Romanian Biotechnological Letters *Vol. 15, No.3, 2010*

ORIGINAL PAPER

Evaluation of antioxidant and free scavenging potential of some Lamiaceae species growing in Romania

Received for publication, October 09, 2009
Accepted, June 5, 2010

A. ARMATU[1], S. COLCERU-MIHUL[1], C. BUBUEANU[1], E. DRAGHICI[1], L. PIRVU[1]
[1]National Institute for Chemical-Pharmaceutical R&D (ICCF-Bucharest), Vitan Road 112, Sector 3, Bucharest, Romania, email: alicearmatu@yahoo.com

Abstract

Antioxidative effects of crude extracts from some Romanian Lamiaceae species have been studied with the use of three in vitro assays - DPPH reduction spectrophotometric assay, phosphomolybdenum method and chemiluminescence assay. Also, HPTLC fingerprints and total phenolic content of the extracts were analyzed in order to establish a relationship between chemical composition and pharmacological activity. All the assays confirmed the good antioxidant potential of the Lamiaceae species and in particular of the Nepetoideae subfamily. The most efficient activity was exhibited by Salvia officinalis L., Rosmarinus officinalis L. and Thymus vulgaris L. extracts, probably due to a high content of polyphenols. On the other hand, Lamioideae species like Lamium album L. and Leonurus cardiaca L. have a polyphenolic low content and, consequently, prove a weak or no antioxidant activity. The results were different depending on the applied test. Although Origanum vulgare L. has the highest content of polyphenols expressed as gallic acid, it showed a good scavenging activity only on DPPH free radical.

Keywords: Lamiaceae, Nepetoideae, Lamioideae, antioxidant, scavenger

Introduction

Lamiaceae (syn. Labiatae) herb family consists of more than 200 genera and 3500 species [1]) and include eight subfamilies: Ajugoideae, Chloranthoideae, Lamioideae, Nepetoideae, Pogostemonoideae, Scutellarioideae, Teucrioideae, and Viticoideae. Over 47% of the Lamiaceae fall within the subfamily Nepetoideae, as do most of the more familiar genera, many of them economically important – *Lavandula, Melissa, Origanum, Rosmarinus, Salvia, Thymus*. The subfamily Lamioideae includes other 2 genera studied in the present paper – *Leonurus* and *Lamium*.

These herbs have been used also in traditional medicine, but are mainly known for their culinary properties. For example oregano, rosemary, sage and thyme are typical seasonings especially in the Mediterranean region. These herbs have a GRAS status given by the U.S. Food and Drug Administration (U.S. Food and Drug Administration, 2006), meaning that they are generally recognized as safe for human consumption without limitations on intake.

Species belonging to the Nepetoideae subfamily produce a great variety of secondary compounds, but are especially renowned for their essential oils. Instead, most species of the Lamioideae produce iridoids. The polyphenols rich content contributes to the beneficial effects of Lamiaceae herbs; the most common group of phenolics are caffeic acid esters and flavonoids. Caffeic acid esters provide important chemosystematic markers at the subfamily level; the two major types are rosmarinic acid and phenylethyl caffeoylglycosides. The presence of rosmarinic acid, a powerful antioxidant, is almost entirely restricted to Nepetoideae[2].

It was found that *O. vulgare* had a higher content of rosmarinic acid (1.6 per cent w/w) than *Rosmarinus* and *Salvia* (SKOULA M. et al (2002)[3]). Nine different phenolic acids have

been reported in the genus *Thymus*, caffeic and rosmarinic acids being those more frequently found (in 29 and 20 species, respectively) [4].

Rosmarinic acid and chlorogenic acid are regularly present also in the leaves of *Lavandula* species; rosmarinic acid was detected in leaves of five out of six species, while chlorogenic acid occurs in four out of the six 5].

As other characteristic of the Lamiaceae, significantly more flavones than flavonols have been found[3].

