IN VITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS FROM SOME *LAVANDULA* SPECIES CULTIVATED IN ROMANIA

SILVIA ROBU[1,2], ANA CLARA APROTOSOAIE[2,*], ANCA MIRON[2], OANA CIOANCĂ[2], URSULA STĂNESCU[2], MONICA HĂNCIANU[2]

[1]*Dunarea de Jos University, Galati, Faculty of Medicine and Pharmacy, 35 Al. I. Cuza Street, Galati, Romania, 800010*
[2]*Faculty of Farmacy, Gr.T.Popa University of Medicine and Pharmacy Iasi, 16 Universitatii Street, Romania, Iasi, 700115*
**corresponding author: anaclara70@yahoo.com*

Abstract

The antioxidant activity of five samples of *Lavandulae flowers* (*L. angustifolia* ssp. *angustifolia*, L. *hybrida*, *L. angustifolia* spp. p*yrenaica*, *L. angustifolia* spp. *angustifolia* cv. Munstead and cv. Hidicote Blue) harvested from Romania was investigated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and ferrous ion chelating assays. Total polyphenolic content (mg/g dried extract) was also determined. In both antioxidant assays, all lavender extracts showed significant and concentration-dependent activities. The ferrous ion chelating effect was superior to DPPH radical scavenging effect. Ethanolic extract from *Lavandula hybrida* was found to be the most active (IC_{50} = 73.53 μg/mL in DPPH scavenging assay and IC_{50} =49.90 μg/mL in ferrous ion chelating assay). Except *Lavandula hybrida* and *Lavandula angustifolia* cv. Munstead, there is no strict positive relationship between the polyphenolic content and antioxidant activity of extracts.

Rezumat

Extracte etanolice din cinci specii de *Lavandula* (*L. angustifolia* ssp. *angustifolia*, *L. hybrida*, *L. angustifolia* spp. *pyrenaica*, *L. angustifolia* spp. *angustifolia* varietatile Munstead şi Hidicote Blue) cultivate în Romania au fost investigate în ceea ce priveşte activitatea antioxidantă şi conţinutul în polifenoli totali. Au fost utilizate testul de scavenger de radicali DPPH (2,2-difenil-1-picrilhidrazil) şi cel de chelatare a ionilor Fe^{2+}. Toate extractele de lavandă prezintă o activitate antioxidantă importantă şi dependentă de concentraţie. Acţiunea de chelatare a ionului feros este superioară celei de *scavenger* de radicali DPPH. Cea mai activă specie este *Lavandula hybrida* (CI_{50} = 73.53 μg/mL şi respectiv, CI_{50} =49.90 μg/mL). Cu excepţia extractelor de *Lavandula hybrida* şi *Lavandula angustifolia* varietatea Munstead, nu există o corelaţie strict pozitivă între conţinutul în polifenoli şi proprietăţile antioxidante ale extractelor de lavandă.

Keywords: antioxidant, *Lavandula* specis, polyphenolic content

Introduction

The oxidative damage of various biomolecules (lipids, proteins, RNA and DNA) in the human body is associated with lipid peroxidation,

cell structural injury, tissue impairment and gene mutation. Free radicals play a crucial role in aging, as well as many diseases conditions (cardiovascular disorders, cancer, neurodegenerative diseases, inflammation) [6]. In addition, lipid oxidation initiated by free radicals is one of the major factors for food deterioration during processing and storage [4].

There is an increasing interest in the antioxidant effects of compounds derived from plants, which could be relevant in relation to their role in health and disease and their nutritional value. Different aromatic herbs and spices have been investigated for their antioxidant activity. Some, particularly those belonging to the *Lamiaceae* family have been found to be very effective with regard to natural antioxidants.

The genus *Lavandula*, an important member of family *Lamiaceae* consists of about 30 species and 100 varieties, mainly cultivated for their essential oils. In addition to the essential oil with great economic value, *Lavandula* plants contain phenolic compounds known for their general antioxidant abilities. In medicine, *Lavandula* is used for the spasmolytic, carminative, stomachic or diuretic properties, and nowadays is applied as a mild sedative and cholagogue in various phytopharmaceuticals [1,2].

