PRELIMINARY RESEARCH ON THE CHARACTERISTICS OF PHENOLS IN *LAVANDULA ANGUSTIFOLIA SP.*

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Abstract: *Starting from the fact that the European Pharmacopoeia allows the use for medicinal purposes only the flowers of Lavandula sp., the main aim of this research was to investigate the chemical composition of Lavandula angustifolia sp. extracts obtained by three extraction methods: ultrasound-assisted extraction, rapid extraction under pressure at 6.7 bar and subcritical fluid extraction. The solvents used for the first two extraction methods were different mixtures of water and alcohol, glycerol or propylene glycol. These extracts were then analyzed for their qualitative composition by high performance thin layer chromatography, attenuated total reflection - Fourier transform infrared and Raman spectrometry as well, and for the total phenolic content using a modified Folin-Ciocalteu method. Subsequent high performance thin layer chromatography analyzes will highlight the main phenolic components in lavender extracts.*

Keywords: *Lavandula sp., extraction, polyphenols, high performance thin layer chromatography.*

1. Introduction

There is ample scientific evidence (Kaka et al. 2016) supporting that Lavandula angustifolia extract protected the neurons against glutamate toxicity (Büyükokuroğlu et al. 2003).

In the last years the beneficial effects of lavender aqueous extract on spatial performance in Alzheimer's disease in rats was evaluated, as well (Kashani et al. 2011). Therefore, according with the evaluation made by the Commitee of Herbal Medicinal Products of European Medicines Agency (EMA/HMPC/143183/2010), Lavandula angustifolia (as flower or leaves extract, and essential oil) has a lot of therapeutical properties, including antiseptic, anti-inflammatory, analgesic, cleansing, balancing, and soothing.

These studies have shown that lavender improve the free radical scavenging activity and reduce the stress hormone, cortisol, which protects the body from oxidative stress (EMA/HMPC/143183/2010; Atsumi and Tonosaki 2007).

The present research deals with the identification of the polyphenols responsible for antioxidant activity of lavender extracts obtained by three extraction methods: ultrasound-assisted extraction, rapid extraction under pressure at 6.7 bar and supercritical fluid extraction. Six different extractive solutions were used for each extract performed using the first two methods. For supercritical fluid extraction 1,1,1,2-tetrafluorethane was used.

The best extracts, in terms of amount and stability of isolated phenolic total extracts were those obtained with supercritical fluid extraction method. This extraction method is a promising alternative to obtain the high amount of phenolic compounds. These phenolics are responsible for a good antioxidant activity and offer the possibility to use them in pharmaceutical formulations linked to the fields of nutrition, cosmetics and drugs (Hossu et al. 2006; Hossu et al. 2009).

2. Materials and methods

For obtaining the extracts, the dried lavender flowers Lavandulae flos from Lavandula angustifolia Mill. and L. species were chosen. The lavender flowers were cut in a plant cutting machine type Herbcut 1340, and dried in a

Miraco dryer with controlled temperature and humidity sensors that trigger the evacuation of humid air only after this moisture remains unchanged a programmable time. Further, dried lavender flowers were crushed in a mill, and then the raw material was sieved at 4 mm.

Ultrasound-assisted extraction was carried out using a Sonomatic Langford ultrasonic bath, equipped with the possibility to control the temperature of the solvent (35 °C) and ultra-sonication time. Rapid extraction under pressure at 6.7 bar on lavender flowers was performed in a Timatic Micro C extractor at 35 °C. The extractive solutions used were as following: 50% water a (pH=5) – alcohol; 50% water b (pH=9) – alcohol; 50% water a (pH=5) – glycerol; 50% water b (pH=9) – glycerol; 50% water a (pH=5) – propylene glycol and 50% water b (pH=9) - propylene glycol (see also Table 1). Waters (a) and (b) were obtained with a Kagen apparatus; glycerol and propylene glycol were of pharmaceutical grade.