It is generally recognized that these vegetal compounds have a remarkable potential of protection from oxidative stress and thus play an important role in the chemoprevention of diseases that has their etiology and pathophysiology in reactive oxygen species. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human disorders.

In the present study, the antioxidant activity of the crude extracts obtained from some Lamiaceae species was investigated using DPPH scavenging assay, chemiluminescence induced by H_2O_2-luminol system and by determining total antioxidant capacity of the extracts. Also, total phenolics content and HPTLC fingerprint profiles were determined.

Materials and methods

Plant material

The dried plant material from following species was studied: *Origanum vulgare* L. (Origani herba), *Melissa officinalis* L. (Melissae herba), *Rosmarinus officinalis* L. (Rosmarini herba), *Salvia officinalis* L. (Salviae herba), *Thymus vulgaris* L. (Thymi herba), *Lamium album* L. (Lamii flos), *Leonurus cardiaca L.* (Leonuri Herba). The materials were obtained from indigenous crops (Bucharest, Romania).

Chemicals and apparatus

Folin-Ciocalteu's phenol reagent, 2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH), luminol, rosmarinic acid, caffeic acid, gallic acid, ascorbic acid, quercetin and rutin were purchased from Sigma Aldrich-Fluka. For absorbance measurements, a Helios Gamma UV/VIS spectrophotometer was used. For chemiluminescence measurements, a Turner Biosystem 20/20 Analyzer was used. A HPTLC scanner with computer system and WinCats Version Software were obtained from Camag (Muttenz, Switzerland). Camag Linomat V was used as applicator. Separation was done on silica gel F254 HPTLC precoated plates purchased from Merck (Germany).

Samples preparation

2 g of raw material in 20 ml of methanol were ultrasonicated for 30 min at 40°C and filtrated. The crude extracts were concentrated under reduced pressure (72-74 mmHg), dissolved in methanol/ 50% ethanol and used for further investigations.

HPTLC analysis

Chromatography was performed on silica gel F254 HPTLC pre-coated plates. Samples were applied on the plates as band of 7mm width using a Camag Linomat V sample applicator at the distance of 14mm from the edge of the plates. The mobile phase was constituted of ethyl acetate-acetic acid-formic acid-water 100:11:11:27 (v/v/v/v). After development, plates were dried and derivatised in NP-PEG reagent.

The fingerprints were evaluated at 366nm in fluorescence mode with a WinCats and VideoScan software. Reference compounds for HPTLC analysis were rosmarinic acid and caffeic acid (0,2mg/mL).

Determination of Total Phenolics

Total phenolics content was determined according to the Folin-Ciocalteu method, using gallic acid as a standard (JAVANMARDI J. et al. (2003) [6]). The aliquots (500 µl) of each extract were added to the test-tubes containing 2.5 ml of Folin–Ciocalteau reagent and 2 ml of 7.5% sodium carbonate.

The absorbance at 740 nm was measured after standing for 30 min. The total phenolic content was expressed as gallic acid equivalents (GAE) by reference to the gallic acid standard calibration curve in milligrams per gram sample.

Antioxidant potential assay

The antioxidant power of the extracts has been assessed with the phosphomolybdenum reduction assay according to Prieto et al. (PRIETO P. et al (1999)[7]). The reagent solution contained ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulfuric acid (0.6 M) mixed with the extracts diluted in 50% ethanol solution at the concentration of 5mg/ml. The samples were incubated for 90 min at 90 °C and the absorbance of the green phosphomolybdenum complex was measured at 695 nm.

For reference, the appropriate solutions (0.2-2mM) of ascorbic acid have been used. The reducing capacity of the extracts has been expressed as the ascorbic acid equivalents (milligrams per gram extract).

Free radical scavenging assay

The extracts were diluted in methanol solution at the concentration of 5mg/mL and 1mg/mL. 50 µl aliquots of the extract were mixed with 2950 µl of the methanolic DPPH solution (0.025g/L).