The aim of this work was to examine five *Lavandula* species from Romania for their *in vitro* possible antioxidant activity. Two assays were carried out on ethanolic extracts: DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging assay and ferrous-chelating assay. The relationship between the antioxidant activity and the total content of polyphenols was also studied.

Material and Methods

Chemicals and apparatus

All chemicals and reagents were of analytical grade and were purchased from Sigma Aldrich Chemical Co. (Germany) and Merck (Germany). All spectrophotometric measurements were performed on ABLE-JASCO V 550 UV-VIS spectrophotometer.

Plant materials

The plant materials used in this study were flowers of lavender plants growing in Botanical Garden from Galati, Romania. Harvest was done in the first half of June 2010, at flowering stage. Collected plant material was air-dried and stored at room temperature without exposure to direct sunlight. The species were identified and the voucher specimens were

deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Gr. T. Popa University of Medicine and Pharmacy, Iasi, Romania. The lavender species involved in this study were: *Lavandula angustifolia* ssp. *angustifolia* (LAA), *Lavandula angustifolia* ssp. *angustifolia* cv. Munstead (LAM), *Lavandula angustifolia* ssp. *angustifolia* cv. Hidicote Blue (LHB), *Lavandula angustifolia* ssp. *pyrenaica* (LAP) and *Lavandula hybrida* (LAH).

Preparation of the extracts

The air-dried flowers of the plants (2 g) were ground into a fine powder in a mill and were extracted with 90 mL ethanol 50% (V/V), at 85°C for 1 h. After cooling, extracts were filtered; the filters were rinsed with 10 mL ethanol 50% (V/V) and the filtrates and rinsings were added in a volumetric flask and diluted to 100 mL with the same solvent. An aliquot of 80 mL from each extract was concentrated using a rotary evaporator (Buchi R 210, Switzerland) to remove the solvent. The residues were dried and stored at 4°C for subsequent analysis.

Determination of total phenolic content

The total phenolic content of each lavender extract was determined with Folin-Ciocalteu reagent according to the Singleton & Rossi method [7] using gallic acid as a standard. 40 µL-aliquot of each extract was diluted with distilled water (3.16 mL) in test tubes. 200 µL of Folin-Ciocalteu reagent was added and the content was mixed. After 5 min, 600 µL of Na_2CO_3 20% was added then the mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 765 nm versus blank sample. The blank consisted of all reagents and solvents, but without Na_2CO_3. A standard curve was calculated using gallic acid concentrations ranging 3-6 µg/mL. The results were expressed as gallic acid equivalents (mg gallic acid per gram of dried extract).

Determination of DPPH free-radical scavenging activity

The antioxidant activity of the extracts based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Malterud et al. [5]. Initially, four dilutions in DMSO (20 mg/mL; 10 mg/mL; 5 mg/mL and 2.5 mg/mL) were carried out with the dried extracts of *Lavandula* flowers. Briefly, an aliquot of each dilution (0.05 mL) was mixed with a solution of DPPH in methanol (4 mg%) (A_{517} = 1.02; 2.95 mL) and the absorbance was measured at 517 nm for 5 min. Gallic acid and quercetin were used as reference standards

and dissolved in DMSO to make solutions within the same range of concentrations (2.5 mg/mL-20 mg/mL). Methanol was used as blank. The DPPH free-radical scavenging activity (%) was calculated as 100 x [(A_{start}-A_{end})/A_{start}], where A_{start} is the absorbance before addition of extract dilution and A_{end} is the absorbance value after 5 min of reaction time. The IC_{50} value (μg/mL), which is the concentration of the extract/standard that reduces 50% of the free-radical concentration, was calculated through linear interpolation between values above and below 50% activity [5,8].

