

Chemical Composition and the Potential of *Lavandula angustifolia* L.Oil as a Skin Depigmentant

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Abstract: Finding non-invasive skin depigmenting agents represent an important goal of cosmetic research and industry. It is now admitted that Tyrosinase inhibitor substances could be the most efficient molecules in this field because Tyrosinase is the key enzyme in the melanogenesis process. In the pathology of macular skin hyperpigmentations it can mention aesthetic problems such as: melasma gravidarum, pigmentation of aged skin, photosensibilisation, hepatic disfunctions and post-inflammatory residual pigmentation. The aim of the study was to find out if the *Lavandula* essential oil has depigmenting properties on human skin, having as theoretical premise its tyrosinase-blocking effect, previously reported in literature for some plant species. By using gas chromatograph coupled with mass spectrometer (GC/MS), a pharmaceutical cream preparation, a protocol of exploration on volunteers (including criteria) and a mexameter, we have highlighted the composition of a sample of *Lavandula angustifolia* essential oil (LEO) and its depigmenting effect on skin melanic macula. The results highlight that the main compounds identified were linalool (26.783%), terpinen-4-ol (22.143%) and 3-carene (21.668%), Terpinen-4-ol is represented as the possible active compound in tyrosinase inhibition. Our dermatocosmetic assay shows that the depigmenting effect of *Lavandula angustifolia* oil is effective after 2 months of daily topical treatment, when the melanine average value (measured by the mexameter) decreases more than a third from the initial value. The study remarks that *Lavandula angustifolia* essential oil (LEO) in daily cutaneous pharmaceutical form application, during two month, reduces the intensity of spot skin melanin, expressed through mexametric values.

Keywords: *Lavandula angustifolia* L.; tyrosinase inhibitor; melanic skin macula. © 2017 ACG Publications. All rights reserved.

1. Introduction

Eliminating or at least reducing hyperpigmented macules – especially facial located - is a major problem in dermatocosmetics. There are two main reasons for trying to solve this skin condition. The first is aesthetic, continuously promoted by commercial advertising and justified by growing demands on the quality of life. In fact the consumers demand represent the research and innovation engine for the

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pharmaceutical industry. In this regard we can mention the aesthetic problems such as melasma gravidarum, senile spots and postacneic (post-inflammatory) residual pigmentation [1]. The second major motivation belongs to the curative medicine because behind most macules of pigmentation may be hidden a real pathology like (phyto)photosensibilization and hepatic dysfunction (because the Melanocyte-stimulating hormone is normally catabolized by the liver) [2]. Ongoing research and innovation for finding new natural depigmenting agents has become a necessity, especially now that the use of hydroquinone as a classic cosmetic ingredient is avoided, since it causes skin irritation, ochronosis and it is a compound that may have cancer-causing action [3]. From the patient point of view, the aesthetic medicine methods (resorcinol or trichloroacetic acid deep peels) are not accepted, for the fear of painful application and side effects: erythema, risk of infections, social isolation, and ironically, even post-procedure pigmentations.

The reassessment of this skin condition came after the elucidation of the mechanism of melanogenesis, physiologically clarified step by step. Today we can say that depigmentants decreasing the synthesis and intraepidermal deposits of melanin are classified as follows: (1) inhibitors of tyrosinase (key enzyme of melanogenesis), (2) DOPA-oxidase blockers, (3) melanosomes transfer inhibitors, in order to block their phagocytosis in neighboring keratinocytes, (4) epidermal turnover accelerating agents, including keratolytics and peeling substances, and (5) the antioxidant substances inhibiting the oxidation of DOPA-quinone [4]. However, when we say melanin depigmentant, we actually understand tyrosinase blockers, because they are the most practically used and in theory they should be the most effective (blocking the first stage in the synthesis of melanin, the start). The pharmaceutical practice still knows many agents employed herein: arbutin, kojic acid, ellagic acid, etc, but the poor efficacy of the products always demand finding new depigmenting agents, more compliant, more active, or substances who increase the efficiency of the first one, used together, synergistically [5].