Subcritical fluid extraction was performed by using a fluid extractor Timatic FC100, in which the volatile components and lipids were removed without affecting the water-soluble components which afterwards may be retrieved.

Table 1 summarizes the composition of the standards used for the identification of phenolics by thin-layer chromatography (continuing the studies in the follow-up stages).

Table 1. *Standards used for the identification by high performance thin layer chromatography*

Standard designation	Standard composition
S1	chlorogenic acid + gallic acid
S2	rutoside + hyperoside + isoquercitroside
S3	luteolin 7-O-glucoside
S4	vitexin + umbelliferone

3. Results and discussion

A first stage, since 2015, has been represented by the obtaining of extracts of fat soluble bioactive compounds from lavender dry flowers within the *EBIOTEFA* Research Center from Transilvania University from Braşov, by extraction of subcritical fluids (fig.1).

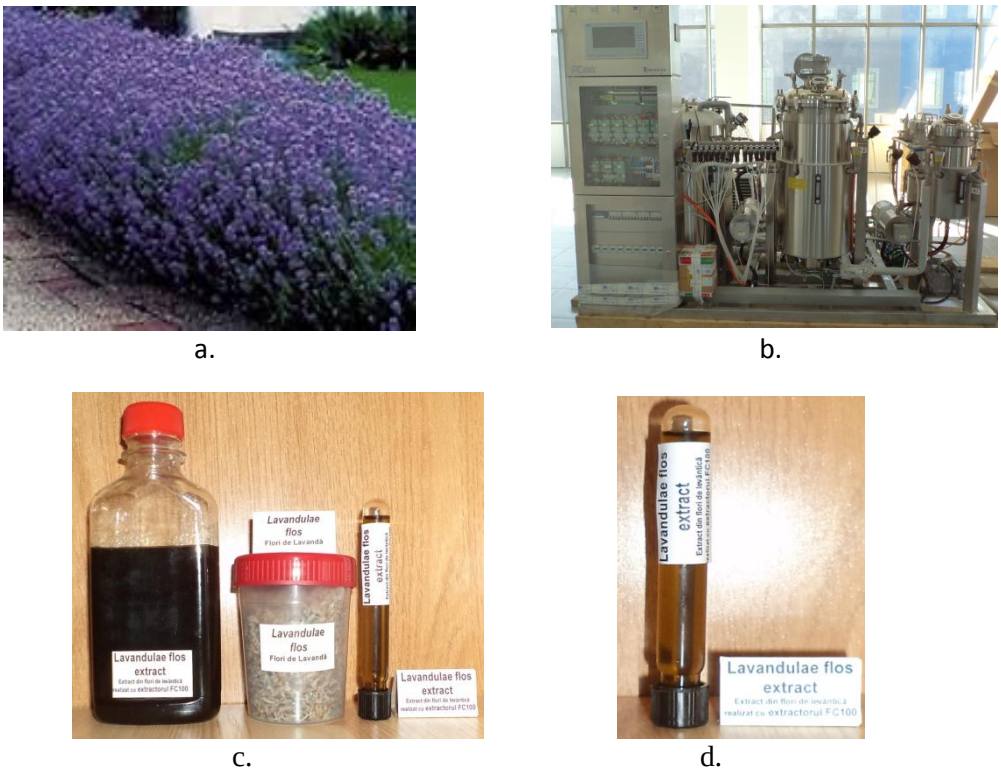

Fig. 1. *The extraction flow starting with lavender pay (a), diverse stages of extraction achieved with the equipment for extraction in fluids at subcritical pressure - the extractor of TIMATIC FC100 model (b) and the obtained extracts (c and d).*

A first chromatographic analysis revealed the presence of main phenolic compounds in different amounts and/or composition in the lavender extracts according to the used extraction methods (i.e. ultrasound-assisted extraction, rapid extraction under pressure at 6.7 bar). By the procedure followed, it seems that the hydro-alcoholic extraction, either with (a) or (b) water, results in more extracted compounds. The chromatograms (Figures 2 and 3) clearly show the presence of chlorogenic acid and umbelliferone in the hydro-alcoholic extracts (Rădulescu, Cristina et al, Valahia University of Targoviste).