Measurement of ferrous ion chelating activity

The iron-chelating abilities of the lavender extracts and standards was estimated by the method of Dinis et al. [3] with minor changes. Four dilutions in DMSO (20 mg/mL, 10 mg/mL, 5 mg/mL and 2.5 mg/mL) were prepared from the dried extracts. Briefly, 0.05 mL of each dilution was added to a 2.7 mL TRIS buffer (pH=7.4). Thereafter, 0.05 mL of 2 mM $FeCl_2$ were added and vortexed for 15 sec. At 30 sec, the reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), the mixture was shaken vigourously at Vortex (Velp Scientifica,UE) for 10 sec. After 1 min beyond addition of $FeCl_2$ solution, absorbance of the solution was measured spectrophotometrically at 562 nm. The ability of extracts to chelate ferrous ion was calculated relative to the control (consisting of TRIS buffer, iron and ferrozine only) using the formula: chelating activity (%) = 100 x [(A_C-A_S)/A_C], where A_C is the absorbance of the control, and A_S is the absorbance of the sample (extract or standard). The IC_{50} value (μg/mL), which is the concentration of the extract/standard that chelate 50% of the ferrous ion, was calculated through linear interpolation between values above and below 50% activity.

All results are presented as means ± standard deviations of three determinations.

Results and Discussion

Total polyphenol content

It has been recognized that the total phenolic content of plant extracts is associated with their antioxidant activities due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. As shown in table I, the total phenolic content (TPC) ranging from 74.98 mg/g to 89.88 mg/g of dry extract. The lowest TPC was found in *Lavandula angustifolia* ssp. *angustifolia* (74.98 mg/g of dry extract).

Table I

The total phenolic content of examined *Lavandula* extracts

Sample	Abbreviation	mg total polyphenols/g of dried extracts
Lavandula angustifolia ssp. *angustifolia*	**LAA**	74.98±0.27
Lavandula angustifolia ssp. *angustifolia* Munstead	**LAM**	80.70 ±0.48
Lavandula angustifolia ssp. *angustifolia* Hidicote Blue	**LHB**	85.76±0.35
Lavandula angustifolia ssp. *pyrenaica*	**LAP**	89.37±0.15
Lavandula hybrida	**LAH**	89.88±0.27

DPPH free-radical scavenging activity

The ability of *Lavandula* extracts to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity test. The antioxidants can react with DPPH, a violet coloured stable free radical, converting it into a yellow coloured α,α-diphenil-β-picrylhydrazine. The discolouration of the reaction mixture can be quantified by measuring the absorbance at 517 nm, which indicates the radical-scavenging ability of the antioxidant.

All the extracts were shown to possess significant DPPH radical-scavenging activity (Table II). At a concentration of 20 mg/mL, all extracts exhibited the highest percent scavenging effects (85.18%-91.39%), while the concentration of 2.5 mg/mL yielded the lowest activity (21.41%-31.72%).

Table II

Free-radical scavenging activity of the *Lavandula* extracts

Sample/Standard	Concentration (mg/mL)				IC_{50} (μg/mL)
	20	10	5	2.5	
LAA	85.41±0.13	66.89±0.72	45.79±0.50	26.87±0.78	95.60±1.70
LAM	85.18±0.20	68.11±0.68	42.79±0.19	21.41±0.20	101.40±0.90
LHB	88.50±0.50	67.60±0.44	45.22±0.48	27.32±0.26	96.53±1.45
LAP	85.98±0.43	65.12±0.97	39.60±0.13	24.40±0.15	110.36±1.40
LAH	91.39±0.50	76.18±0.77	51.91±0.75	31.72±0.36	73.53±1.25
Gallic acid	97.34±0.06	97.23±0.07	97.21±0.01	96.24±0.34	1.50±0.00
Quercetin	96.25±0.35	96.04±0.08	95.54±0.47	94.02±0.03	3.40±0.00

The scavenging effect increased with increasing concentration of the extract. However, scavenging activity of gallic acid and quercetin, well-known antioxidants, were relatively more pronounced than that of extracts (figure 1).

