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LAVENDER EXTRACT WITH TETRAFLOUROETHANE - CHEMICAL COMPOSITION, ANTIMICROBIAL ACTIVITY AND APPLICATION IN COSMETICS

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Abstract. *The chemical composition of extract from lavender (Lavandula angustifolia Mill.) by extraction with tetrafluoroethane was analyzed using GC and GC/MS. The main compounds (concentration higher than 3%) of extract were: linalool (32.48%), linalyl acetate (22.98%), borneol (5.12%), cis-linaloloxide (4.49%), (E)-β-farnesene (4.10%), lavandulol (4.22%) and β-caryophyllene (3.34%). The extract is used in model cosmetic emulsions O/W type. The studied extract demonstrated antimicrobial activity against Gram-positive and Gram-negative bacteria.*

Keywords: *lavender, tetrafluoroethane extraction*

Introduction

Lavender (*Lavandula angustifolia* Mill. = *L. officinalis* Ch.= *L. vera* D.C.) is plant from the family Lamiaceae. Lavender oil is produced by steam distillation of freshly cut, flowering tops and the oil is produced in a yield of 1 %. The oil is used primary in perfumery and cosmetics. Lavender oil is a pale, yellow, amber-tinged liquid with a fresh, sweet, herbaceous odor on a woodly balsamic base with high ester content (Bauer et al., 2001; Lawrence, 1979, 1988, 1991, 1993, 1996, 2000, 2004; Lis-Balchin and Hart, 1999; Lis-Balchin, 2002; Schmidt, 2003; Tucker et al., 1984). The essential oil has the antimicrobial activity (Adam et al., 1998; Bourrel et al., 1995a, 1995b; Deans, 2002; Gabrielli et al., 1988; Hammer et al., 1996; Jain and Kar, 1971). Lavender extract is also commercially important and is produced by solvent extraction with n-hexane.

Essential oil of lavender, one of the most popular oils, has for many years been produced in Bulgaria. About 70 components have been identified in the oil from Bulgaria, among which dominate linalyl acetate (24,7 – 51,6 %), linalool (16,1 – 42,5 %), terpinene-1-ol (4,5 – 8,7 %), 1,8-cineole, cis and trans-ocimene and lavandulol (Baser et. al., 2005; Georgiev, 1989; Konakchiev and Tsankova, 2004; Ognyanov, 1984; Schmidt, 2003; Stoyanova, 2007). Bulgarian oil possesses well-defined antimicrobial properties (Kumanova, 1988; Burkova and Markovsla, 1982; Stoyanova, 2007), on account of which it finds extensive application in medicine and cosmetics (Savova et al., 1990a, 1990b; Kumanova, 1988).

In Bulgaria the aromatic products are processed by extraction with 1,1,1,2-tetrafluorethane (Nenov, 2006; Nenov et al., 2008). The produced extracts are harmless that's why they can be widely used in food and flavour industry, cosmetics and medicine. The installation is used for processing of different essential oil bearing plants in Bulgaria.

The aim of present study is producing of new plant extracts from lavender growing in Bulgaria, by using liquefied freon 134a (1,1,1,2-tetrafluorethane) in laboratory installation and determination of their chemical composition and characteristics for possible application in cosmetics products.

Material and methods

Obtaining of extract:

The air-dried plants was ground separately in an attrition mill to a size of 0.15 - 0.25 mm and the extract obtained by a 1 dm^3 volume $C_2H_2F_4$ (1,1,1,2-tetrafluorethane) laboratory-extractor under following conditions: temperature 20 - 25°C, pressure 5,7 - 6,5 bar, time 90 min.

The physical-chemical properties of extracts were measured according to Russian Pharmacopoeia, 1990.

Determination of chemical composition.

GC analysis was performed using gas chromatograph Agilent 7890A; column HP-5 ms (30m x 250μm x 0.25μm); temperature: 35°C/3 min, 5°C/min to 250°C for 3min, total 49min; carrier gas helium 1ml/min constant speed; split ratio 30:1.