The reduction of the DPPH free radical was measured by reading the absorbance at 517nm and related to the absorbance of the control without the herbal drugs. Inhibition ratio (percent) was calculated from the following equation: % inhibition [(absorbance of control – absorbance of sample)/absorbance of control] x 100%. Quercetin and rutin methanolic solution were used as positive controls.

Chemiluminescence assay

The extracts were diluted in 50% ethanol solution at the concentrations of: 5mg/mL, 1mg/mL and 1µg/mL. The assay contained sodium carbonate buffer (0.1 M, pH 7.8), 8mM luminol together with 5mM hydrogen peroxide and the respective vegetal extract or standard compound (rutin at the concentration of 0.2mg/mL). The chemiluminescence (CL) was measured on a Turner Biosystem 20/20 Analyzer and related to the chemiluminescence of the control without the herbal drugs (corresponding 100%).

Results and discussions

HPTLC analysis

The fingerprint of the constituents present in samples was recorded using Camag TLC visualizer and WinCats Software.

The chromatograms (Figure 1) showed spots which are characteristic for several Lamiaceae species. In particular, rosmarinic acid and caffeic acid appear as intense fluorescent blue spots (Rf-value 0,91 and 0.96, respectively) on tracks corresponding to Nepetoideae subfamily members. *Origanum vulgare, Melissa officinalis, Salvia officinalis* and *Rosmarinus officinalis* extracts contain high amounts of rosmarinic acid comparing to *Lamium album* and *Leonurus cardiaca* extracts, members of Lamioideae subfamily (profiles comparison in Figures 2 and 3).