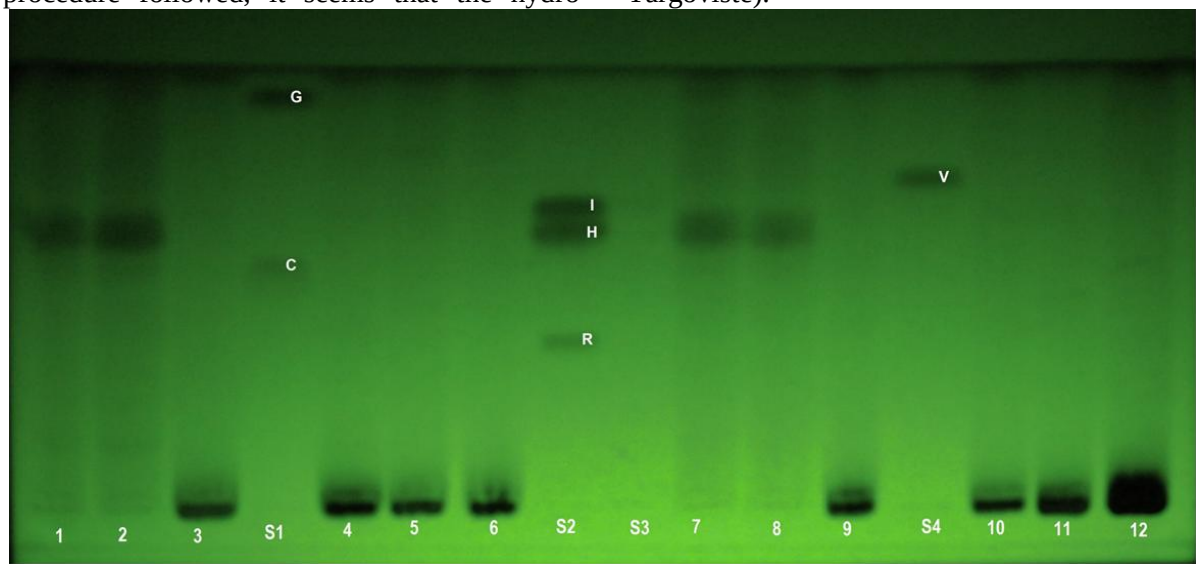

Fig. 2. *High performance thin layer chromatography image of selected chromatographic plates obtained for the extracts – visualized with ultraviolet light at 254 nm. Samples are named according to Table 1. Samples 1, 2, 7 and 8 were concentrated and samples 4 to 6 and 9 to 12 were concentrated and purified using silica gel cartridges (see main text). Standards are marked as following: C=chlorogenic acid; G=gallic acid; R=rutoside; H=hyperoside; I=isoquercitroside; L=luteolin 7-O-glucoside; V=vitexin; U=umbelliferone. The silica gel plate was developed with ethyl acetate: acetic acid anhydrous: formic acid: water (72:7:7:14) and derivatized with 2-aminoethyl diphenyl borate.*