GC/MS analysis was carried out on a mass spectrometer Agilent 5975C, carrier gas helium, column and temperature as the same as the GC analysis.

The identification of chemical compounds is made by comparison to their relative retention time and library data. The identified components are arranged in order to the retention time and quantity in percentage.

Determination of antimicrobial activity.

Antimicrobial activity of extract was determined against pathogenic and spoilage bacteria and yeasts from clinical and food isolates and also against reference strains. The used test microorganisms and their origins are listed in Table 3. The strains are deposited in the microbial culture collection of Department "Biochemistry and Microbiology", "Paisii Hilendarski" University of Plovdiv, Bulgaria.

Minimal Inhibitory Concentration (MIC, μg/ml) and Minimal Bactericidal Concentration (MBC, μg/ml) of extract were determined by reference methods for broth dilution antimicrobial susceptibility tests for bacteria that grow aerobically and reference method for broth dilution antifungal susceptibility testing of yeasts (CLSI, 2008) A stock solution to be tested was prepared by diluting the respective extract sample in 5% DMSO (Sigma-Aldrich Co.). Antimicrobial activity of the extract was determined in concentrations ranging from 0.00025 to 1.6 % (w/v).

Evaluation of microbial safety of natural cosmetic product (cream) with lavender extract as natural preservative.

To evaluate the possibilities for application of obtained lavender extract as natural cosmetic preservative two different recipies for cream were prepared – one control sample without lavender extract and another with 1 % (w/w) lavender extract. Microbial safety of cosmetic preparation is defined as the ability of the studied preservative to inhibit the initial microbial contamination of the product. Evaluation of the microbial safety of prepared cosmetic products was carried out by reference method BNS EN ISO 11930, 2012, also known as challange test. In accordance with the applied method the antimicrobial and preservation activity of the lavender extract is estimated based on the its ability to inhibits the growth of *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 6535, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027 into cosmetic preparation. Both samples, control and the sample supplemented with lavender extracts was inoculated with standardized microbial inoculum of the above mentioned microorganisms and the number of viable cells into the cream was evaluated on the 3rd, 7th, 14th, 28th day of the storage and on the 3rd, 6th and 9th month of the storage.

Results and discussion

The produced extract is liquids with characteristic odour. The yield and some of physical and chemical characteristics of the extract are shown in Table 1. As seen the results for the yields of extract is comparable with literature data and values of the physical and chemical characteristics of the extract are almost equal with these for the essential oils.

Table 1. Yield and physical-chemical properties of lavender extract.

Properties	Lavender extract
Yield, %	1.2
Color	Light yellow
Dry substance, % (105°C)	20.5
Refractive index (n_D^{20})	1.4800
Acid number (mg KOH/g extract)	1.2

Chemical compositions of the extract is listed in Table 2. As seen 36 components representing 94.98% of the total content were identified. Ten of them were in concentrations over 1 % and the rest 26 constituents were in concentrations under 1%. The main compounds (concentration higher than 3%) of extract were: linalool (32.48%), linalyl acetate (22.98%), borneol (5.12%), cis-linaloloxide

(4.49%), (E)-β-farnesene (4.10%), lavandulol (4.22%) and β-caryophyllene (3.34%). According to qualitative and quantitative content of the major constituents the produced extract is equal to the essential oils, published by above listed authors.

Table 2. Chemical composition of lavender extract.