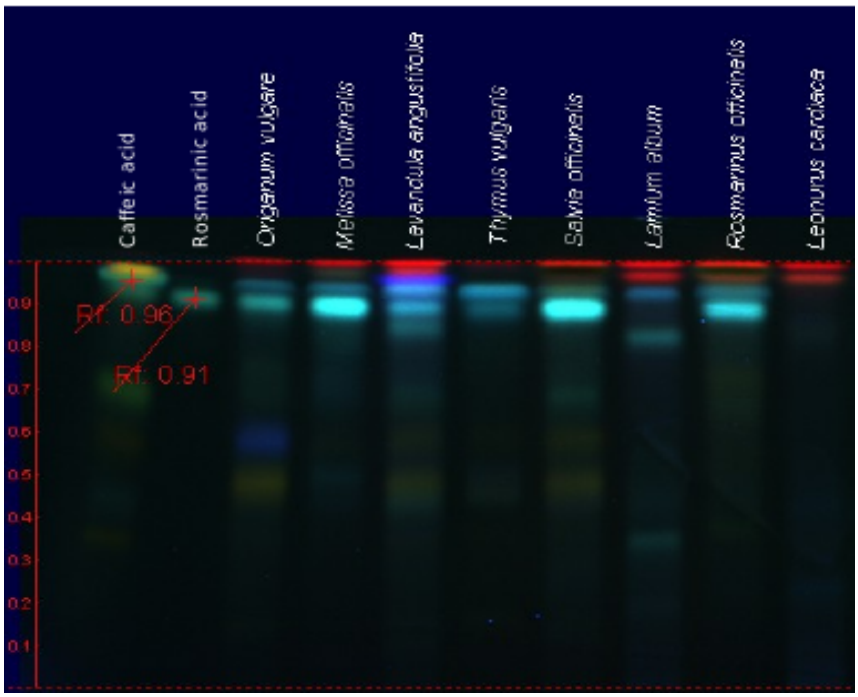
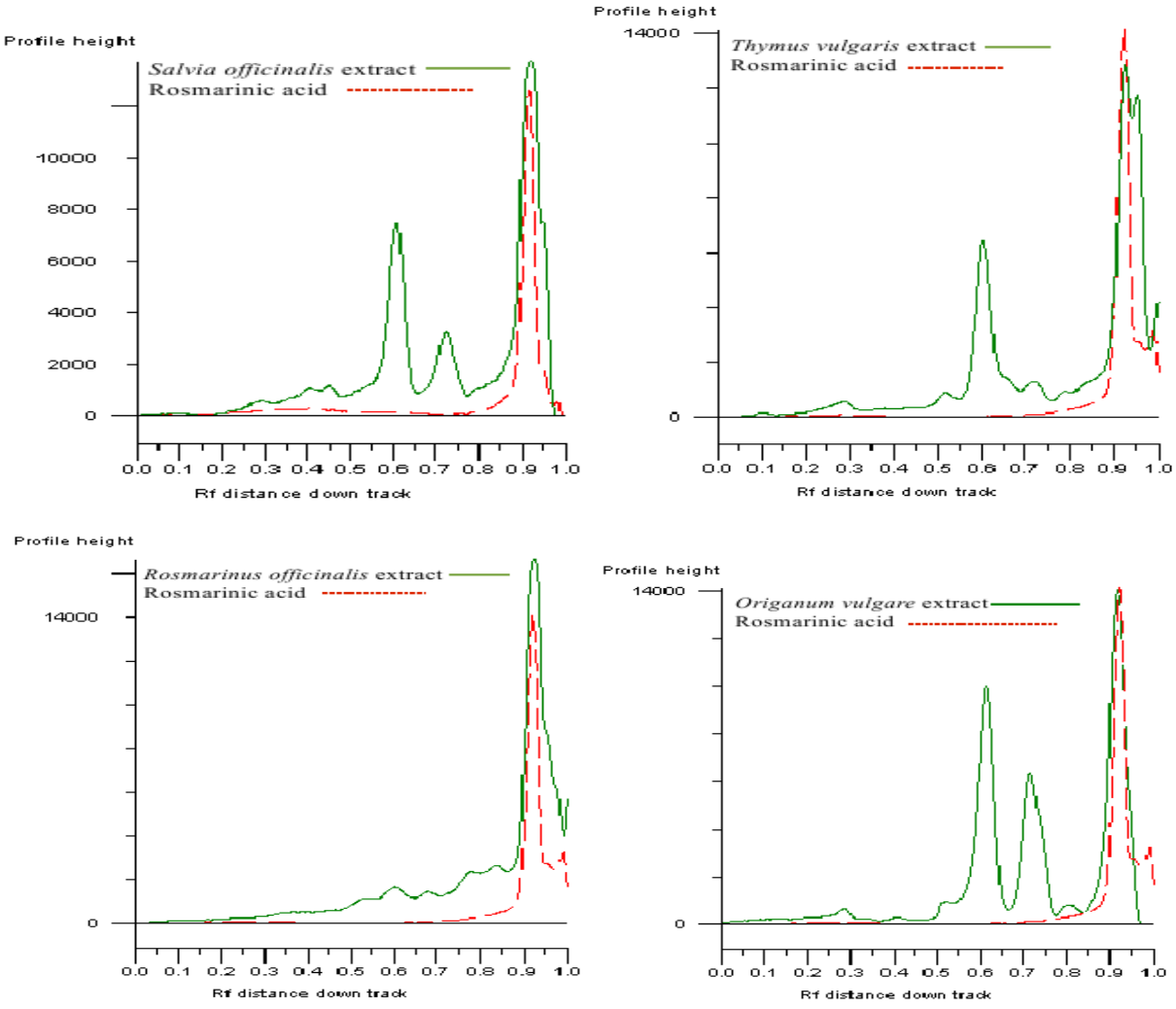


Figure 1. HPTLC fingerprint of some Lamiaceae species, after derivatization with NP-PEG, 366nm



