ORIGINAL ARTICLE

LAVANDULA HYBRIDA: MICROSCOPIC CHARACTERIZATION AND THE EVALUATION OF THE ESSENTIAL OIL

SILVIA ROBU[1], BIANCA IOANA CHESARU[1]*, CAMELIA DIACONU[1], OLIMPIA DUMITRIU-BUZIA[1], DANA TUTUNARU[1], URSULA STANESCU[2], ELENA LACRAMIOARA LISA[1]

[1]*Faculty of Medicine and Pharmacy, "Dunărea de Jos" University, Galați, Romania*
[2]*Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Romania*

**corresponding author: dr_chesaru@yahoo.com*

Manuscript received: November 2015

Abstract

Lavandin (*Lavandula hybrida* Reverchon) is related to *Lavandula angustifolia,* but the species cultivated in Romania are poorly studied in terms of their secondary metabolites and their uses in therapy. The purpose of the study was mainly to reveal the microscopic characteristics of Romanian lavandin grown in Piatra Neamț, Romania. Moreover, the biological potential of volatile fractions isolated from lavender (used for comparison) and lavandin were assessed especially on standard microorganisms. The results indicated similar microscopic elements, and some common chemical characteristics to *Lavandula angustifolia*, but also an important variation regarding the antimicrobial activity of the essential oils.

Rezumat

Lavandina (*Lavandula hybrida* Reverchon) este o specie înrudită cu *Lavandula angustifolia*, dar cercetările asupra speciilor cultivate în România sunt puține în ceea ce privește metaboliții secundari și utilizarea terapeutică a acestora. Ne-am propus să evidențiem în primul rând elementele microscopice ale lavandinei de proveniență românească cultivate în Piatra Neamț, România. În plus, a fost evaluat potențialul antimicrobian al fracțiunilor volatile izolate din lavandă (utilizată ca martor) și al lavandinei asupra unor tulpini standard de microorganisme. Rezultatele au indicat similitudini de ordin microscopic și chimic cu *Lavandula angustifolia*, precum și diferențe exprimate într-o variație importantă a acțiunii antibacteriene a celor două uleiuri volatile.

Keywords: microscopy, TLC, GC-MS, essential oil, antimicrobial

Introduction

Lavender flowers and its essential oil are widely used in aromatherapy [6], both in our country, and especially in the European Community. In modern times, the oil and the lavender flowers have a wide range of uses: therapeutic aromatherapy, perfumery, cosmetics, pharmaceuticals, soap, detergent, and even food industry. Various studies in the last decade resulted in placing on the market Lasea® that contains *Lavandulae aetheroleum* enriched with linalool [3], known today as Silexan. The product addresses to the treatment of generalized anxiety syndrome and its sub-syndromal form [7].

The most known representatives of *Lavandula Genus* are: *Lavandula angustifolia* (*L. officinalis*), *L. latifolia*, *L. stoechas*, *L. burnatii*, *L. dentata*, *L. canariensies*, *L. abrotanoides*, *L. lanata*, *L. multifida*, *L. pinnata*, *L. viridis*, *L. x intermedia*, *L. luisierii*. *L. hybida* (sin. *L. x intermedia*) is common in Romania and has a highly productivity in regards of essential oil (60 - 150 kg essential oil/ha).

Previous studies indicated that different extracts isolated from aromatic plants, members of *Lamiaceae* family, possess important antioxidant properties [8, 10]. Thus, the aim of the present study was to evaluate the microscopic characteristics of the flowers, the chemical composition and the antibacterial properties of Romanian origin lavandin essential oil.

Materials and Methods

Plant material and essential oil isolation

Dried plant product, originating from the Biological Research Centre in Piatra Neamț, Romania, was crushed and extracted with water vapours. The essential oil was obtained throw hydro-distillation for 3 hours in a Clevenger-type apparatus. The essential oil was dried on anhydrous sodium sulphate and stored at 4°C until analysis. One voucher specimen of the investigated plant material was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, "Dunărea de Jos" University, Galați, Romania.