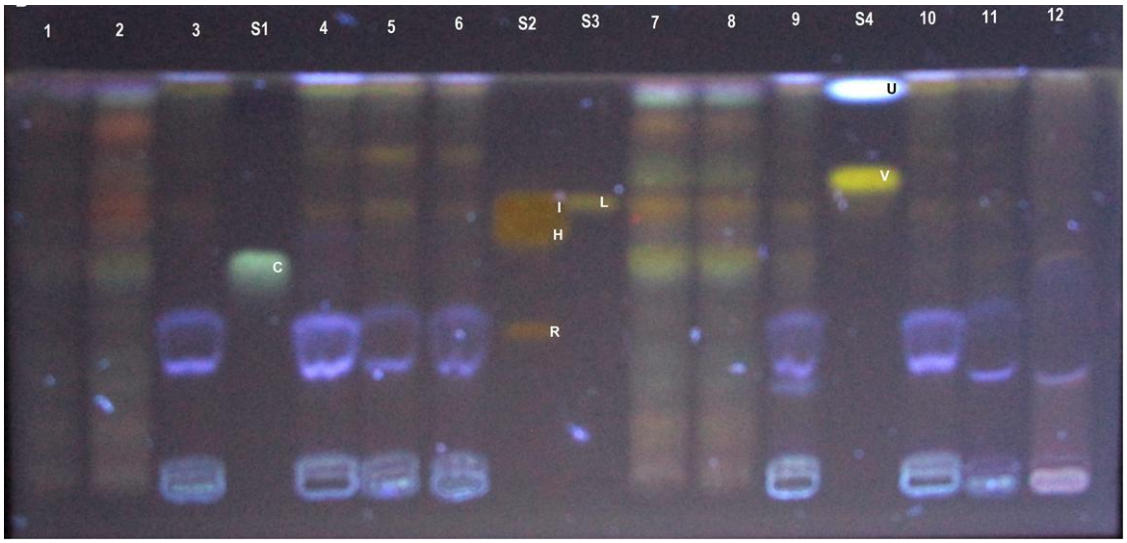

Fig. 3. *High performance thin layer chromatography image of selected chromatographic plates obtained for the extracts – visualized with ultraviolet light at 365 nm. Samples are named according to Table 1. Samples 1, 2, 7 and 8 were concentrated and samples 4 to 6 and 9 to 12 were concentrated and purified using silica gel cartridges (see main text). Standards are marked as following: C=chlorogenic acid; G=gallic acid; R=rutoside; H=hyperoside; I=isoquercitroside; L=luteolin 7-O-glucoside; V=vitexin; U=umbelliferone. The silica gel plate was developed with ethyl acetate: acetic acid anhydrous: formic acid: water (72:7:7:14) and derivatized with 2-aminoethyl diphenyl borate.*

High performance thin-layer chromatograpy analysis showed (Figures 1 and 2, Table 3) that the hydro-alcoholic extracts obtained by rapid extraction under pressure at 6.7 bar (samples 7 and 8), contain chlorogenic acid and umbelliferone in higher concentrations compared with the same extracts obtained by ultrasound-assisted extraction method (i.e. extracts 1 and 2). Isoquercitroside (3,3',4',5,7-pentahydroxi-3-beta-D-glucofuranoside flavone) could be identified in all the extracts, except for samples 1 and 12.

The highest yield of total flavonoids from concentrated lavender extract obtained was obtained by supercritical fluid extraction. Good values was obtained for hydro-alcoholic lavender extract (i.e. extracts 7 and 8) obtained by rapid extraction under pressure at 6.7 bar (Table 2).

Table 2. *Qualitative analysis of total phenolic compounds in lavender extracts*

Sample	Total flavonoids [µg/mg total extract]	
	Mean	Standard Deviation
Ultrasound-assisted extraction		
1	7.245	0.034
2	11.433	0.026
3	1.209	0.011
4	1.062	0.043
5	1.734	0.026
6	1.712	0.012
Rapid extraction under pressure at 6.7 bar		
7	32.673	0.804
8	31.972	0.756
9	1.562	0.018
10	1.762	0.012
11	1.145	0.024
12	1.435	0.035
Supercritical fluid extraction		
Extract in 1,1,1,2-tetrafluorethane	78.345	0.982

Conclusions

In the present study three types of extracts from flowers of Lavandula angustifolia sp. were prepared by different extraction methods (i.e. ultrasound-assisted extraction, rapid extraction under pressure at 6.7 bar and supercritical fluid extraction). The main phenolic compounds identified in analyzed extracts were as follows: chlorogenic acid, gallic acid, umbelliferone, luteolin 7-O-glucoside, vitexin and isoquercitroside (completing the studies will lead to the conclusion being finalized).

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