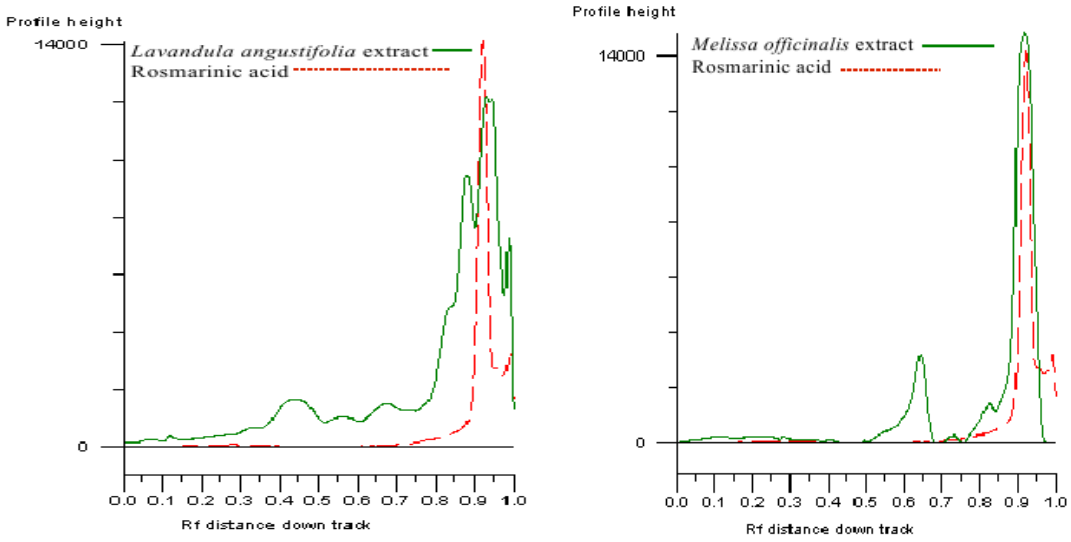


Figure 2. HPTLC profiles for each studied Nepetoideae species

Rosmarinic acid is not characteristic for Lamioideae subfamily – *Leonurus cardiaca* and *Lamium album* as it is showed in figure 3.

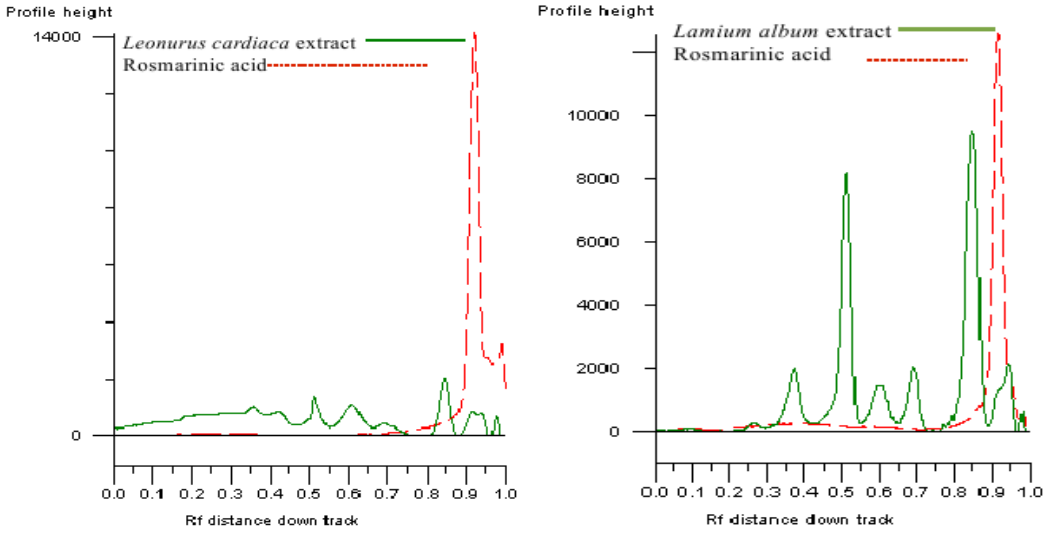


Figure 3. HPTLC profiles for each studied Lamioideae species

Determination of Total Phenolics

The results of the Folin–Ciocalteu total phenols photometric assay are presented in table 1.