The microscopic identification

The microscopic pharmacognostic analysis was led by classical methods, when the powdered flowers were clarified with 80% chloral hydrate, as indicated by the Romanian and European Pharmacopoeias [1, 2]. The specific elements were

photographed with a Canon PS A540 camera and an Optika microscope.

The chemical analysis

Initially, the qualitative characteristics of the essential oil were established by thin layer chromatography (TLC) as compared to *Lavandula officinalis aether.*, linalool and linalyl acetate as standards. The chromatographic conditions were as indicated by European Pharmacopoeia [1], in visible light and using vanillin solution as developer.

The semi quantitative analysis employed GC-MS-FID (gas chromatography - mass spectrometry - flame ionisation detector) techniques (Agilent Technologies 6890N/5975) with the following parameters: injection volume 1 µL (Column: HP, 5MS bonded phase 5% phenylmethylsiloxane; 0.25 mm i.d.; 30 m length; 0.25 µm film thickness); ratio 1:100, carrier gas Helium, temperatures: injector 250°C, detector 280°C, column 50°C, 2 min; 10°C/min to 250°C for 10 min. The identification of the volatile compounds was based on the comparison of their retention indices (RI), and mass spectra with those obtained from authentic samples and/or National Institute of Standards and Technology/National Bureau of Statistics, Wiley libraries and literature.

Antimicrobial assay

The antimicrobial activity of the essential oil (10 µL) was measured by the disc-diffusion method, in Mueller - Hinton agar, on the following strains: *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231). The analysis was in accordance to National Committee for Clinical Laboratory Standards (NCCLS) 2009.

All results were assessed three times and the yield was considered for discussions.

Results and Discussion

The structural elements that were observed were: epidermal cells with sinuous walls, characteristic glandular and surface trichomes, small oxalate crystals, papilla on the surface of corolla, and pollen grains (Figure 1).

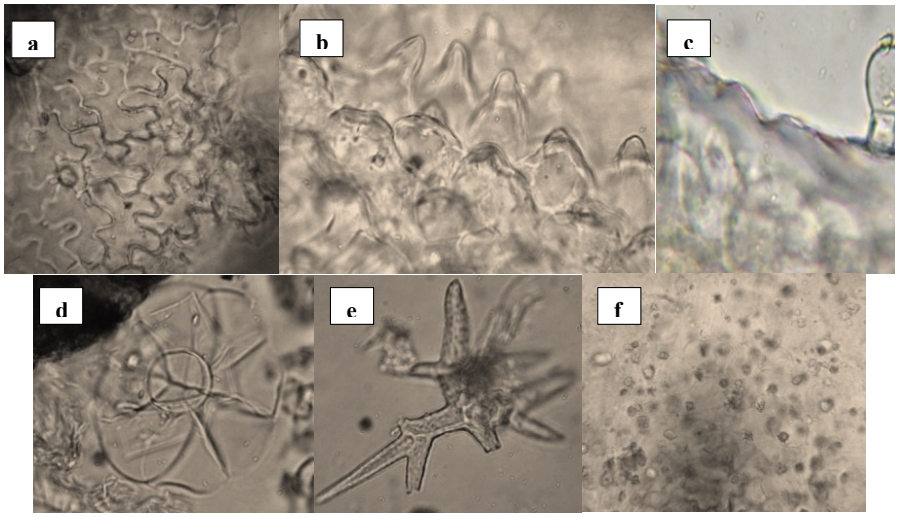

Figure 1.

Microscopic features of *Lavandula hybrida* flowers

Legend: epidermal cells (a), corolla with papilla (b), glandular trichomes (c, d), surface hairs (e), oxalate crystals (f)

The TLC results showed that lavandin essential oil has a similar spectrum to lavender essential oil (EO) (Figure 2), however the GC analysis indicated the presence of the compounds included in Table I.

