purposes and possess several pharmacological and biological activities. Lavandula genus (*Lamiaceae*) comprises 28 species, it provides valuable essential oils mainly for the food (flavouring), perfumery and cos-

metic industries, and is also very popular in aromatherapy (6). Lavandula oils have been reported to have sedative and antispasmodic properties (7), antimicrobial (8) and antioxidant activities (9). Lavenders (*Lavandula officinalis*) of the genus *Lavandula* are extensively found in the Mediterranean region and are often cultivated in Spain, France and Italy (10). Among different species of *Lamiaceae* in Montenegro, Lavandula species are cultivated as commercial crops but also exist freely growing sub-introduced species.

The main constituents of lavender dried blossoms essential oil are linalool, linalyl acetate, cineole, camphor and βocimene (7). The plant also contains a high percentage of tannins up to 12 %. (11). According to the U.S. Food and Drug Administration, *L. officinalis* has been classified as safe and has been included in the safe substances list, commonly known as "Generally Recognised as Safe" (GRAS) (11, 12).

Therefore, the aim of this investigation was to analyse the chemical composition and antimicrobial activity of lavender essential oils from Montenegro. The inhibitory effects of the essential oils towards Gram-negative and Gram-positive bacteria were studied. Moreover, the effect of lavender oil on the autonomic nervous system and emotional state is the subject of this research too. Our study is designed to examine influence of trans-dermal lavender oil application on heart rate, blood pressure, skin temperature and mood assessment of volunteer respondents.

Materials and Methods

Sampling and oils isolations

The plant samples of *Lavandula officinalis* were collected from different sites in the south of Montenegro (Budva, Bar and Ulcinj). The samples were collected in 2016, when all plant samples were in full bloom. Essential oils (EO) from dried flowers of *Lavandula officinalis* were prepared by water-distillation and provided from the commercial company Flowers-Katani from Ulcinj, Montenegro. The obtained oil was dried over anhydrous sodium sulphate, measured, poured into hermetically sealed dark-glass containers and stored in refrigerator at 4 °C until analysed by GC-MS.

Chemical analysis

The analyses were performed on a gas chromatograph-mass spectrometer, GCMS QP 2010 plus, Shimadzu, equipped with split-splitless injector and a ZB-5MS capillary column (30m × 0.25 mm; 0.25 μm film thickness). The chromatographic conditions were as mentioned in the preceding paragraph. Injector was heated at 260 °C, detector (MSD) was heated at 260 °C, while the column temperature was linearly programmed from 35 to 270 °C (5.0 °C min-1). The EI MS spectra (70 eV) were obtained in the scan mode in m/z range 50–500.

Antimicrobial activity

To assess the antimicrobial properties of the lavender oil, nine strains of pathogenic bacteria were used in the study: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Salmonella enteritidis* ATCC 13076, *Listeria monocytogenes* ATCC 19111, *Proteus mirabilis* ATCC 25933, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* ATCC 19433 and *Candida albicans* ATCC 10231. All microorganisms were derived from the culture collection of the Institute of the Health of Montenegro and Department of Biology, Faculty of Sciences, University of Montenegro. The minimal inhibitory concentrations (MICs) were determined by the broth dilution method. All the tests were performed in Müller-Hinton broth and Nutrient broth for bacterial strains and in Sabouraud dextrose broth for *C. albicans*. Overnight broth cultures of each strain were prepared in a final concentration of 2×10^6 CFU/mL for bacteria, and 2×10^5 CFU/mL for *C. albicans*. The essential oils were tested in concentrations range from final concentrations of 71, 35, 14, 7, 3.5, 1.4 μg/ml. Microbial growth was determined after incubation at 37°C for 24 h (for bacteria), and at 26°C for 48 h (for *C. albicans*). The MIC was defined as the lowest concentration of the oil or the extract at which the microorganism does not demonstrate visible growth (13). All the tests were performed in duplicate.

Autonomic nervous system (ANS) and mood measurement

Total of 20 healthy subjects (10 men and 10 women), aged 19 to 28 years (mean age 21 ± 2.95 years) and body mass index (BMI) 18 to 30 (mean BMI 23.8 ±

1.69) was included in this study. We have taken into account that obesity may affect the parameters of the measurement of the autonomic nervous system (14). None of the subjects had any abnormalities affecting smell, had a cardiovascular disease or took any medication or smoked, or had a history of epilepsy, neurological diseases, allergies, diabetes, infections and sleep disorders. Menstruating women were excluded from the study (14, 15). Demographic data of the participants is presented in Table 1. Subjects were given a full explanation of the research and a written informed consent of all aspects of the present study and were free to withdraw at any time.