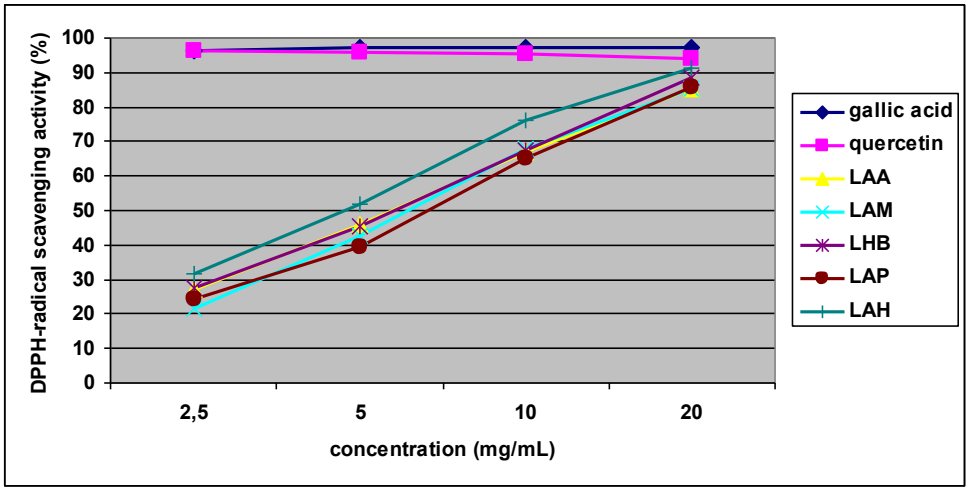

Figure 1
DPPH scavenging activity (%) of *Lavandula* extracts and standards

Ferrous ion chelating activity

Ferrous ions are one of the most effective pro-oxidants; their interaction with hydrogen peroxide in biological systems can lead to formation of highly reactive hydroxyl radicals. Ferrozine is a ferroin compound that can quantitavely form stable magenta-coloured complexes with ferrous ion (Fe^{2+}). In the presence of other chelating agents, the complex formation is disrupted and the colour of the complex decreases. Measurement of the rate of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator. In this study, the iron-chelating capacity assay was used to evaluate the ability of lavender antioxidants to disrupt the formation of the complexes or to prevent interaction between metal and lipids.

All extracts interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity. The strongest iron chelating activity was noticed at a concentration of 20 mg/mL (96.72%-98.30%), while the concentration of 2.5 mg/mL exhibited the lowest activity (36.81%-42.75%) (Table III). As shown in figure 2, the ferrous ion chelating activity increased with the increasing concentration. Unlike the DPPH assay, the iron chelating ability of lavender extracts is more pronounced even for concentrations of 5 mg/mL. At a concentration of 20

mg/mL, all extracts exhibited activity values close or slightly higher to those the gallic acid and quercetin.

Table III

Ferrous chelating capacity (%) of lavender extracts

Sample/Standard	Concentration (mg/mL)				IC_{50} (µg/mL)
	20	10	5	2.5	
LAA	97.95±0.13	96.80±0.22	70.91±0.33	36.81±0.57	54.46±0.55
LAM	96.96±0.64	94.07±0.54	80.11±0.87	38.32±0.34	50.60±0.40
LHB	98.21±0.10	97.24±0.24	73.00±0.42	42.75±0.93	49.93±0.75
LAP	96.72±0.42	93.84±0.08	50.32±0.32	37.10±0.33	81.90±1.40
LAH	98.30±0.29	97.10±0.24	73.00±0.42	41.94±0.93	49.90±0.90
Gallic acid	98.61±0.16	97.36±0.12	97.09±0.41	97.03±0.03	3.36±0.05
Quercetin	97.39 ±0.36	96.60 ±0.30	93.36±0.89	91.98±0.35	6.03±0.0.0