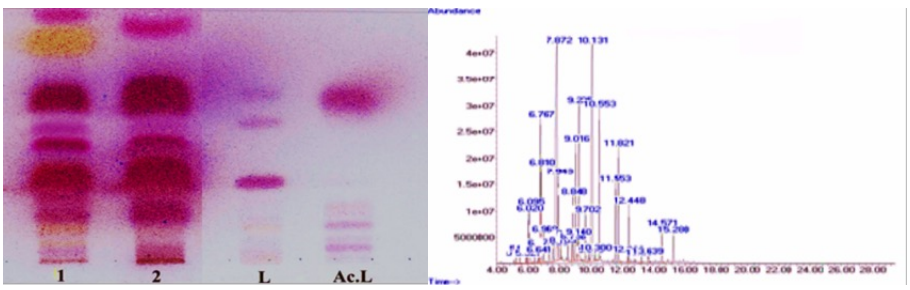

Figure 2.

TLC and GC-MS chromatograms for the investigated samples

Legend: 1 – EO L. hybrida; 2- EO L. angustifolia; L – linalool; AcL-linalyl acetate

The GC-MS analysis indicated the presence of 45 compounds that belong to monoterpene hydrocarbons (myrcene, *p*-cymene), monoterpene alcohols (linalool, lavandulol), monoterpene esters (neryl- and lavandulyl acetate, aliphatic compounds and sesquiterpenes. Nevertheless, the most quantitatively important components identified in lavandin essential oil were: linalool (21.5%), linalyl acetate (22.5%), lavandulyl acetate (8.4%), 4-terpinen-ol (16.7%), α-terpineol (7.5%), borneol (1.33%), nerol (2.24%), neryl acetate (1.54%), myrcene (1.13%), and *p*-cymene (1.35%). Most of the identified compounds were in compliance with the European Pharmacopoeia requirements for *Lavandula aetheroleum*.

Table I

Selective compounds identified in *Lavandula hybrida* essential oil

Compound	Requirement Ph. Eur.	Area %
limonene	less than 1.0 %	0.8
cineol	less than 2.5 %	tr*
camphor	less than 1.2 %	tr
linalool	20.0 - 45.0 %	21.5
linalyl acetate	25.0 - 46.0 %	22.5
4-terpinen-ol	0.1 - 6.0 %	16.7
lavandulyl acetate	more than 0.2 %	8.4
lavandulol	more than 0.1 %	tr
α-terpineol	less than 2.0 %	7.5

* tr - traces

Our results were in concordance with other scientific data, lavandin usually has a different profile that *L. angustifolia,* although lavender is considered the parent plant for lavandin [3, 4, 11]. Moreover, the presence of such components (4-terpinen-ol, α-terpineol) may suggest the existence of antibacterial properties [8, 9]. In addition, formulation of lavandin oil might increase its availability, allowing the concentration of active terpenes on the bacterial membrane thus enhancing its antibacterial activity [12].

In regards to the antimicrobial properties the investigated essential oil showed no activity against Gram-negative strains. Moreover, our results showed that the antistaphylococcal activity is reduced, while there is a moderate antifungal activity. Arldogan *et al.* have also found that *Lavandula hybrida* essential oil exhibits a mild potential only against *Staphylococcus aureus*. Nevertheless, the chemical composition of their investigated sample contained camphor, bisabolene and geraniol in higher amounts than our essential oil [5].

The values recorded for our tested oils are presented in Table II, and the lavandin essential oil is shown as compared to the lavender essential oil and three synthetic standards (ampicillin, chloramphenicol and nystatin).

The calculated values against *Candida albicans* strain were situated slightly below the measured values for nystatin used as standard. Since the investigated sample did not show a marked antibacterial action, although it contains components such potential (especially α-terpineol), we might presume that the ratio between each individual substance is of great importance in expressing the biological potential.