The research was approved by the Ethics Committee, Faculty of Medicine, University of Montenegro in accordance with the Helsinki Declaration. The study was conducted in the morning (8.00-12.00 AM), to minimize circadian variations. All activities were performed in a silent room with an ambient temperature of 23 ± 1°C and 40-50% humidity. During the experiment, the subjects evaluated the pleasantness on a 5-point Likert scale, after a trans-dermal application, 0.5 ml of lavender oil in the area of the abdomen, at determined time intervals. This scale described their subjective affective feelings based on the following 5 factors - attention (good / bad), alertness (active /drowsy), calm (relax/ stress), sensuality (romantic/frustrated), opacity (fresh/ disgust). Before measurement beginning, the researcher clearly informed the participants of the procedure, and then the participants signed an Informed Consent Form describing the present study and their rights. ANS parameters, systolic and diastolic blood pressure, heart rate, skin temperature, and mood state, were recorded at the same time. The test protocol consisted of three parts: first parts served as a base line (resting period) and took ten minutes. After completion of the first part, the subjects were asked to rate their mood state scales and the ANS parameters were measured for the subjects.

Table 1. Demographic data for the volunteers

Parameters	N	Minimum	Maximum	Mean	SD
Age	20	19	28	21.25	4.13
Height (cm)	20	165	197	177.3	7.22
Weight (kg)	20	50	113.7	74.05	6.38
Body mass index	20	18.4	29.3	23.8	2.04

The second part took 20 minutes (transdermal application). After the transdermal application of lavender oil, the mood state and the ANS parameters were measured for the subjects. All the steps in this experiment were similarly conducted as per the previous study recorded on the effects of rosemary oil inhalation (15).

Anthropometric measurements

Weight measurement was performed on barefoot subjects in light clothes on a digital scale accurate to 0.1 kg (SECA, model SE 808). Body height was measured with a stadiometer accurate to 0.5 cm (GIMA, code 27328). Body mass index (BMI) was measured with a body composition analyser (TANITA, BC-420MA). An Omron HEM 907 XL oscillometric monitor was used for measurement of blood pressure. The measurement was performed in a quiet room, in a sitting position, and on a non-dominant arm, after a rest of 5 min. Three measurements with a 1 min interval were performed after calibration of the measuring system.

Data and statistical analysis

The STATISTICA statistical package was used for data analysis by a paired t-test for dependent variables and regression analysis.

Results

Chemical composition

The yield of the lavender essential oil (*L. officinalis*) on a dry-weight basis was 0.66 %. Results obtained from GC and GC-MS analyses of the essential oil of *L. officinalis* are shown in Table 2. The chemical composition of essential oil was characterized by the presence of 31 compounds, which accounted for 96.88% of the total oil for *L. officinalis* respectively. Oxygenated monoterpenes are highly predominant in *L. officinalis* essential oil with 85.24 %, followed by acyclic oxygenated monoterpenes (48.88%) and cyclic oxygenated monoterpenes with 36.36%. Then follow monoterpenic hydrocarbons (7%). The sesquiterpene hydrocarbons and oxygenated sesquiterpenes accounted for only 1.82% and 0.11% of the total oil, respectively. The essential oil of *L. officinalis* from Montenegro has been characterized by a high amount of

β-linalool (24.84%). The other major components are: linalyl acetate (22.39%), 1.8-cineol (18.13%), camphor (12.88) and borneol (3.02%).

The main components for *L. officinalis* EO were detected in other investigations (12) and they reported that dominant constituent is linalool (20.1-65.9%), followed by borneol (6.3-32%) and camphor (2.4-13.5%).

Table 2. Chemical composition (% of compound) of the essential oils from *L. officinalis*