The *Lavandula* species are one of the most popular medicinal herbs. Lavanda, enjoys a history of over 2000-2500 years, beginning with Theophrastus (370-285 BC), until the first monograph 1780. *Lavandula* volatile oils have been on the market since 1976, to our days when in 2009, it is also conditioned as capsules, an authorized product in Germany, for the anxiety treatment [6]. Recently, there are researches regarding the inhibitory effect of *Lavandula angustifolia* essential oil (LEO) on fungal tyrosinase. In the fungi, tyrosinase plays a different biological role than in the human body. In plants and fungi, tyrosinase can oxidize a variety of phenolic compounds, leading to the undesirable effect of blackening of fruits and vegetables, causing a degradation both on nutritional quality and on the commercial value of commodities [7]. Because of this, depigmenting agents are highly sought after in agriculture and the food technology industry.

Volatile spike lavender oil (*Lavandula spica* L.) has been investigated by a team of Italian researchers evaluating the inhibitory activity on fungal tyrosinase. Their composition was evaluated (described) by gas chromatography and mass spectrometry. Both oils have inhibitory effect on fungal tyrosinase in a dose-dependent manner [8].

Extrapolating the encouraging result of Fiocco et al 2011 and 2016 [7,8] and knowing that in the human body, tyrosinase is the key enzyme of melanogenesis, the present study attempts to highlight whether this biochemical property (inhibition of tyrosinase by lavender oil) would also have a beneficial impact on melanogenesis. The result could be encouraging and favorable, because after the worldwide avoidance of hydroquinone (due to its significant toxic potential), the other potential natural blockers of tyrosinase have a mediocre clinical effect, requiring continued and stronger, or synergistic use of those highlighted to date.

The aim of this study was: i) to determine the proximate composition of *Lavandula angustifolia* flowers; ii) to obtain and characterize the LEO; iii) to demonstrate, on human subjects, the potential to diminishing the melanin of the skin, at the level of unreliable melanic spots (macules of diverse causes) by the mexametry method.

2. Materials and Methods

2.1. Plant Prelevation and LEO Extraction

The *Lavandula angustifolia* (LA) flower was taken in June 2016 from the private culture obtained in the western part of Romania. Species identification has been confirmed by the Department of Herbs and Medicinal Plants, USAMVB Timisoara and a voucher sample has been retained (Herbarium number LA5). The plants were dried at room temperature, grunted with GrindomixRetsch GM 2000 Laboratory Mills, and the oil was extracted by water distillation using the Clevenger Equipment.

2.2 Plant Material Characterisation

The characterization of the plant material was achieved by determining the proximate composition using the official AOAC methods (AOAC, 1997): protein (method 954.01); Lipids (920.39); Ash (923.03) and humidity (925.09). Macro and microelements analysis was done using atomic absorption spectrophotometry (SAA).

2.3. LEO Component Analysis Using GC-MS

The chemical composition of oil was determined using a Agilent Technology 7820A (Agilent Scientific, USA) gas chromatograph (GC/MS) coupled with MSD 5975 mass spectrometer and equipped with a capillary column with the following characteristics: DB WAX (30 m x 250 μm x 0.25 μm). The carrier gas was helium at a flow rate of 1 mL/min. For the separation of compounds, the following program was used: 40°C for 1 min, rate of 5°C/min to 210°C where was maintained for 5 min. Injector and ion source temperatures were 250°C and 150°C respectively. The injection volume was 1 μL at a ratio of 1:20. The NIST 17 Mass spectral Library database was used to identify volatile compounds. The Linear retention indexes (LRI) wererecalculated using n-alkanes C8-C20 and compared with NIST databasereported on DB column.

2.4. Preparation of a Pharmaceutical form with LEO

A pharmaceutical product was prepared according to the formulas listed in the table S1 and the Composition according to INCI (International Nomenclature of Cosmetic Ingredients) in the Table S2 of the Supporting Information.