Compounds	RI	%	Compounds	RI	%
α-Thujene	931	0.09	Linalool	1097	32.48
α-Pinene	939	0.13	1-Octen-3-yl-acetate	1113	0.21
Camphene	954	0.49	Camphor	1146	0.17
1-Octen-3-ol	979	0.28	Borneol	1169	5.19
3-Octanone	984	0.11	Lavandulol	1173	4.22
6-Methyl-5-heptene-2-one	986	0.93	Terpinene-4-ol	1177	3.08
β-Myrcene	991	0.21	α-Terpineol	1189	0.58
Sabinene	975	0.09	Hexyl butyrate	1191	0.21
β-Pinene	979	0.07	Geraniol	1253	0.08
p-Cymene	1025	0.20	Linalyl acetate	1257	22.98
Limonene	1029	0.34	Bornyl acetate	1285	0.11
Eucalyptole	1031	0.54	(±)-Lavandulyl acetate	1290	0.16
(Z)-β-Ocimene	1039	0.56	Geranyl acetate	1382	0.79
(E)-β-Ocimene	1049	0.89	β-Caryophyllene	1419	3.34
γ-Terpinene	1060	0.18	(E)-β-Farnesene	1458	4.10
trans-Sabinene hydrate	1070	0.22	Germacrene D	1464	1.41
cis-Linalool oxide	1073	4.49	Caryophyllene oxide	1580	0.50
trans-Linalool oxide	1078	0.77	Farnesol	1725	4.78

Distribution of major groups of aroma substances in the extract is shown in Figure 1. Oxygenated monoterpenes are the dominant group in the extract, followed by sesquiterpene hydrocarbons and oxygenated sesquiterpenes.

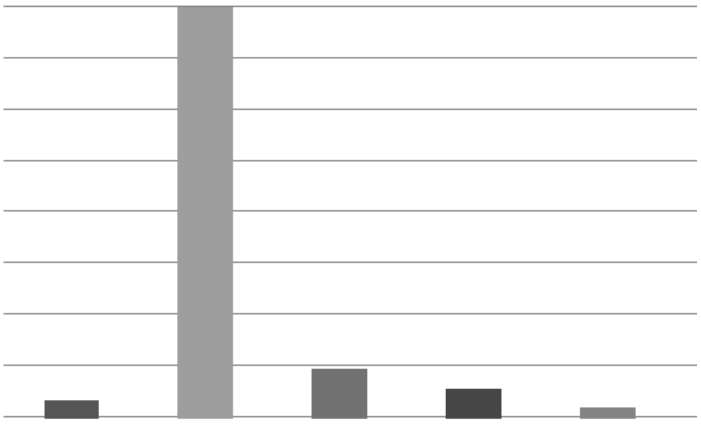

Fig. 1. Group of components in lavender extract, %.
1-monoterpene hydrocarbons (3.21 %), 2-oxygenated monoterpenes (79.87%), 3-sesquiterpene hydrocarbons (9.32 %), 4-oxygenated sesquiterpenes (5.56%), 5-oxygenated aliphatic (1.83 %), 6-phenyl propanoids (0.021 %).

The results of antimicrobial testing are presented in Table 3. The studied lavender extract demonstrated antibacterial activity against used test bacterial cultures, with the exception of both species belonging to the genus *Pseudomonas* tested by Agar Disc Diffusion Test. Probably it is due either to the diffusion limitations into the agar medium or to the increased resistance of these bacterial species. It is well known that Serial Broth Dilution Method is more reliable and reproducible and overcome the disadvantages of the Agar Diffusion Method. For these reasons it was applied parallel to the diffusion test. The results obtained by both methods shown that Gram-positive bacteria belonging to the species *Bacillus*

and *Staphylococcus* are more sensitive in comparison with Gram-negative species *Citrobacter, Escherichia* and *Pseudomonas*. The different antimicrobial resistance of both types of bacteria is due to the different chemical composition and structure of the cell wall of Gram-positive and Gram-negative bacteria. Probably the so called "*external membrane*" which is typical for the Gram-negative bacteria deteriorated the diffusion of the extract from the nutritive medium trough the cell wall and membrane into the cytoplasma. The lavender extract also demonstrated antimicrobial activity against three species of medically important yeasts belonging to genus *Candida*. Non albicans candida (NAC) species *C. glabrata* and *C. parapsilosis* were more resistible in comparison with *C. albicans*.