Number	Compounds	RT	RI	%
1	alpha-thujene	11.05	931	0.04
2	alpha-pinene	11.49	939	0.96
3	camphene	12.09	953	1.22
4	sabinene	12.99	976	0.23
5	beta-pinene	13.14	980	0.80
6	3-octanone	13.50	986	0.18
7	beta-myrcene	13.65	991	0.40
8	hexyl acetate	14.48	1008	0.18
9	para-cymene	14.85	1026	0.30
10	limonene	15.04	1031	1.06
11	1,8-cineole	15.13	1033	18.13
12	beta-trans-ocimene	15.29	1040	0.99
13	beta-cis-ocimene	15.65	1050	1.00
14	linalool oxide-cis	16.44	1074	1.96
15	linalool oxide-trans	16.97	1088	1.63
16	beta-linalool	17.48	1098	24.84
17	1-octen-3-yl acetate	17.67	1110	0.47
18	camphor	18.93	1143	12.88
19	borneol	19.72	1165	3.02
20	terpinen-4-ol	19.97	1177	0.37
21	1-hexyl butyrate	20.27	1191	0.25
22	linalyl acetate	22.02	1257	22.39
23	lavandulyl acetate	22.94	1289	1.55
24	neryl acetate	24.99	1365	0.03
25	geranyl acetate	25.51	1383	0.07
26	trans-caryophyllene	26.73	1404	1.33
27	alpha.-bergamotene	27.03	1436	0.15
28	beta-farnesene	27.47	1458	0.13
29	germacrene d	28.29	1480	0.11
30	gamma-cadinene	29.08	1513	0.10
31	caryophyllene oxide	30.77	1581	0.11
Total				**96.88**

Antimicrobial potential

In this study, the antimicrobial efficacy of *L. officinalis* essential oil from Montenegro was investigated against 9 pathogens whereby MIC values of 1.4 μg/mL were predominantly observed against the tested pathogens. A few exceptions (*Bacillus subtilis and Pseudomonas aeruginosa* with an MIC of 71μg/mL, and *Streptococcus faecalis, Candida albicans* with an MIC of 14.0 μg/mL) were noted. The results of antibacterial activity of essential oils and MIC tested are presented in Table 3. The inhibitory effect of lavender oil on the majority of the investigated bacterial cultures was confirmed in the research (3), but MIC values in this research for most pathogens are lower.

Autonomic nervous system parameters

In this study we examined the effects of trans-dermal applied lavender oil on the human autonomic nervous system. Emotional measurement and ANS parameters (blood pressure, heart rate and skin temperature) were recorded as the indicators of the arousal level of the nervous system. The mean and Standard Deviation (SD) values of autonomic parameters in the experiment are presented in Table 4.

The data on various autonomic parameters were compared during resting and following the trans-dermal application of lavender oil. The subjects had significantly decreased diastolic pressure and heart rate (p-value < 0.05) during the lavender oil treatment compared with those of the resting period. Following the trans-dermal application of lavender oil in male

Table 3. Minimal inhibitory concentrations (MIC) (μg/mL) of *Lavandula officinalis* essential oils against bacteria using dilution method

Test organisms	**MIC** (μg/mL)
Escherichia coli ATCC25922	1.4
Staphylococcus aureus ATCC25923	1.4
Bacillus subtilis ATCC6633	71
Salmonella enteritidis ATCC13076	7.1
Listeria monocytogenes ATCC19111	1.4
Proteus mirabilis ATCC25933	1.4
Pseudomonas aeruginosa ATCC27853	71
Streptococcus faecalis ATCC19433	14
Candida albicans ATCC10231	1.4

Table 4. Mean and standard deviation of ANS parameters during resting condition and transdermal application of lavender oils

Parameters	n	Rest		LO		p-value rest and LO
		Mean	SD	Mean	SD	
Systolic blood pressure (mmHg)	20	127.6	9.60	124.5	9.95	0.530; p <0.05
Diastolic blood pressure (mmHg)	20	65.0	7.30	59.5	9.94	0.04; p<0.05*
Heart rate (bmp)	20	92.58	16.7	84.25	19.1	0.017; p<0.05*
Skin temperature (°C)	20	36.4	1.69	36.7	1.94	0.150; p <0.05

*Significant difference, p-value <0.05; LO = lavender oils

subjects, the effect on pulse was recorded sooner than in female subjects.

The mean and SD of emotional state response are shown in Table 5. After trans-dermal application of lavender, the subjects felt they had significant increases in pleasant emotions: good and romantic (p-value < 0.05). Furthermore, the active, relaxed and fresh feelings were not statistically significant (p-value <0.05).

Discussion

The Lavandula genus is distributed in all Mediterranean regions and consists of about 20 species of small evergreen shrubs with aromatic foliage and flowers (4). The differences in the lavender essential oil composition might arise from several environmental (climatic, seasonal, geographical) and genetic differences (16). Lavender essential oil from Montenegro is characterized by greater percentages of camphor (12.88%) compared to lavender oil from Turkey (not detected) (17), Spain (0.31%) (18) and France (1.22%) (19).