The quantities of ingredients were separated in 4 phases:

Phase A: The lipidic ingredients were heated in a patented water bath at 70-80 ° C: 10.0 g of rice germ oil 3.0 g of mango butter. Montanov™ 68 was then added in a quantity of 3.0 g. This is a non-ionic, liquid crystal emulsifier based on the HLB system. Liquid crystal emulsifiers create a lamellar or bilayered structure that mimics the lipid bilayers in our stratum corneum, which may help actives or lipids from our lotions penetrate our skin easier. They also assist in reducing transepidermal water loss as the lotions hold more water in contact with the skin for a longer period of time. In the heated oil phase it was included Montanov™ 68, which does best with a 25% oil phase and works to emulsify natural oils as well as silicones and esters. Including a fatty alcohol like Cetyl or Cetearyl Alcohol can help increase the liquid crystal formation in the lotion. It is compatible with cationic ingredients, like Honeyquat and Cetrimonium Chloride. And it works well from pH 3 to 12, so it's a good choice for making lotions as diverse as light facial moisturizers with AHAs and bleaching or depilatory creams. Lotions made with Montanov™ 68 are described as being lighter and more moisturizing than those made with non-liquid crystal emulsifiers, like Polawax™ or Emulsifying Wax NF, this being the main reason for choosing him as an ingredient. The O/W emulsifier is versatile and can be used for natural or "green" concepts and offers variable consistency ranging from spray to thick creams with a

light texture. Appearance: white flakes. Stability: stable between pH 3 – 12. HLB:10. Country of Origin: France, Seppic. Approved by ECOCERT, COSMOS and Natrue, preservative-free, synthesised without solvent.

Phase B: 60.0 g of water, 2.2 g of Caolin then 0.5 g Sanfteen was added, which is a nonionic sugar surfactant from coconut oil and sucrose. It is a tough, whitish mass with a somewhat soapy odor, which tends to become liquid at temperatures above 25 ° C. Gentle because of its very good skin compatibility (mild and irritation free) is usually worked as co-surfactant in shampoos, shower gels, foam baths and cleansing formulations. The mucosal tolerance is improved by others, with processed surfactants. At the same time, it has a moisturizing and nourishing effect with high fine foaming ability. Therefore it is particularly recommended for sensitive skin and baby products. The pH is between 7 and 8, and the wash-active substance (WAS) is 65%. The cosmetic additive was ordered from the German producer Spinnrad.

Phase C: 9.8 g of glycerol 0.5 g of xanthan

Phase D: 1.0 g Rokonsal™ BSB-N, Lactic acid (Q. S) and the Essential oil of *Lavandula angustifolia*. Rokonsal™ BSB-N is a combination of Benzoic and Sorbic acids in Benzyl alcohol. The ingredients of Rokonsal™ BSB conform to the German BDIH recommendation for Natural Cosmetic Products and the recommendations of the "Council of Europe". Due to the organic acids, Rokonsal™ BSB may be classified as a "mild preservative", provided the pH of the final product is not higher than 5. It is effective against Gram-positive and Gram-negative bacteria, yeasts and moulds at acid pH values. It is produced by Ashland, advancing skin with science. The company develops and produces a comprehensive line of biofunctionals, fixative and styling polymers, bioadhesives, rheology and conditioning polymers for the Personal Care industry. To help formulators create blockbuster innovations, Ashland focuses on breakthrough chemistries that deliver advanced performance properties in hair care, skin care, sun care and oral care products.

2.4.1. Method of Preparation

2.4.1.1. *Phase A:* The solid butter was weighed, then melted to 75 ° C, oil was added and then the pot was withdrawn from the hot plate of the heater.

2.4.1.2. *Phase B:* In water heated to 75° C, Sanfteen and kaolin was added and mixed until homogeneous. Then phase B was added in phase A, emulsifying for 1-4 minutes.

2.4.1.3. *Phase C:* Xanthan is dispersed in glycerine, added to phase A / B, and soon emulsified, then gently mixed while it cools, until homogeneous.

2.4.1.4. *Phase D:* The preservative emulsion is added gradually, the pH is checked and adjusted if necessary to pH 5 to 5.4, gently mixing until well incorporated. Only lavender oil will be added at the end.