Table 3. Antimicrobial activity of lavender extract.

Test microorganisms	Origin	Lavender extract		
		IZ, mm	MIC, µg/ml	MBC, µg/ml
B. cereus	ATCC 11778	24	256	512
C. diversis	Clinical isolate	12	512	1024
E. coli	ATCC 8739	12	512	1024
E. coli	Food isolate	12	512	1024
P. aeruginosa	ATCC 9627	0	1024	2048
P. aeruginosa	Clinical isolate	0	1024	2048
P. fluorescens	Food isolate	0	1024	2048
S. abony	Clinical isolate	10	1024	1024
S. abony	ATCC 6017	10	512	1024
S. aureus	ATCC 6538	24	256	512
S. aureus	Food isolate	23	256	256
S. epidermidis	Clinical isolate	24	256	512
C. albicans	*ATCC 10231*	22	256	512
C. albicans	Clinical isolate	23	256	512
C. glabrata	ATCC 90030	12	512	1024
C. glabrata	Clinical isolate	13	1024	1024
C. parapsilosis	Clinical isolate	14	512	1024

The next step of the investigation has been devoted to studies on the possibility of including the resulting flavors in various cosmetic preparations. Process parameters in the preparation of each product are established in accordance with the relevant production technology for formulations that are copyrighted. The raw materials are written according to the requirements of the International Nomenclature of Cosmetic Ingredients (INCI). Extracts are incorporated in the emulsion type o/w. This type of cosmetic preparations are suitable to nourish all skin types - dry, oily, sensitive, normal, toning, moisturizing. The cosmetic formulation is a cream which can be applied not only on a daily basis, but also be used for removal of light daily makeup. Finished formulations were tested by five persons by their position indicated in the appropriate place in the text. Preparation of model type cream o/w is presented in Table. 4. The quantity of extract is between 0.5 and 2% - at higher concentrations products are with a dark color undesired by the users, and at lower - no beneficial effects on the skin are detected. Developed is also a control sample, without extract.

Some of the physical parameters of the composition are presented in Table. 5. The determination of the studied physical performance after four months of storage showed no significant deviations from the initial values.

The carried out challange test for evaluating of microbial safety of the creams inoculated with *C. albicans* ATCC 10231, *Staphylococcus aureus* ATCC 6535, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027 demonstrated that even on the 7th day of the storage no viable cell were detected into the cream sample suplemented with lavender extract. The cream was microbially safe during the whole 9 month storage period. The obtained results shown that the lavender extract is effective antimicrobial agent and can be used not only as aroma composition of cosmetic preparations, but even as effective natural preservative.

Conclusion

For the first time in Bulgaria new extract from lavender (*Lavandula angustifolia* Mill.) was produced by extraction with tetrafluoroethane. The optimal conditions for extraction procedure were determined. The produced extract was almost equal with essential oils from the essential oil bearing

plants, according to its chemical composition and antimicrobial activity. The extract is prospective for possible application in cosmetics products.

Table 4. Composition of the o/w cream.

Groups of compounds	INCI	%
A	Glyceryl Stearate SE	10.0 – 13.0
	Paraffinum Liquidum	3.0 – 5.0
	Dimethicone	2.0 - 5.0
	Cetyl Palmitate	2.0 - 3.0
	Ethylhexyl Stearate	1.0- 2.0
	Stearic Acid	0.5 - 2.0
	Propyl Paraben	0.1 - 0.2
	Methyl Paraben	0.2 - 0.3
B	PEG-8	1.0 - 3.0
	Glycerin	3.0 - 5.0
	Aqua	ad 100
C	Methylisothiazolinone	0.05 - 0.1
D	Lavandula Angustifolia Extract	0.5 - 2.0

Table 5. Physical parameters of the o/w cream.

Parameters	Control o/w cream	Cream with lavender extract
Color	white	
Odor	odorless	specific
pH	5.58	5.60

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