Table 1. Polyphenol content in alcoholic extracts expressed as mg of gallic acid equivalents per g extract

Origanum vulgare	*Melissa officinalis*	*Lamium album*	*Salvia officinalis*	*Rosmarinus officinalis*	*Thymus vulgaris*	*Lavandula officinalis*	*Leonurus cardiaca*
180	22	4.6	32	22	32	7.8	2.8

Antioxidant potential assay

Total antioxidant capacity of the methanol extract is given in Figure 4. Total antioxidant capacity of the extracts is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. All the extracts showed increase in antioxidant capacity with

increase in dose. At 5mg/mL concentration, *Rosmarinus officinalis, Salvia officinalis, Origanum vulgare, Thymus vulgaris* and *Melissa officinalis* extracts exhibited significant antioxidant activity and the effect was maintained at 1mg/mL, especially regarding *Thymus vulgaris* extract. *Leonurus cardiaca* and *Lamium album* showed weak or no antioxidant activity.

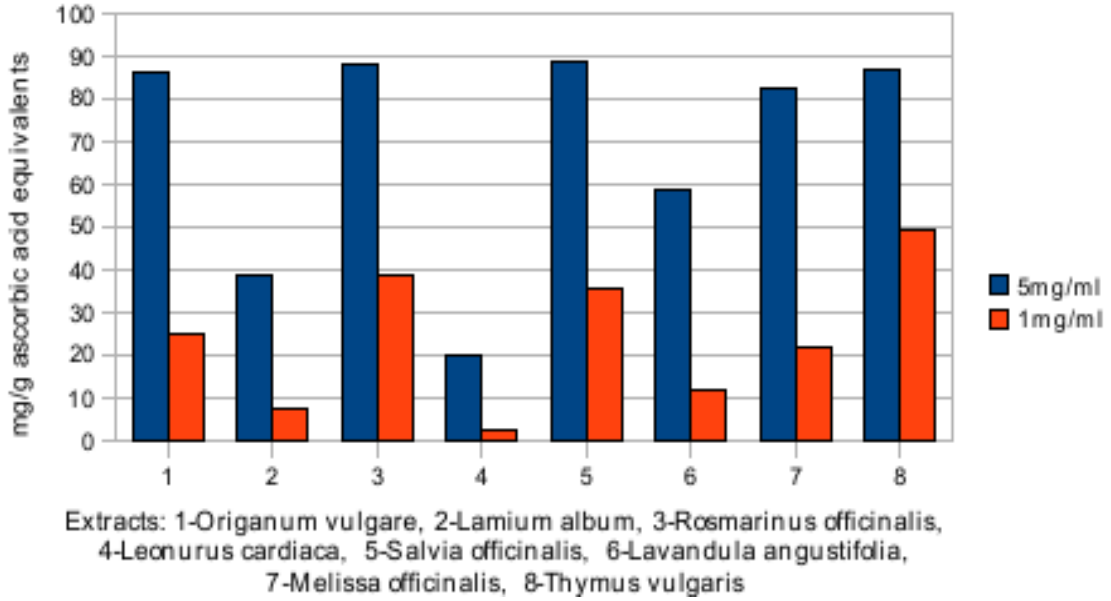


Figure 4. Total antioxidant capacity of the Lamiaceae extracts at different doses

Free radical scavenging assay

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. Comparison of the antioxidant activity of the extracts (at a dose of 5mg/mL and 1mg/ mL, respectively) and various reference standards (at doses of 0.2mg/mL and 0.04mg/mL) is shown in Figure 5. The methanol extract of *Rosmarinus officinalis, Salvia officinalis* and *Thymus vulgaris* exhibited an efficient (88.5%, 88.8% and 87.3%, respectively) inhibition of DPPH free radical. Reference standards rutin and quercetin showed significant inhibition activity – 84% and 89%, respectively.