The ointment contains an increased percentage of lipophilic components and water. This is why this formulation includes: stabilizers, antioxidants, antimicrobial preservatives, emulgators, surfactants and finally plant extracts. *Lavandula angustifolia* was chosen rather than *Lavandula x intermedia* which can be found and harvested in the western part of Romania, for the chemical composition and antimicrobial properties of the essential oil[9].

In the preparation of the emollient ointment, it should be paid attention at the fact that the heating of the lipophilic components is at a temperature of 70-80°C. The emulsion of the aqueous phase in the lipophilic phase is recommended at the same temperature. Only the volatile oil was added after cooling, in order to avoid changing its structure with increasing temperature.

Mixing a O/W alkylpolyglucoside emulsifier of natural origin, it can be obtained a rich, easy-to-spread cream. As a promoter of liquid crystals which biomimic the lipid bilayer of the cellular membrane, it helps to stabilize the emulsion, provides the restructuring effect (reduction of TEWL) and the moisturizing effect.

The obtained O/W emulsion contains a fatty phase of almost 23% requiring minimum 3% of emulsifying factors. This creamy white emulsion with lavender smell is extremely easy to apply and

non-irritant, even for sensitive, dry and mature skin. It can be appreciated that the lavender oil can be not only a flavouring but an active cosmetic ingredient in a higher concentration[10].

2.5. The Dermocosmetic Assay

The mexametric assay was performed on 15 volunteers, all women, aged between 22 and 64 years, which accepted to applied 2 times a day the cream prepared in our laboratory, for a 2-month horizon. *The criteria for including the volunteers in the study* were: (1) Written consent of the volunteers after they understood the test procedure, (2) Diagnostic of the melanic macula, without a tegumentary relief (melasma, spot of photosensibilisation, senile or postacneic (post-inflammatory) residual pigmentations), (3) Lack of any pathological conditions on the explored tegumentary area (eg. acne, seborrheic dermatitis, psoriasis)(4) Elimination of cutaneous intolerance, after a preliminary test of the cream at a topical skin application.

The intensity of the melanin colour (and the possible diminishing of skin coloration) we used the Mexameter MX 18, produced by produced by Courage Khazaka (DE). This is a very simple, fast and economical tool used to measure the two components, mainly responsible for skin color: melanin and hemoglobin (erythema).

The ethics committee of the Faculty of Pharmacy in Timișoara approved the study on volunteers with the mexametry, the noninvasive method for evaluation of skin coloring (Protocol number 6/22.02.2017).

2.6. Statistical Analysis

In order to observe if our results have significances we used the ANOVA one way method. Additionally, layout of the design and statistical analysis is simple. A framework for one-way ANOVA following a single factor with more data levels has been found to be ideal. Results are presented as means \pm standard deviation (SD) of triplicate measurements. Analysis of variance was performed on the data recorded using statistical data analysis, from EXCEL.

3. Results and Discussion

3.1. Chemical composition

The proximate composition and the content of macro and microelements of *Lavandula angustifolia* flowers are presented in table S3 and 1, while the chemical composition of LEO are shown in Table 2.

Table 1. Macro and microelements composition of *Lavandula angustifolia**

K	Ca	Mg	Na	Mn	Fe	Zn	Ni	Cu
3437 \pm 0.62	5000 \pm 0.20	598 \pm 0.15	92.65 \pm 0.50	24.72 \pm 2.25	186 \pm 0.5	4.632 \pm 0.25	0.145 \pm 0.33	0.72 \pm 0.40

*Results are given in ppm

The values we found in the composition of *Lavandula angustifolia* are in accord with previously data reported in the literature: 8.71% moisture, 9.16% ash, 6.82% protein, 6.89% lipids and 61.37% carbohydrates [11,12]. Experimental results on elemental composition reveal that calcium was the most abundant macro element, followed by potassium, magnesium and sodium. Similar values of macro and microelement content in *Lavandula angustifolia* have been reported previously [13,14].

In *Lavandula angustifolia* essential oil (LEO) were identified 11 major compounds, in concentration over 0.3%. The main compounds identified were linalool, (26.783%), terpinen-4-ol (22.143%) and 3-carene(21.668%), representing 70.594% from all analysed compounds (table 2). Also, other compounds as: thymol (11.104%), o-cymene (6.238%), β -farnesene (6.587%), and Lavandulyl acetate (3.291%) were found in percentage over 3% from total.