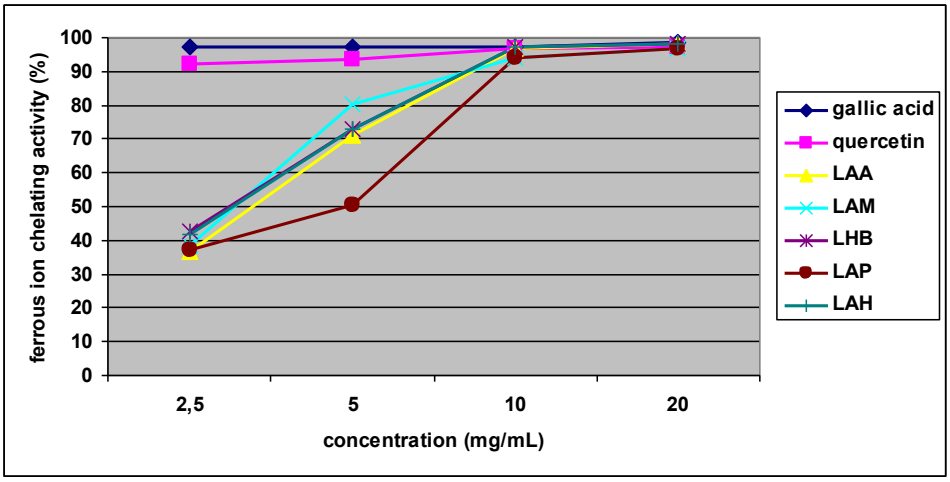

Figure 2

Iron-chelating activity (%) of lavender extracts and standards (LAH and LHB curves are superimposed; in 10-20 mg/mL concentration range, LAH, LHB, LAM and LAA samples showed overlapped curves)

In both *in vitro* assays, the most active plant was found to be *Lavandula hybrida* (IC_{50} = 73.53 µg/mL in DPPH scavenging assay and IC_{50} =49.90 µg/mL in ferrous ion chelating assay) (Tables II and III).

The ethanolic extract of *Lavandula hybrida*, which had highest polyphenol content (89.88 mg/g extract) exhibited the strongest antioxidant activity, particularly in the chelating of ferrous-ion. However, there is not

always a positive relationship between total polyphenolic content of extracts and their antioxidant abilities.

Conclusions

All ethanolic lavender extracts exhibited significant and concentration-dependent DPPH radical scavenging and iron-chelating activities. The ferrous ion chelating effect was superior to the DPPH radical scavenging effect.

The most active plant was found to be *Lavandula hybrida* (IC_{50} = 73.53 μg/mL in DPPH scavenging assay and IC_{50} = 49.90 μg/mL in ferrous ion chelating assay). Except *Lavandula hybrida* and *Lavandula angustifolia* cv. Munstead, there is not a strict positive relationship between total polyphenolic content of extracts and their antioxidant abilities. This suggests that in addition to polyphenolic content, the spectrum of polyphenolic compounds and the ratio between them are equally important for determining the antioxidant potential.

References

1. Basch E., Foppa I., Liebowitz R., Lavender (Lavandula angustifolia Miller), *J Herb Pharmacother*, 2004, 4(2), 63-68
2. Cavanagah H.M., Wilkinson J.M., Biological activities of lavender essential oil, *Phytother Res*, 2002, 16(4), 301-308
3. Dinis T. C. P., Madeira V. M. C., Almeida L. M., Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and peroxyl radical scavengers, *Archives of Biochemistry and Biophysics*, 1994, 315 (1), 161-169
4. Donnelly J.K., Robinson D.S., Free Radicals in foods, *Free Radic Res*, 1995, 22 (2), 147-176
5. Malterud K.E., Farbrot T.L., Huse A.E., Sund R.B., Antioxidant and radical scavenging effects of anthraquinones and anthrones, *Pharmacology*, (1993). 47, 77-85
6. Pham-Huy L.A., He H., Pham-Huy C., Free radicals, antioxidants in disease and health, *Int J Biomed Sci*, 2008, 4 (2), 89-96
7. Singleton V.L., Rossi J.A., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am J Enol Vitic*, 1965, 37, 144-158
8. Wangensteen H., Samuelsen A.B., Malterud K.E., Antioxidant activity in extracts from coriander, *Food Chem,* 2004, 88, 293-297.

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