Interestingly, in the essential oil of lavender Montenegro, 1,8 cineole accounts for 18.13% compared to lavender essential oil in the Mediterranean area which ranges from 1.7-9.6% (17-19). High values of cineole were recorded in *L. officinalis* species in the region of Iran (20). Also, the proportion of camphor is high 12.88% compared to EO originating in Spain, Turkey and France (Mediterranean region) and similar to EO originating in Croatian and Italy. The high values of camphor of over 20% were recorded in species *Lavandula pedanculata* and *Lavandula dentata* from Morocco (21). According to ISO standard 3515, several components in lavender oil from Montenegro stand out from standards like 1,8 cineol (ISO 0-15%), limonene (ISO 0-0.5%), camphor (ISO 0-0.5%). Also, according to Ph. Eur. 8.0 camphor and 1, 8- cineole did not meet the corresponding requirements.

Although a number of *in vitro* studies have been conducted on the antimicrobial activity of *L. officinalis* essential oil against a wide variety of microorganisms, many studies have used MIC assay to quantify antimicrobial activity (13, 22). According to the general literature, *L. officinalis* has been used as an antibiotic or antiseptic in combination with a number of other oils (bitter orange, caraway, cedarwood, chamomile, geranium, grapefruit, lemon, marjoram, patchouli, rosemary, sage, sweet orange, and ylang-ylang) (23, 13). In this investigation lavender oil recorded high antimicrobial potential on *E. coli*, *S. aureus*, *L. monocytogenes* and *P. mirabilis*. The inhibitory effect of lavender oil on the majority of

Table 5. Mean and standard deviation of emotional state during resting period and transdermal application of lavender oils

Emotion	n	Rest		LO		p-value rest and LO
		Mean	SD	Mean	SD	
Good	20	4.50	0.75	4.87	0.35	0.079 *
Active	20	4.08	0.90	3.58	1.08	0.214
Relax	20	4.33	1.07	4.16	1.02	0.338
Romantic	20	3.75	1.28	4.12	1.24	0.079 *
Fresh	20	4.16	1.02	4.41	0.79	0.274

*Significant difference, p-value <0.05; LO = lavender oil

the investigated bacterial cultures was confirmed in the research (3), but MIC values in this research for most pathogens are lower. Contrary to the investigation by Rapper et al. (13) which reported that MIC for lavender oil against *Pseudomonas aeruginosa* is 2 μg/mL, in this investigation *Pseudomonas aeruginosa* was less sensitive (MIC 71 μg/mL). Lavender oil contained numerous components of comparable percentage as shown in Tab. 2. but linalool was assumed to be a major contributor to the bioactivity. Linalool is a natural plant product, an essential oil with known antifungal and antimicrobial (24). Addition of linalool to essential oil lavender may significantly enhance her antimicrobial effectiveness (synergistic and additive effects) (25).

The stimulatory effects on the autonomic nervous system may be explained by the abundance of oxides (1, 8-cineole) and linalool that are present in lavender oil. Furthermore, 1,8-cineole was reported to inhibit the activity of acetylcholinesterase (AChE), a nervous system enzyme that catalyses the hydrolysis of the neurotransmitter acetylcholine to transmit nerve impulses (16). 1,8-cineole, a monoterpene, has been reported to have several biological effects including an anxiolytic effect (21). Moreover, 1,8-cineole has been reported to be a hypotensive agent and smooth muscle relaxant as well as a modulator of neural firing in areas of the olfactory lobe. Several authors have found that linalool inhibits acetylcholine release and afters ion channel function at the neuromuscular junction (7). Also, linalool and linalyl acetate are rapidly absorbed through the skin after topical application with massage and are thought to be able to cause central nervous system depression (26). The results of the present study support previous studies (14) indicating lavender odour can influence relaxing after inhalation. Previous studies using a foot bath containing lavender oil also supports the positive effects on the neural activity of lavender oil. Also, study by Hoseini et al. (27) showed that inhalation of lavender could reduce the level of anxiety and cortisol in open-heart surgery patients before surgery.

Conclusion

Our results revealed that β-linalool, linalyl acetate and 1,8 cineole are the major components of *Lavandula officinalis* essential oil from Montenegro. The lavender oils possess rather a significant activity against different microorganisms. A stronger antidepressant effect of lavender oils from Montenegro was not recorded in this study compared to other lavender oils, despite higher concentrations of 1,8 cineole and camphor. Furthermore, it was shown that trans-dermal application of the lavender oils in this investigation yields a significant statistical effect on ANS parameters, as well as application by inhalation (14).

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