The presence of compounds belonging to a specific class of secondary metabolites, within a species or even within a single plant may serve as chemotaxonomic markers indicating the biosynthetic pathways and the functional properties of plant [15]. Secondary metabolites are the compounds that usually perform non-essential functions in the plants [16]. Terpenoids represent secondary metabolites found in essential oils of plants widely utilized for chemotaxonomic classification. These groups of compounds exhibit a wide variation in chemical diversity, distribution and function [16].

In the analyzed oil, the monoterpene oxygenated compounds are predominant (60.419%), followed by monoterpene hydrocarbonates (29.704%) and sesquiterpene oxygenated (6.587%) from all chemical compounds. The group of Smigielski *et al.*, 2009 identified a similar distribution of oxygenated monoterpene that represents 73% of all compounds, hydrocarbonate monoterpene (10.3%) and oxygenated sesquiterpene (4.8%) [17].

Table 2. Chemical composition of *Lavandula angustifolia* essential oil

No.crt.	Compounds	LRI reported in literature*	Linear Retention index (LRI)**	%
1	3-carene	997–1027	1011	21.67
2	Linalool	1074–1098	1086	26.78
3	Terpinen-4-ol	1148–1180	1177	22.18
4	o-cymene	1009–1076	1197	6.24
5	Thymol	1260–1289	1290	11.10
6	Lavandulyl acetate	1268–1276	1293	3.29
7	β -farnesene	1428–1458	1449	6.59
8	α -muurolene	1486–1496	1460	0.44
9	α -caryophyllene	1396–1419	1462	0.99
10	γ -muurolene	1467–1479	1482	0.37
11	α -bisabolol	1649–1686	1690	0.35
Total				100
Monoterpene hydrocarbonates				29.70
Sesquiterpene oxygenated				6.59
Monoterpene oxygenated				63.71
Yield of essential oil % (w/v)				3.4

* Linear retention indices (LRI) according to [15],[16],[18]

** Linear retention indices (LRI) calculated against n-alkanes (C8-C27) for DB WAX

The essential oil's composition is different depending on genetic, environmental and maybe agrotechniques factors [19]. Previous studies indicate the linalool and linalyl acetate as major compounds [20-23], in *Lavandula angustifolia* essential oil. Opposite, L Hui *et al.*, 2010 identified linalyl butyrate as main compound (43.73%), followed by α -Ocymene (25.10%), Eucalyptol (7.32%) and Camphor (3.79%) [23]. In *Lavandula angustifolia* essential oil prelevated from west Romania were identified caryophyllene (24.12%) and eucalyptol (15.69%) [9].

The chemical composition influences the biological properties of the essential oils. Closely related with skin and hair pigmentation in mammals, tyrosinase represents the key enzyme of melanogenesis, and its activity can be influenced by the composition of EOs. Kinetic studies of tyrosinase inhibitory activity of 19 essential oils extracted from medicinal plants revealed the high activity of *Lavandula angustifolia* essential oil [8]. Other studies explain the tyrosinase inhibitory activity in close connection with the chemical composition of the essential oils [24,25].

Fiocco, D. et al., 2011 which constituted our premise, has not yet advanced any explanation on the relationship between chemical structure and antityrosinase activity, namely, it does not express which substance in the composition of *Lavandula angustifolia* essential oil inhibits or blocks tyrosinase [7].

Our research, identifying components in the *Lavandula angustifolia* essential oil, attempts to compare them with other similar components for which anti-tyrosinase activity is certain. In support of this reasoning is the study of Korner A. and Pawelek J., 1982 which highlights the fact that volatile oil of *Eucalyptus camaldulensis* flowers, has tyrosinase inhibitory properties, and therefore antimelanogenic [26].

By comparing the chemical compositions of the two volatile oils, the "suspects circle" of antityrosinase substances is limited to terpinen-4-ol, found in high concentration in *Lavandula angustifolia* and *Eucalyptus camaldulensis* oils [27].