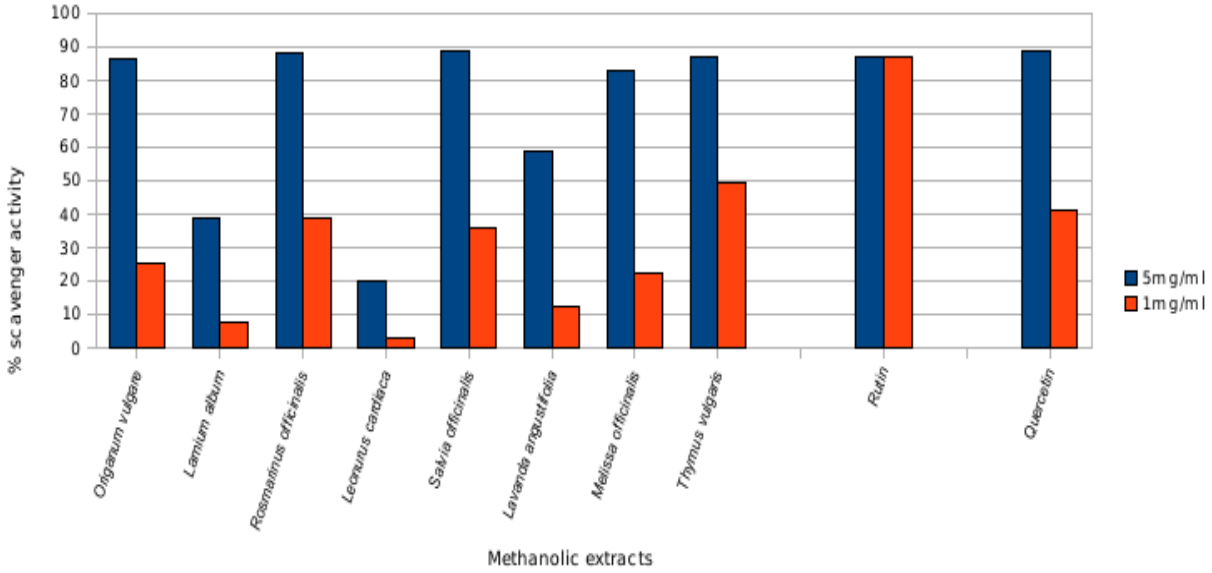


Figure 5. DPPH scavenging activity of the Lamiaceae extracts and standards at different doses

Chemiluminescence assay

All the extracts showed dose-dependent antioxidant activity (Figure 6). Again, the most potent were *Rosmarinus officinalis, Thymus vulgaris* and *Salvia officinalis* extracts. Also, a good inhibitory effect on free radicals had *Lavandula angustifolia* extract. At the opposite pole, *Leonurus cardiaca* exhibited a weak activity(47.8% at 5mg/mL).