Table II

In vitro antimicrobial activity of *Lavandula* sp. essential oils (EOs)

Sample/standard	Inhibition area (mm)				
	S. aureus **ATCC 25923**	***B. subtilis*** **ATCC 6633**	***B. cereus*** **ATCC 14579**	***E. coli*** **ATCC 25922**	***C. albicans*** **ATCC 10231**
Lavandula hybrida EO	7	12	13	0	17
Lavandula angustifolia EO	14	18	15	0	5
Ampicillin 10 μg	27	32	25	22	nt
Chloramphenicol 30 μg	25	30	28	24	nt
Nystatin 100 μg	nt*	nt	nt	nt	24

*nt = not tested

To comprehend the intensity of action better we calculated also the minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The registered values were MIC 80% *L. hybrida* = 0.5 μL/mL and MBC *L. hybrida* = 1.00 μL/mL respectively, whereas MIC 80% *L. angustifolia* = 0.25 μL/mL and MBC *L. angustifolia* = 0.25 μL/mL. As a general assessment *L. angustifolia* essential oil is more active on certain strains, but *L. hybrida* essential oil possible induces different mechanisms against fungi. These results confirm other findings in which the differences in the cell membrane of bacterial groups influence the sensitiveness of each strain to a certain proportion and mixture of active compounds [12].

Conclusions

Our research revealed that a lavandin essential oil is not recommended as an anti-microbial in serious infections. Considering all aspects regarding possible antidepressant and anxiolytic effects, one might sustain that *L. hybrida* essential oil of Romanian origin can be used in rooms with ventilation systems or air conditioning to reduce stress condition and to give comfort, possibly

reducing bacterial contamination in the spreading area.

Conflict of interest

The authors declare that they have no potential conflicts of interest to disclose.

References

1. ***European Pharmacopoeia, 6th edition, EDQM Stuttgart, 2008.
2. ***Romanian Pharmacopoeia, 10th edition, Editura Medicală, Bucureşti, 1993.
3. Abuhamdah S., Chazot P.L., Lemon Balm and Lavender herbal essential oils: Old and new ways to treat emotional disorders?. *Current Anaesthesia & Critical Care*, 2008; 19: 221-226.
4. Arabaci O., Bayram E., Baydar H., Tahsin A.F., Ozay N., Chemical composition, yield and contents of essential oil of *Lavandula hybrida* Reverchon grown under different nitrogen fertilizer, plant density and location. *Asian J. of Chemistry*, 2007; 19(3): 2184.
5. Andoğan B.C., Baydar H., Kaya S., Demirci M., Özbaşar D., Mumcu E., Antimicrobial activity and chemical composition of some essential oils. *Archives of Pharmacol. Research*, 2002; 25(6): 860-864.
6. Barocelli E., Calcina F., Chiavarini M., Impicciatore M., Bruni R., Bianchi A., Ballabeni V., Antinociceptive and gastroprotective effects of inhaled and orally administered *Lavandula hybrida* Reverchon "Grosso" essential oil. *Life Sciences*, 2004; 76(2): 213-223.
7. Brüning E., Lavendelöl (Lasea) gegen Angst?. *Arznei-Telegramm*, 2011; 42(3): 31-32.
8. Cioanca O., Mircea C., Hritcu L., Trifan A., Mihasan M., Aprotosoaie A.C., Robu S., Gille E., Hancianu M., *In vitro–in vivo* correlation of the antioxidant capacity of *Salviae aetheroleum* essential oil. *Farmacia*. 2015; 63(1): 34-39.
9. Giordani R., Regli P., Kaloustian J., Mikail C., Abou L., Portugal H., Antifungal effect of various essential oils against *Candida albicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris*. *Phytotherapy Research*, 2004; 18(12): 990-995.
10. Miftode A.A., Ștefanache A., Șpac A., Dorneanu V., *In vitro* evaluation model for total antioxidant activity evaluation wit applications on biological products. *Farmacia*, 2015; 63(2): 301-305.
11. Varona S., Martin A., Cocero M.J., Gamse T., Supercritical carbon dioxide fractionation of Lavandin essential oil: Experiments and modelling. *The Journal of Supercritical Fluids*, 2008; 45(2): 181-188.
12. Varona S., Rojo S.R., Martín Á., Cocero M.J., Serra A.T., Crespo T., Duarte C.M., Antimicrobial activity of lavandin essential oil formulations against three pathogenic food-borne bacteria. *Industrial Crops and Products*, 2013; 42: 243-250.