The future use of *Lavandula angustifolia* essential oil as a depigmenting natural product would be a great advantage for the cosmetics industry [28]. *Lavandula* is more widely spread than *eucalyptus* (for example) and essential lavender oil is a patient-friendly product and well received by the consumer.

3.2. Results Concerning the Dermocosmetic Assay

The melanine values for the 15 volunteers were calculated as arithmetic average, for 2 cutaneous areas: near (outside) the melanine macula and respectively inside the melanine macula. The melanin concentration inside the stain is higher compared to the outside, reacting more strongly in the positive sense to the applied product. Those results are shown in the table 3.

Table 3. The values of melanine in the melanine macula, during the mexametry assay

Melanine inside vs. outside of the macula		
	Outside	Inside
1	99.67	174.67
2	93.32	172.30
3	92.27	170.33
4	88.11	152.12
5	89.15	147.27
6	89.72	132.26
7	92.12	112.92
8	97.25	103.71
n	8	8
X	92.50	145.41
s	4.10	27.62
X_a	118.96	

Literature finds similar results for plant species such as *Glycyrrhiza glabra* (as a tyrosinase inhibitor), *Arctostaphylosuva-ursi* containing arbutin and kojic acid from fungal sources, as well as *Glycine max* extracts as an inhibitor of the melanosomal transfer [29].

Against all of them, *Lavanda angustifolia* is more geographically spread and more attractive to patients who see *Lavanda* essential oil, in their subconscious, as a fragrant product. In addition, Soy extracts are controversial because consumers associate them with the idea of a genetically modified plant. In fact, the pharmaceutical research does not seek the supremacy of a species at the expense of another, but the identification of several extracts that, by mixing them in a cosmetic product, reducing the concentration of each and avoiding hydroquinone.

Using the software that analyzed all input data, its own statistical analysis table has resulted and is presented in the tabel 4. Detailed, specialized explanations follow it.

Table 4. Statistical analysis of the data

source	df	SS	MS	F	P-value
		11.199			
treatments	14	0.460	14.9900.460	2870.248	0.011.565
error	1	458.428	389.888		
total	15	14.6570.888			

F distribution have been used in this given situation, depending on the number of degrees of freedom. Once the appropriate F distribution was determined, we rejected the null hypothesis. The total Sum of the Squares (SS) is the sum of the squares of the deviation of individual observations taken together and the Mean Square (MS) is due to the treatment. When these calculations have been performed within the group, it was evident that our assumption was true. Also the P value being typically < 0.05 is significant, meaning that our results indicate a melanin decrease worth of consideration, especially inside the melanine macula

This evolution is presented in the Figure S1 in Supporting information file.

Lavandula angustifolia essential oil was cited in literature for its tyrosinasis inhibitory property [7, 8, 22, 23]. This effect was demonstrated for the botanical world, on fungi. Extrapolating this effect on the human melanine synthesis, and taken in consideration that tyrosinasis is the key enzyme in melanogenesis, this study shows very clear that the depigmenting effect is visible, and its decreasing can be measured by mexametry.

In our study, the depigmenting effect was effective only after minimum 2 months of daily treatment, when the melanine average value decreases more than a third from the initial value, but close to the natural skin melanine average value.

This diminution of melanine inside the spot is independent of the natural evolution of the healthy skin (tanning, e.g). For the flat evolution for the first 3 weeks, it is suspected that the epidermis keeps the mature melanosomes inside it at least for a keratinocyte turnover (for a cycle cell time). For the epidermis, the cycle cell is about 28 days. After this time horizon, the epiderm acquires chances to diminish the excessive, the pathological melanine amount. In order to optimise this effect, we **recomand** the association of *Lavandula* oil with other tyrosinasis inhibitors, but most important with keratolitic agents.

Seeing this new property highlighted in the present study for *Lavandula angustifolia* essential oil (LEO), as well as the rich composition reported by other authors [30], we consider now that *Lavandula angustifolia* deserves to be reconsidered from the classic aromoterapic plant status to the pharmacologically and dermocosmetic active species.

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/RNP>

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