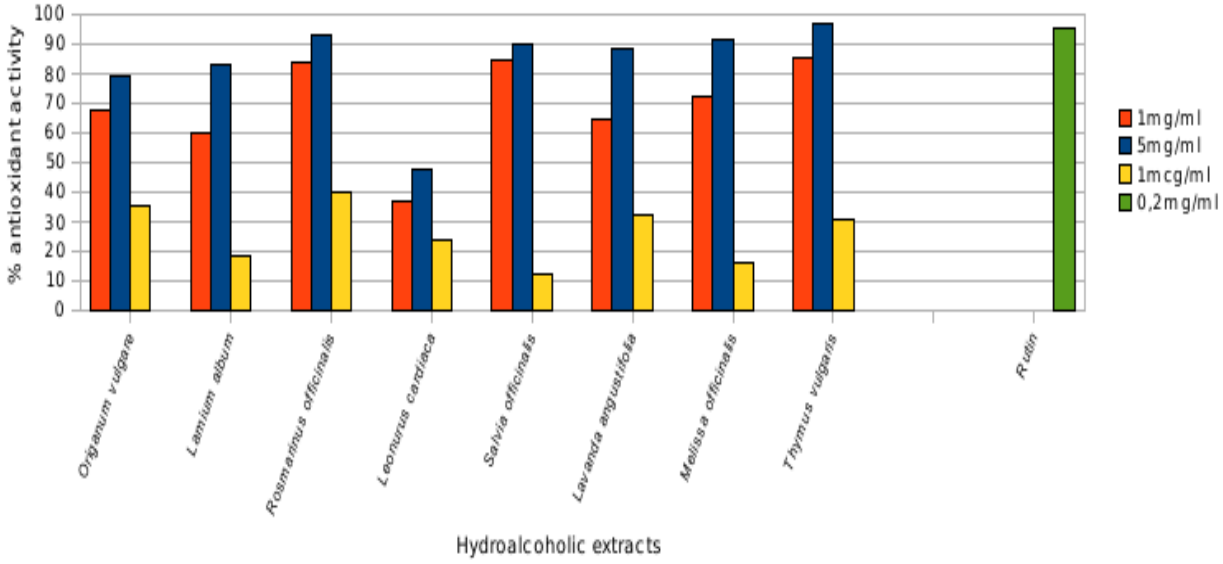


Figure 6. Inhibition of luminol-dependent chemiluminescence by Lamiaceae extracts and standards at different doses

Discussions

All the assays confirmed the good antioxidant potential of the Lamiaceae species and in particular of the Nepetoideae subfamily. The most efficient activity was exhibited by *Salvia officinalis, Rosmarinus officinalis* and *Thymus vulgaris* extracts, probably due to a high content of polyphenols. Although *Origanum vulgare* has the highest content of polyphenols expressed as gallic acid, it shows a good scavenging activity only on DPPH free radical. This fact proves that the antioxidant activity is given not only by flavones and polyphenolcarboxylic acids but it is attributed also to the contribution of other compounds, volatile oils, tri- and diterpenes. Lamioideae species like *Lamium album* and *Leonurus cardiaca* have a polyphenolic low content and, consequently, prove a weak or no antioxidant activity.

The high antioxidant potential of some Lamiaceae species can broaden their applications towards the prevention of degenerative diseases of various organs, but the research needs to be continued in order to analyze the role of different classes of vegetal compounds, other than polyphenols, in the antioxidant protection.

References

1. O. SHARMA, *Plant Taxonomy,* Tata McGraw-Hill Ed. 1993, pp. 358
2. R. HARLEY, S. ATKINS, A. BUDANTSEV, P. CANTINO, B. CONN, R. GRAYER, M. HARLEY, R. de KOK, T. KRESTOVSKAJA, R. MORALES, A. PATON, O. RYDING, T. UPSON, *The families and genera of vascular plants, vol. 7- Lamiales,* J. Kadereit, Ed, Springer Ed. 2004, pp. 182
3. SKOULA M, HARBORNE J., *Medicinal and Aromatic Plants – Industrial Profiles, vol. 25-Origanum,* S. Kintzios, Ed., Taylor&Francis Ed. 2002, pp. 95, 96
4. VILA R, *Medicinal and Aromatic Plants – Industrial Profiles, vol. 24-Thyme,* E. Stahl-Biskup and F. Saez, Eds, Taylor&Francis Ed. 2002, pp. 149
5. HARBORNE J, WILLIAMS C, *Medicinal and Aromatic Plants – Industrial Profiles, vol. 29-Lavandula,* M. Lis-Balchin, Ed, Taylor&Francis Ed. 2002, pp. 92
6. J. JAVANMARDI, C. STUSHNOFF, E. LOCKE, J. VIVANCO, *Food Chem* 83:547 2003
7. P. PRIETO, M. PINEDA, M. AGUILAR. *Anal Biochem* 269:337 1999.